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COMPARISON OF ADSORPTIVE WITH EXTRACTIVE STRIPPING VOLTAMMETRY IN ELECTROCHEMICAL DETERMINATION OF RETINOL

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ABSTRACT

Adsorptive stripping voltammetry (AdSV) of retinol at solid glassy carbon electrode (GCE), carbon paste electrode (CPE) covered by thin layer of multi-wall carbon nanotubes (CPE/MWCNTs) and carbon paste electrode covered by thin layer of single layer graphene (CPE/Graphene) was compared with an extractive stripping voltammetry (ExSV) into silicone oil (SO) as lipophilic binder of glassy carbon paste electrode (GCPE). All types of selected working electrodes were characterized by a scanning electron microscopy to determine overall morphology of electrode surfaces together with spatial arrangement of used carbon particles. The retinol, also known as vitamin A_1, was chosen as a model analyte because it is the most biologically active representative of retinoids which are classified as a significant group of lipophilic vitamins. Based on this comparison, it was observed that electrochemical method with high sensitivity (ExSV at GPCE) is generally characterized by shorter linear range of the calibration curve than in case of AdSV at CPE/MWCNTs or CPE/Graphene. Unlike AdSV at solid GCE, all other tested electrochemical methods could represent suitable analytical tools for monitoring of retinoids in different types of foodstuffs. Especially, content of retinol up to tenths milligrams can be easily determined using ExSV. Additionally, negative interference of chemical species present in real samples is minimal in comparison with direct voltammetric methods performed in supporting electrolytes based on organic solvents due to application of accumulation step in "ex-situ" mode.

Keywords: adsorptive voltammetry; carbon nanotubes; extractive voltammetry; glassy carbon; graphene; retinol

INTRODUCTION

In food analysis, stripping voltammetry in combination with some pulse electrochemical techniques may offer up ultratrace electroanalytical method comparable to the inductively coupled plasma mass spectrometry (ICP-MS) **(Abdel-Galeil et al., 2014)**, especially in determination of heavy metals **(Švancara et al., 2010)**.

According to different variants of deposition steps, analytical methods based on stripping voltammetry can be devided to several variants such as conventional stripping voltammetry with an electroplating step when the accumulation of an analyte on electrode surface is controlled by electrolysis **(Hočevar et al, 2007)** (i), adsorptive stripping voltammetry (AdSV) when the mentioned accumulation is realized by an adsorption of an analyte on electrode surface **(Kalvoda and Kopanica, 1989)** (ii), extractive stripping voltammetry (ExSV) where the analyte is extracted from a sample matrix into a paste liquid of a heterogeneous electrode **(Wang and Freiha, 1984)** (iii) stripping voltammetry based on specific reaction at chemically modified electrodes **(Murray et al., 1987)** (iv). From an electroanalytical point of view, it can be assumed that all these variants of stripping voltammetry exist in two opposite modes; anodic (ASV) or cathodic stripping voltammetry (CSV).

But it is important to note that the above adsorption on the electrode surface is always preceded by extraction into the interior of the electrode material (adsorptive/extractive accumulation process). It is major reason why you can meet a term adsorptive/extractive stripping voltammetry in the scientific literature **(Wang et al., 1985; Tuzhi et al., 1990**; **Cookeas et al., 1992)**.

As a further example of using the ExSV can be considered voltammetric determination of iodide where corresponding authors called the extraction of the iodide into a carbon paste electrode (CPE) based on tricresyl phosphate (TCP) as synergistic type of accumulation **(Švancara et al., 1998)**.

In this case, it is very difficult to determine where an electrochemical reaction of extracted analyte takes place, mostly at the interface between selected paste liquid and an aqueous supporting electrolyte. The discipline dealing with this issue is known as so-called liquid/liquid interfaces electrochemistry **(Samec, 2004)**.

In this contribution, comparison of AdSV at solid GCE, CPE covered by thin layer of multi-wall carbon nanotubes

(CPE/MWNTs) and CPE covered by thin layer of single layer graphene (CPE/Graphene) with ExSV at glassy carbon paste electrode (GCPE) containing silicone oil (SO) as paste binder in determination of retinol is presented.

The retinol has the highest biological activity of a series of carotenoids which belong to lipophilic vitamins **(Goodman, 1984)**. They can be classificed as fat-soluble chemical compounds essential for human health **(Webster, 2012)**. Due to insolubility in aqueous solutions, their electrochemical determination by voltammetric techniques is rather complicated.

