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Potravinarstvo® Scientific Journal for Food Industry
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Potravinarstvo, vol. 10, 2016, no. 1, p. 260-264
doi:10.5219/587
Received: 9 February 2016. Accepted: 11 April 2016.
Available online: 14 June 2016 at www.potravinarstvo.com

ISSN 1337-0960 (online)

ADSORPTIVE STRIPPING VOLTAMMETRY IN LIPOPHILIC VITAMINS DETERMINATION

Milan Sýs, Simona Žabčíková, Libor Červenka, Karel Vytřas

ABSTRACT

The aim of this contribution was to check if adsorptive stripping differential pulse voltammetry (AdSDPV) is suitable tool for sensitive simultenous electrochemical detection of lipophilic vitamins. Retinol (vitamin A_1), cholecalciferol (vitamin D_3), α-tocopherol (vitamin E) and phylloquinone (vitamin K_1) were selected as representatives. All electrochemical measurements were performed in two separate steps due to the lipophilic character of the analytes. In the first step, an accumulation of lipophilic vitamin on the surface of glassy carbon electrode (GCE) was done by immersing working electrode into the aqueous-acetonitrile solutions (50%, *v/v*) of each vitamin (50.0 μmol.L^{-1}) at 400 rpm for 5 min. In the second one, differential pulse voltammetry of accumulated vitamins was performed in 0.01 mol.L^{-1} acetate (pH 4.5) buffer at potential step (E_{step}) 5 mV, potential of amplitude (E_{ampl}) 25 mV, interval time (t) 0.1 s and scan rate (v) 50 mV.s^{-1}. It was observed that electrochemical behaviour of lipophilic vitamins adsorbed on surface of solid GCE in the aqueous electrolyte was very similar to those performed in organic/aqueous electrolyte in literature. Due to reversible electrochemical behaviour of vitamin K_1 (phylloquinone/phyllohydroquinone redox couple), it was possible to detect all lipophilic vitamins only in one analysis. Observed values of peak potentials (E_p) were sufficiently different for their recognition which was confirmed by the analysis of real sample. The results obtained in this study showed that simultaneous determination of some lipophilic vitamins is possible requiring further optimization study. For this reason, it is necessary to understand this work as an initial step in simultaneous determination of lipophilic vitamins without application of any chromatographic technique.

Keywords: lipophilic vitamin; glassy carbon electrode; adsorptive voltammetry; margarine analysis

INTRODUCTION

It is known that lipophilic vitamins are nonpolar organic compounds essential for proper functioning of the human metabolism which have to be received through diet **(Cockburn, 2003)**. Thus, their detection and quantification in different kinds of samples in a great importance in nutrition, medicine, cosmetics and food technology **(Gonnet et al., 2010)**. Unfortunately, analysis of lipophilic vitamins is quite complicated and time consuming due to their lipophilic character. The main disadvetage is the use of organic solvents.

The determination of lipophilic vitamins is not practically possible without using chromatographic techniques, especially by high performance liquid chromatography (HPLC) followed by extraction of lipophilic vitamins into organic solvent. It is necessary to remind that HPLC analysis of fats may take up to several hours.

All lipophilic vitamins contain conjugated system of double bonds in their structures, therefore a normal-phased HPLC with combination of UV detection is common way of their determination **(Kamal-Eldin et al., 2000)**. Moreover, they were also determined in human serum by reversed-phase HPLC with electrochemical detection **(Wang et al., 2001)**.

Generally, lipophilic vitamins are classified in four main groups **(Webster, 2012)**. In our experiment, the most biologically active forms (all-trans-retinol; vitamin A_1, cholecalciferol; vitamin D3, α-tocopherol, vitamin E and phylloquinone, vitamin K_1) were selected as standards to explore if an adsorptive stripping differential pulse voltammetry (AdSDPV) is suitable electrochemical method for their sensitive simultanenous detection in model sample and selected margarine.

Adsorptive stripping voltammetry (AdSV) is similar to anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) with the preconcentration step being not controlled by electrolysis **(Wang, 1990)**. In our case, the preconcentration step is controlled by adsorption of analytes on solid glassy carbon electrode (GCE). Their electrochemical detection was performed by differential pulse voltammetry (DPV) which is the most commonly used electrochemical technique for simultaneous determinations **(Baranowska et al., 2008)**.

