

ELECTROCHEMICAL STUDY OF FACTORS AFFECTING REPRODUCIBILITY OF CAPSAICIN EXTRACTION INTO CARBON PASTES

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Abstract

According to the results obtained, it was observed that extraction reproducibility of lipophilic analytes into carbon pastes usually depends on many factors which reflect the homogeneous distribution of carbon particles in corresponding paste, shape and size of present carbon particles, height of the paste column in an electrode holder and presence of solid contaminants or air bubbles on electrode surface. The effect of all the factors mentioned on the reproducibility of capsaicin extraction was studied by square wave voltammetry. It can be concluded that an extraction of electroactive lipophilic compounds can provide satisfactory reproducibility, thus allowing development of electrochemical methods based on similar kind of accumulation.

Souhrn

Na základě získaných výsledků bylo zjištěno, že reprodukovatelnost extrakce lipofilních analytů do uhlíkové pasty je obvykle ovlivněna mnoha faktory, které odrážejí homogenní distribuci uhlíkových částic v příslušné pastě, pak tvarem a velikostí přítomných uhlíkových částic, výškou sloupce pasty v elektrodovém držáku, přítomností pevných cizorodých částic nebo vzduchových bublin na povrchu elektrody. Vliv výše zmíněných faktorů na reprodukovatelnost extrakce kapsaicinu byl studován pomocí square wave voltametrie. Závěrem lze konstatovat, že extrakce elektroaktivních lipofilních sloučenin může být dostatečně reprodukovatelná, tudíž umožňující vývoj elektrochemických metod založených na podobném principu akumulace.

1. Introduction

Generally, electroanalytical “*ex situ*” methods take place in two separate steps. In the first step, an analyte is accumulated from sample matrix and its electrochemical detection is performed in second following step. Electroplating is a typical accumulation step controlled by constant voltage of the working electrode. It is frequently used in anodic stripping voltammetry for determination of heavy metals [1]. However, accumulation based on other principles may occur as well. For example, an extraction of lipophilic analytes into an electrode material is known, but it is used sporadically due to insufficient reproducibility. For that reason, only a few scientific papers deal with this topic [2,3].

Carbon paste electrodes are typical heterogeneous sensors which can be used for this kind of accumulation because they can be calcified as homogeneous composition of some lipophilic binder and carbon powder [4]. For example, a CPE surface can be modified by thin layer of a surfactant [5], then corresponding CPEs are usable for sensitive determination of numerous significant bioactive compounds with lipophilic properties such as alkaloids [6,7], vitamins [8-10] and drugs [11].

At the beginning, one must remember that extraction of some lipophilic analytes into the CPE is reproducible regardless working conditions such as extraction time, presence of organic solvents, amount of analyte, speed of stirring, amount of paste binder and temperature. Parameters mentioned above are important for the extraction efficiency, e. g., for sensitivity of the final stripping voltammetric measurements. It follows that selected working conditions of the electrochemical technique applied (square wave voltammetry; SWV) influence the sensitivity significantly as well.

In this contribution, different factors affecting the reproducibility of capsaicin extraction into CPE were studied. Capsaicin is a lipophilic alkaloid which is responsible for hot taste of spicy meals [12]. It was chosen as a model analyte because of its electrochemical behaviour at carbon-based electrodes in neutral electrolytes, already described in details (see Fig. 1) [13,14].

From statistical point of view, the reproducibility of extraction carried at various kinds of CPEs is characterized by small range of results obtained within several repetitions (n) at each