Direct voltammetric determination of selected lipophilic vitamins at solid glassy carbon electrode (GCE) in 0.05 mol.L^{-1} lithium perchlorate in a methanol/benzene (2:1) mixture **(Atuma et al., 1975)**, at a rotating GCE in methanolic solution using lithium perchlorate **(Hernández Méndez et al, 1988)**, at CPE in aqueous-organic mixtures with presence of detergent Triton X-100 **(Jaiswal et al., 2001),** at GCE modified MWCNTs in 0.1 mol.L^{-1} $HClO_4$ with acetonitrile (ACN) **(Ziyatdinova et al, 2012)** etc. has two main disadvantages, namely high consumption of organic solvents and relatively low sensitivity.

The first our contribution published in this scientific journal was focused on explanation of anodic AdSV application in simultaneous electrochemical detection of lipophilic vitamins in the margarine within one analysis. In this paper, it was unfortunately reported that the AdSV at bare GCE is not sensitive analytical method for determination of all lipophilic vitamins **(Sýs et al., 2016)**, especially retinol (vitamin A_1) and cholecalciferol (vitamin D_3).

Based on this finding, the retinol as a model analyte was chosen to investigate whether the sensitivity in the electrochemical determination of lipophilic vitamins can be significantly increased by adsorption of selected carbon nanomaterials (CNs) characterized by high specific surface area, conductivity and lipophilic properties **(Komarov and Mironov, 2004; Lu, et al., 2012)** or by extraction into paste binder.

A reaction mechanism of retinol electrochemical oxidation has not been completely elucidated yet. The electrochemical oxidation of retinol with formation of retinal with participation of 2 e^- and 2 H^+ was proposed based on observation only one oxidation peak at 0.85 V during cyclic voltammetry at GCE in 0.1 mol.L^{-1} $LiClO_4$ in acetonitrile-ethanol media **(Ziyatdinova et al., 2010)**.

However, this statement is incorrect because similar electrochemical behaviour was found for the esters of retinol which devoid a free hydroxyl group **(Tan et al., 2014)**. According to electron density of retinyl propionate, it was predicted that electrochemical oxidation probably takes place on C3-position of cyclohexene ring with participation of 2 e^- and H^+ **(Masek et al., 2014)**.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Synthetic retinol (crystalline) of purity ≥95%, *N,N*-dimethylformamide (DMF) and 99.8% acetonitrile (ACN) were purchased from Sigma Aldrich (Praha, Czech Republic). A stock solution of 0.01 mol.L^{-1} retinol was prepared by dissolving the appropriate amount of this substance in pure ACN. Due to chemical instability of the retinol, this solution had to be stored in a freezer at -5 °C. Glacial acetic acid and sodium acetate from Merck (Darmastadt, Germany) together with ultrapure water (ρ = 18.3 MΩ cm; Milli-Q system, Millipore) were used for preparing 0.1 mol.L^{-1} acetate (pH 4.5) buffer.

Sodium hydroxide, potassium hydroxide, potassium chloride, 65% nitric acid, 35% hydrochloric acid, 96% sulfuric acid from Lach Ner (Neratovice, Czech Republic) and sodium phosphate, potassium phosphate from PENTA (Prague, Czech Republic) were necessary for preparation of other supporting electrolytes which were tested in pH study.

Apparatus

All electrochemical measurements were carried out in a 50 mL glass cell at 25 °C. Conventional three-electrode system consisting always one of tested working electrodes, 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 version 1.11. Scanning electron microscopy (SEM) used for characterization of all tested working electrodes was performed at VEGA3 SB (TESCAN, Czech Republic).

Preparation of working electrodes

Pretreatment of glassy carbon electrode

Surface of solid GCE (diameter 2 mm) from already mentioned Metrohm (Prague, Czech Republic) was renovated on polishing pad with presence of wet Al_2O_3 powder (particle size 1.0 µm) for 30 s and subsequently placed in an ultrasonic bath for 5 minutes. After subsequent rinsing of the surface by distilled water, the GCE was ready for new electrochemical experiment.

Preparation of glassy carbon paste electrode

The GCPE was prepared by mixing of 0.3 g glassy carbon powder Sigradur-G (distribution of particle size 5 – 20 µm) from HTW Hochtemperatur-Werkstoffe GmbH (Maintingen, Germany) and 0.052 g silicone oil MV 8000 (SO) from Lučební závody (Kolín, Czech Republic) in ceramic mortal for 20 min. Resulting homogenous paste was pressed into the cavity (diameter 3 mm) of Teflon piston-like electrode holder. It is very important to note that the height of column in the cavity must be less than 2 cm due to difficult extrusion of glassy carbon paste.