For comparison, the declared contents of lipophilic vitamins in selected traditional Czech margarines are shown in Table 1. Contents of all present lipophilic vitamins were only copied from nutrition facts of corresponding labels. Additionally, it was observed that tested margarines always contained a mixture of several plant (palm, sunflower and rapeseed) oils whose volume ratios were not surprisingly listed.

Table 1 Declared contents of lipophilic vitamins in several traditional Czech margarines.

Margarines (types)	*Vitamin A (μg/100g)*	*Vitamin D (μg/ 100g)*	*Vitamin E (mg/ 100g)*
Flora light	*800*	*7.5*	*10*
Flora gold	*800*	*7.5*	*—*
Flora original	*800*	*7.5*	*14*
Flora pro-active	*800*	*7.5*	*11*
Perla plus vitamíny	*800*	*7.5*	*—*
Perla tip	*800*	*7.5*	*—*
Rama classic	*800*	*7.5*	*9.2*
Stella	*800*	*3.5*	*—*

It is evident that concentration levels of present lipophilic vitamins are mutually very different (~10 mg E, ~1 mg A and ~0.01 mg D). Therefore, it can be assumed that simultaneous electrochemical determination of lipophilic vitamins in real samples, especially in margarines, remains a challenge for further scientific research.

MATERIAL AND METHODOLOGY

Standards of lipophilic vitamins

Vitamin A_1 as retinol (crystalline), vitamin E as (+)-*α*-tocopherol (from vegetable oil; 1000 IU.g^{-1}), vitamin K_1 as phylloquinone (viscous liquid) and acetonitrile (ACN) of HPLC purity (99.8%) were purchased from Sigma Aldrich (Vieanna, Austria). Vitamin D_3 as cholecalciferol (40×10^6 IU.g^{-1}; crystalline) was obtained from Merk (Darmstadt, Germany).

Instrumentation

All electrochemical measurements were carried out at conventional three-electrode system consisting solid GCE with surface diameter 2 mm from, Ag/AgCl and 3.0 mol.L^{-1} KCl as salt bridge (reference) and platinum wire (auxiliary) electrode which were together connected to potentiostat Autolab PGSTAT101 from Metrohm (Prague, Czech Republic) which is also compatible with software Nova (Prague, Czech Republic).

Pretreatment of glassy carbon electrode

Surface of solid GCE was renovated by polishing pad with presence of wet Al_2O_3 powder for 30 s. After subsequent rinsing of the surface by distilled water, the GCE was ready for new electrochemical experiment.

Sample preparation

The sample preparation is consisted only by dissolving of 2 g margarine type "Perla plus vitamíny" from UNILEVER ČR, spol. s r.o. (Prague, Czech Republic) in pure ACN and filled to the mark of 50 mL volumetric flask.

Procedure

Adsorptive stripping voltammetry of lipophilic vitamins was performed in two separate steps. In the first step, the analytes adsorption on GCE surface was done by immersing working electrode in aqueous-acetonitrile solutions (50% content of ACN) containing 50 μmol.L^{-1} of each vitamin 10 min at 400 rpm. In second one, repettive CV of accumulated lipophilic vitamins in 0.01 mol.L^{-1} acetate (pH 4.5) buffer was done to examine their electrochemical behaviours at potential step (E_{step}) 5 mV, scan rate (v) 50 mV.s^{-1} and fivecycles repetition.

Analogically, DPV of accumulated vitamins (100 μmol.L^{-1} of each vitamin in 25% ACN at 400 rpm for 10 min) was performed in 0.01 mol.L^{-1} acetate (pH 4.5) buffer with deposition potential (E_{dep}) –0.6 V for 120s, potential step (E_{step}) 5 mV, potential of amplitude (E_{ampl}) 25 mV, interval time (t) 0.1 s and scan rate (v) 50 mV.s^{-1}.

RESULTS AND DISCUSSION

Cyclic voltammetry of accumulated vitamins

Electrochemistry of retinol (vitamin A) film deposited on GCE surface

Vitamin A1 deposited on surface GCE provided only one sensitive oxidation peak at +0.708 V whose current response dramatically decreased with the number of cycles. For demonstration, typical repetitive CV of vitamin A1 accumulated at GCE in acetate buffer is shown in Figure 1.