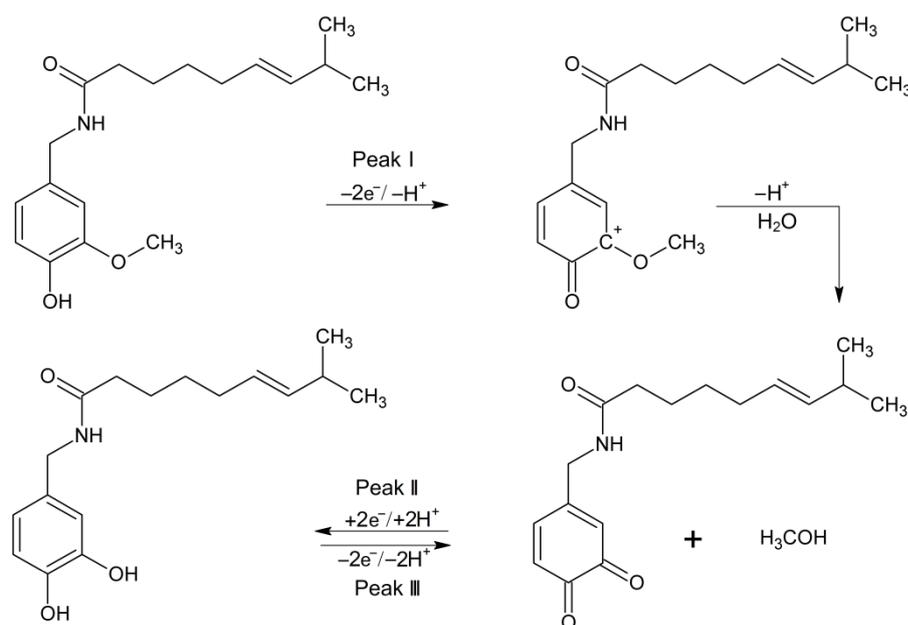


Figure 1. Electrochemical behaviour of capsaicin [13].

electrode under test. Indices of reproducibility (r) defined by formula (1) were calculated from corresponding arithmetic means (\bar{x}) and standard deviations (σ); values higher than 95% are assumed as optimum for significance level $\alpha = 0.05$.

$$r = 100 - (\sigma / \bar{x}) \times 100 \quad (1)$$

2. Experimental

2.1. Chemicals and reagents

The capsaicin (8-methyl-N-vanillyl-*trans*-6-nonenamide) together with paraffin oil (PO) was purchased from Sigma-Aldrich (Prague, Czech Republic). Glassy carbon powder type Sigradur G (particle size $\sim 5 \mu\text{m}$) was from HTW Hochttemperatur-Werkstoffe (Thierhaupten, Germany). Graphite powders types CR-2 (particle size $< 2 \mu\text{m}$) and CR-5 (particle size $< 5 \mu\text{m}$) were supplied from Maziva Týn (Týn nad Vltavou, Czech Republic). Chemicals of p.a. purity, $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ and KH_2PO_4 needed for preparation of 0.01 M phosphate (pH 7.0) buffer as supporting electrolyte were from Lachema (Neratovice, Czech Republic).

2.2. Instrumentation

Conventional three-electrode system which consisted some kind of CPE (working), Ag/AgCl/3.0 M KCl (reference) and platinum sheet (auxiliary) electrode, was used for all electrochemical measurements. These electrodes were connected to potentiostat EmStat (PalmSens, Houten, The Netherlands) which operated with software PSTrace 3.0. Each value of potential shown in this work is referred to the above mentioned reference electrode.

2.3. Preparation of working electrodes

Various kinds of CPEs were prepared by mixing of corresponding carbon powder (0.3 g) and PO (0.075 g) in ceramic mortar for 10 min. The homogenous mixture was always pressed into a Teflon piston-like electrode holder ($\text{Ø} = 3 \text{mm}$) with conductive screw which was developed by electrochemical group in University of Pardubice. If not in use, prepared CPEs were stored dry at laboratory conditions.

2.4. Procedure

An extraction of capsaicin into paraffin oil as paste liquid started when corresponding CPE was immersed into 50 μM capsaicin solution with low content of ethanol ($\sim 2.5\%$) at 400 rpm for 120 s (see Fig. 2A). After that, the enriched CPE together with other electrodes was used at anodic SWV performed in 0.01 M phosphate pH 7.0 buffer with following

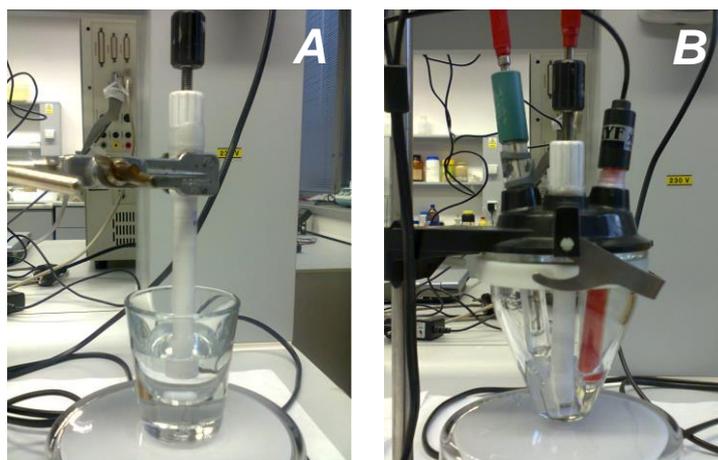


Figure 2. Open circle extraction of capsaicin (A) and conventional three electrode system (B). parameters: potential window, 0 to +0.8 V; potential step (E_{step}), 5 mV; potential of amplitude (E_{ampl}), 50 mV; frequency (f), 25 Hz (for arrangement, see Fig. 2B).