Similar electrochemical behaviour was observed at GCE in a methanol/acetate (pH 5.0) buffer at scan rate 50 mV.s^{-1} **(Wring et al., 1988)** which corresponds to irreversible electrochemical oxidation of retinol to the retinaldehyde with participation of two protons and electrons **(Ziyatdinova et al., 2010)**.

However, it is important to note that a background

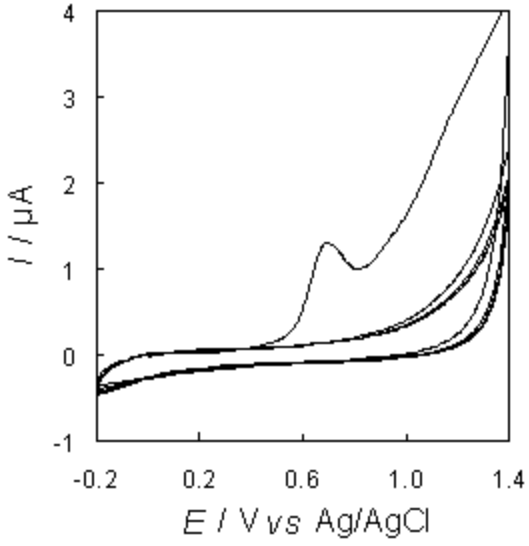

Figure 1 Repetitive cyclic voltammetry of vitamin A_1 (50.0 μmol.L^{-1}) in 0.01 mol.L^{-1} acetate (pH 4.5) buffer at v = 50 mV.s^{-1}.

current increased after oxidation of vitamin A1, probably due to adsorption of the oxidized products. Unfortunately, this phenomenon can negatively affect an electrochemical detection of other lipophilic vitamins which could be oxidized at higher values of potential than present vitamin A1.

Electrochemistry of cholecalciferol (vitamin D_3) film deposited on GCE surface

Electrochemically similar behaviour as in previous situation was observed also for vitamin D3 which also provided only one oxidation peak at +1.032 V which was not visible under following repetitions. According to obtained cyclic voltammogram shown in Figure 2, the oxidation process of cholecalciferol appeared to be irreversible. In fact, the same electrochemical behaviour has been obtained at GCE in in a methanol/acetate (pH 6.0) buffer at scan rate 50 $mV.s^{-1}$ **(Hart et al., 1992)**.

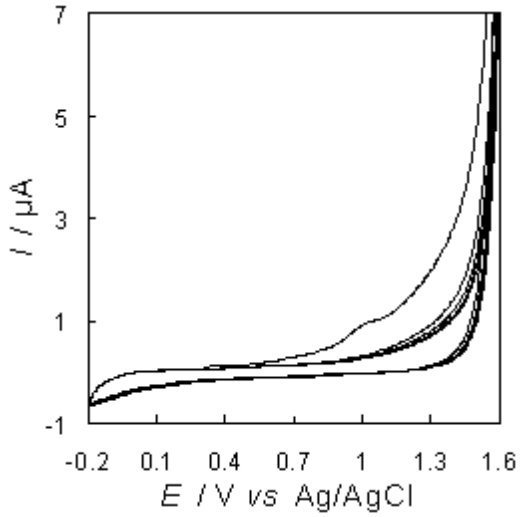

Figure 2 Repetitive cyclic voltammetry of vitamin D_3 (50.0 $\mu mol.L^{-1}$) in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer at v = 50 $mV.s^{-1}$.

Electrochemistry of α-tocopherol (vitamin E) film deposited on GCE surface

Thin layer electrochemistry of α-tocopherol (α-TOH), known as the most active form of vitamin E in aqueous electrolytes was investigated resulting in formation of lipid multilayer **(Yao et al., 2009)** or modification of carbon paste **(Kim and Kusuda, 1994)**. Electrochemical behaviour of α-TOH deposited on surface of solid GCE in aqueous electrolytes was also published by our research group **(Sýs et al., 2016)**.

Electrochemistry of phylloquinone (vitamin K_1) film deposited on GCE surface

In comparison to previous measurements, cyclic voltammetry of vitamin K_1 always began with cathodic scan due to content of quinone unit in its structure **(Wang et al., 1994)**. Thus, the electrochemical behaviour of vitamin K_1 was very similar to redox couple quinone/hydroquinone. According to Figure 3, the vitamin K_1 provided typical two reversible electrochemical peaks at –0.325 and –0.006 V. Moreover, another sensitive cathodic peak was observed at –0.832 V.