3. Results and discussion

In phosphate pH 7.0 buffer, capsaicin extracted into the CPE provides only one sensitive oxidation peak (I) at 0.365 V (0.405 V at GCPE). Another secondary oxidation peaks (II) were obtained at 0.150 V (CPE) and/or 0.175 V (GCPE) using repetitive SVW. This electrochemical behaviour of capsaicin corresponds with data already published [13,14]. The first oxidation is not affected by any previous electrochemical reaction; therefore the current response (peak height; I_p) and its peak area (A_p) were used for study of extraction reproducibility. During experiments, it was also observed that the capsaicin was accumulated easily into electrode materials which may be attributed to the presence of long amide alkyl chain in its structure. Moreover, it was observed that accumulated amount of capsaicin was not oxidized completely during one anodic scan (from 0 to +0.8 V). This fact was confirmed using a repetitive SWV at CPE (CR-2 with 20% PO), shown in Fig. 3A.

An application of positive potential +0.8 V at speed of stirring 400 rpm for 120 s as cleaning step cannot be used because oxidation products of capsaicin remain dissolved in PO and they block free places for new accumulation. Following this finding, the electrode surface was refreshed by extruding the carbon paste from cavity of electrode holder; usually longer than 3 mm column of the paste had to be removed. Insufficient restoration of carbon paste was indicated by observation of secondary oxidation peak (II) which was not obtained at freshly prepared CPE in the first anodic scan (solid line in Fig. 3A). For five repetitions of one experiment, approximately 15 mm of carbon paste is consumed which is unfortunately not a negligible amount.

From chemical point of view, carbon pastes are dispersions of carbon particles in viscous lipophilic binders. It has to be clear that an amount of extracted capsaicin increases with increasing amount of present binder. It is a reason why the homogeneity of the carbon paste is important factor which have significant effect on extraction reproducibility.

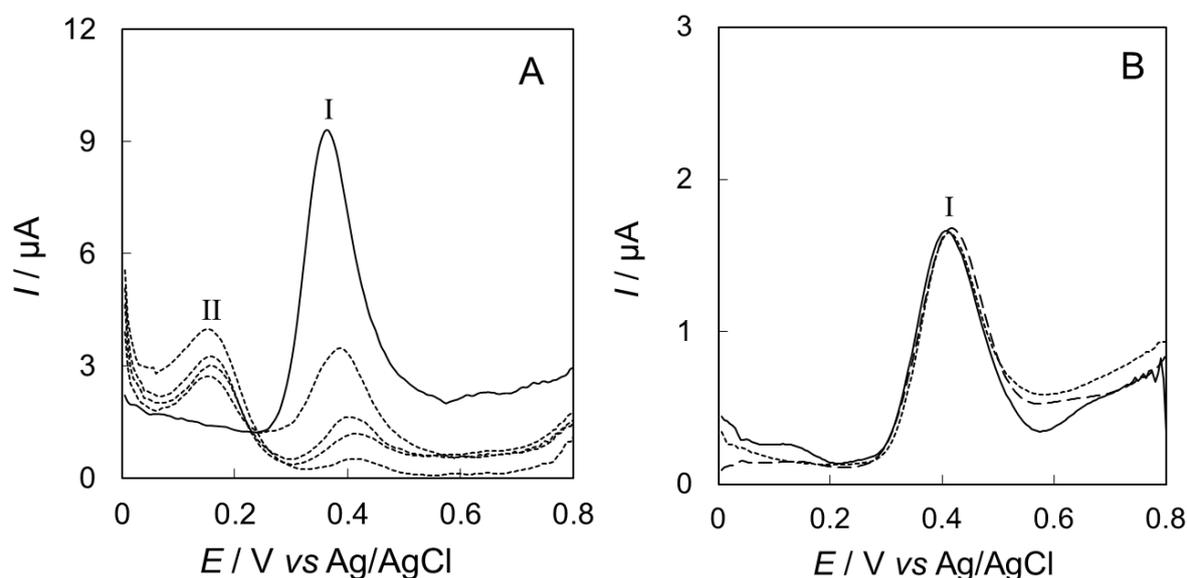


Figure 3. Repetitive SWV at CPE of accumulated capsaicin into CPE, solid line: the first anodic scan and dashed lines: following scans (A). Three times measured SVW of completely new extracted capsaicin into GCPE always with perfectly renewed electrode surface (B).

Various types of CPEs were prepared to recognize if size and shape of corresponding carbon particles can influence the reproducibility of capsaicin extraction significantly (see Table 1). Thus, CPEs of the same surface diameter containing the same amount of PO were used. It is very important to remember that an interface between two immiscible phases (*liquid-liquid extraction*) is located on electrode surface. It has to be accepted that the amount of accumulated capsaicin definitely decreases with depth of carbon paste.

Carbon powders types CR-2 and CR-5 are small sheets of the same graphite with different sizes. For this reason, they were chosen as ideal tool for study of particle size influence on extraction reproducibility. Actually, present carbon particles block the penetration of the analyte into the interior of carbon paste and new renewal of electrode surface significantly changes the structure of the electrode surface. It is evident that these changes dramatically increase with increasing particle size and they significantly decrease the extraction reproducibility.

Table 1. Comparison of various kinds of carbon paste electrodes.

<i>n</i>	CPE (CR-5)		CPE (CR-2)		GCPE (Sigradur G)	
	$I_p / \mu\text{A}$	$A_p / \mu\text{A V}$	$I_p / \mu\text{A}$	$A_p / \mu\text{A V}$	$I_p / \mu\text{A}$	$A_p / \mu\text{A V}$
1	5.954	0.759	7.865	0.915	1.127	0.148
2	5.551	0.674	7.548	0.859	1.309	0.152
3	7.610	0.821	7.922	0.972	1.041	0.155
4	7.486	0.824	7.025	0.802	1.233	0.149
5	5.407	0.599	5.965	0.742	1.229	0.172
\bar{x}	6.402	0.735	7.265	0.858	1.188	0.155
σ	0.917	0.079	0.616	0.069	0.083	0.007
<i>r</i>	85.67	89.23	91.52	91.97	93.01	95.57

I_p ; peak height, A_p ; peak area, \bar{x} arithmetic mean; σ ; relative standard deviation and *r*; reproducibility

CPEs based on common graphite powders provided better sensitivity than GCPE but worse reproducibility (see Fig. 3B). An explanation can be found in different shape of corresponding carbon particles. Generally, an electric contact between sheets of graphite particles is better than between beads of glassy carbon because each particle of glassy carbon is covered by thin layer of paste binder. It is reason why glassy carbon pastes are suitable (stable) electrode material for electrochemical measurements in aqueous-organic mixtures [10]. In fact, glassy carbon paste inside cavity of electrode holder is a column full of small beads where free spaces between glassy carbon particles are filled by sticky lipophilic binder. Thus, there are not any changes in structure after each restoration and polishing of electrode surface which results in satisfactory reproducibility.

Any contaminants of air bubbles can not block the transport of analyte to electrode surface and they have to be removed, because it was observed that presence of mentioned bubbles caused decreasing of reproducibility more than 15% for CPE which was prepared using graphite powder CR-2 and 20% of paraffin oil (*w/w*). Solid contaminants can be easily removed by filtration but an elimination of air bubbles by shaking of electrode is usually quite difficult because the interface between two immiscible liquid phases is located just on electrode surface.

4. Conclusion

According comparison of various kinds of conventional CPEs and GCPE, it is seems that not only homogeneity of carbon paste but also shape of used carbon particles can have significant effect on extraction reproducibility. Moreover, it was also observed that the reproducibility increases with decreasing of particle size. This fact was confirmed

by comparison of two CPEs which were prepared from the same graphite powders differing only in their particle sizes. This short contribution also shows that GCPEs can suitably be applied as analytical devices for isolation of lipophilic analytes and for their consecutive electrochemical determination in pure aqueous electrolytes because the reproducibility of extraction was found a little bit higher than 95 % for values of peak area.

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