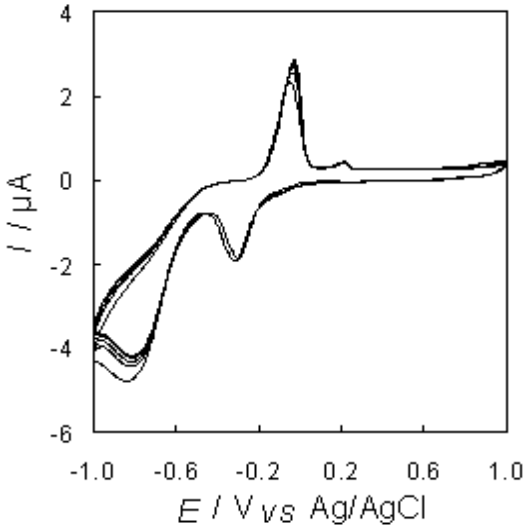

Figure 3 Repetitive cyclic voltammetry of vitamin K_1 (50.0 $\mu mol.L^{-1}$) in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer at v = 50 $mV.s^{-1}$.

Simultenous differential pulse voltammetry of accumulated vitamins

Form the previous section, it states that only vitamin K_1 can not be electrochemically oxidized because it usually occurs in its oxidation form as naphthoquinone with long alkyl chain. Based on this finding, it was necessary to reduce the phylloquinone to phyllohydroquinone with participation of two protons and electrons. Additional lipophilic vitamins accumulated together with phylloquinone on surface of working electrode were present in their corresponding reduction forms. Therefore, applying of deposition potential –0.6 V for 120 s did not cause any electrochemical changes of these vitamins (A_1, D_3 and E).

Only after electrochemical reduction of vitamin K_1, anodic DPV can be used for simultaneous electrochemical detection of all presented lipophilic vitamins in potential window from –0.6 to +1.4 V. The evidence that all selected lipophilic vitamins can be determined together in one analysis is demonstrated in Figure 4. Moreover, it shows that distance of individual voltammetric peak was satisfactory for their sufficient resolution without using any chromatographic technique due to sufficiently different values of the appropriate peak potentials.

It is very important to realize that the electrochemical method presented in this contribution has not been optimized yet. It can be assumed that whole optimization will be very time consuming because it is always based on the finding the optimal working conditions to obtain high sensitivity such as selection of suitable electrode material, organic solvent and many others.

For example, an amount of deposited analytes on solid electrode material is limited by surface area. Therefore, it is obvious that linearity range of developed analytical method will be very narrow and the sensitivity will be completely dependent on the time of accumulation. The

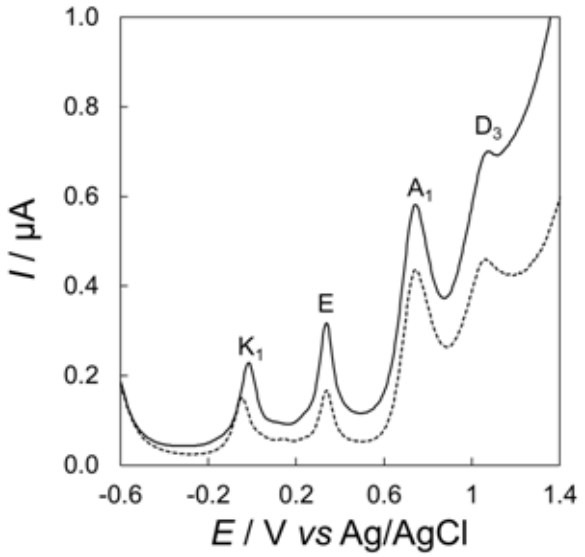

Figure 4 Simultaneous adsorptive stripping voltammetry of lipophilic vitamins deposited on GCE surface from their 100 µmol.L^{-1} solution containing 25% ACN at 400 rpm for 10 min; then detected by DPV in 0.01 mol.L^{-1} acetate (pH 4.5) buffer at E_{dep} = –0.6 V at 120 s, E_{step} = 5 mV, E_{ampl} = 0.025 V, v = 25 mV.s^{-1} (dashed line) and v = 50 mV.s^{-1} (solid line).

solution can be found in using of suitable kind of carbon paste electrode (CPE) which can be classified from physical point of view as a dispersion of solid carbon powder particles in a viscous lipophilic binder **(Švancara et al., 1996)**. In this case, the amount of accumulated analyte is controlled by corresponding extraction equilibrium.

According to publication **(Žabčíková and Červenka, 2015)**, carbon paste can be prepared from plant oils which are commonly used in technology of margarines. Thus, it can be another way how lipophilic vitamins also could be electrochemically detected.

Using carbon nanomaterials offers another possibility. Especially, carbon nanotubes (CNTs) immobilized on some carbon-based electrode material usually cause dramatical increasing of electrode surface due to their specific physical properties **(Volder et al., 2013)**.

Analysis of margarine

Analysis of margarine (Perla plus vitamíny) was only based on qualitative determination of present lipophilic vitamins. Therefore, any sophisticated statistical treatment was not necessary to use. Values of peak potentials are usually presented as arithmetic mean ($\bar{x}$) of minimally five repetitions (n) and corresponding standard deviations (σ) were less than 2% due to polishing of electrode surface after each measurement.

In this case, 2.5 mL sample solution was added into the 7.5 mL pure water in order to obtain 25% ACN in total volume. After that, GCE was immersed into resulting solution and deposition occurred at 400 rpm for 20 min. Obtained voltammograms of accumulated analytes performed in acetate buffer is shown in Figure 5.

It is interesting that only vitamin K_1 and vitamin E were qualitatively determined in the sample of margarine, although these vitamins were not listed on the product label. An explanation lies in the basic ingredients of all margarines. From physical point of view, they can be defined as emulsions of water in edible plant oil which are natural resources of these lipophilic vitamins **(Piironen et al., 1997)**. According to manufacturer, the analyzed margarine contains sunflower and rapeseed oils.

Mentioned rapeseed oil usually contains relatively high amounts of oleic and linoleic acids **(Frančáková et al., 2015)** which are very important like lipophilic vitamins. It is maybe reason why these compounds beneficial for health are very often abused in commercials.

It is clear from Figure 5 that second peak at +0.332 V (anodic oxidation of vitamin E) is not symmetric like oxidation peak of vitamin K_1 at –0.080 V. It is necessary to remember that vitamin E is not chemical individual but group of eight isomers known as tocopherols **(Gliszczyńska-Świgło1 et al., 2007)** which have similar electrochemical properties. Therefore it is quite possible that not only α-TOH was present in the sample of the margarine.

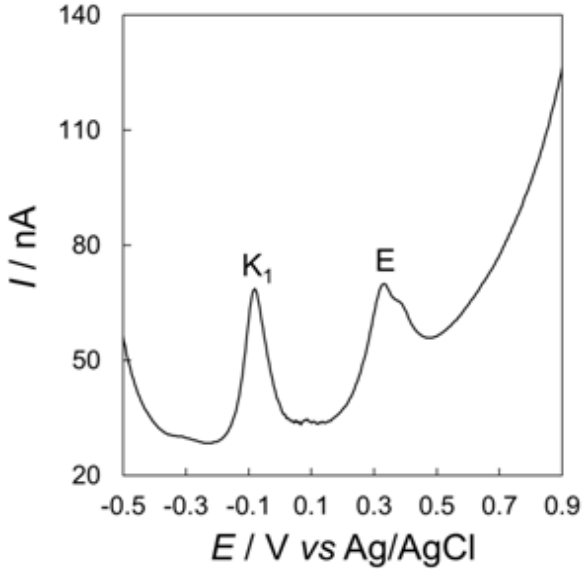

Figure 5 Qualitative determinations of lipophilic vitamins in margarine (Perla plus vitamíny) by adsorptive stripping voltammetry of at solid GCE.

CONCLUSION

According to our experimental results, it may be concluded that simultaneous qualitative determination of lipophilic vitamins is possible using adsorptive stripping differential pulse voltammetry. Unfortunately, it is clear that deposition of analytes on solid glassy carbon electrode and their following electrochemical detection does not provide satisfactory sensitivity, especially, in determination of vitamin D_3. However, it can be assumed that the sensitivity to all lipophilic vitamins can be improved using carbon nanomaterials or heterogeneous carbon materials which are known as carbon pastes. It is necessary to understand this work as an initial step in simultaneous determination of lipophilic vitamins.

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Acknowledgments:

Financial support from the Ministry of Education, Youth, and Sports of the Czech Republic (Project CZ.1.07/2.3.00/30.0021) is gratefully acknowledged.

Contact address:

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

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

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

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