Freshly made paste electrodes should not be employed generally in any experiments due to their rather unstable electrochemical behavior caused by an incomplete homogenization. Thus, it is recommended to leave both used types of paste electrodes (GCPE and conventional CPE) at the laboratory conditions for one day. Only after this self-homogenization process, paste electrodes are really suitable for following voltametric measurements **(Mikysek et al., 2009)**.

Preparation of conventional carbon paste electrode

Conventional CPE was prepared by the same way like previously mentioned GCPE; only different carbon powder (graphite) CR-2 type (particle size <2 µm) from Maziva

Týn nad Vltavou (Týn nad Vltavou, Czech Republic) was used. Unlike GCPE, resulting carbon paste based on graphite powder may occupy the entire cavity of the electrode holder. Extrusion of carbon paste can be carried out without problems due to shape of graphite particles resembling sheets.

Immobilization of selected carbon nanomaterials

Conventional CPE was covered by thin layer of graphene (resistivity ≤0.30 Ω cm**;** specific surface area 400 – 1000 $m^2.g^{-1}$) from ACS Material, LLC (Medford, USA) or MWCNTs (diameter 10 – 30 nm; length 5 – 15 µm; specific surface area 40 – 300 $m^2.g^{-1}$) from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) by simple immobilization which is consisted by applying of 20 µL corresponding dispersion of the carbon nanomaterial (CN) in DMF (2 $mg.mL^{-1}$) on the surface of the CPE at laboratory temperature for 24 hours.

It should be noted that before the immobilization of CNs, these two dispersions of MWCNTs without any pre-treatment and single layer graphene with high surface area had to be homogenised by ultrasound at laboratory temperature for 60 min **(Sýs et al., 2015)**.

Procedure

AdSV or ExSV of retinol were performed in two separate steps. In the first step, the adsorption of analyte on GCE, CPE/MWCNTs and CPE/Graphene surfaces or its extraction into SO of GCPE were realized by immersing of selected working electrode in an aqueous-acetonitrile solutions (25% content of ACN) containing 100 $µmol.L^{-1}$ of the retinol at 400 rpm for 10 min. In second one, differential pulse voltammetry (DPV) of accumulated retinol was performed in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer from 0 to +1.4 V 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 $mV.s^{-1}$. Otherwise, all changes in the experimental conditions described above are specified below, in the legends of the corresponding figures.

Statistical analysis

Evidently, one of the most important criterion of an analytical method is its sensitivity **(Hallett, 1960)**. Generally, the sensitivity of every analytical method is given by a slope of corresponding calibration curve. From anyltical point of view, each analytical method is characterized by other parameters such as linearity of calibration curve, limit of quantification (LOQ) and limit of detection (LOD).

This LOD is usually defined by the lowest value of an analyte concentration which can be reliably detected. It is usually assessed by linear regression of the calibration curve applying the following equation 1 **(MacDougall and Crummett, 1980)**,

$$\mathrm{LOD} = \frac{3\sigma}{S} \qquad (1)$$

where: the σ presents the standard deviation of the response and S is slope of the calibration curve. In a narrower sense, the standard deviation is usually calculated from the lowest signal responses obtained for five repetitions (n) minimally. This procedure is used especially in voltammetric methods where the theoretical value of the LOD calculated from a baseline of background current response would not probably corresponded to reality.

RESULTS AND DISCUSSION

Characterisation of working electrode surfaces

Nowadays, scanning electron microscopy (SEM) is already routine tools for characterisation of electrode surfaces morphology. Due to particle size of glassy carbon powder around 5 µm, scale of magnification 50 µm was used. In Figure 1, SEM images of working electrode surfaces (CPE/MWCNTs; A, CPE/Graphene; B and GCPE; C) are shown (magnification scale bar of 50 µm). At the first glance, it is evident that the structures of these electrode surfaces are significantly different.

Unlike the smooth surface of solid GCE (not shown SEM) and other working electrodes, the surface of CPE/MWCNTs is the least homogeneous. It can be assumed that the homogeneity of each surface structure depends on the arrangement of used carbon particles (nanotubes). For example, immobilized MWCNTs create irregular skeins of various sizes and multifaceted range of interconnection. Moreover, no functionalized MWCNTs by a preatretmant in mixture of strong inorganic acids **(Sun et al., 2002; Kuzmany et al., 2004)** are characterized by high lipophilicity which can cause significant increasing of retinol adsorption. On the other hand, it has to be clear unfortunately that mentioned weak homogeneity can have dominant effect on adsorption reproducibility, therefore significantly reduce sensitivity.

CPE/Graphene electrode

Surface of the CPE covered by thin layer of graphene reminds a structure of a coarse cloth probably due to mutual impaction of the graphene nanosheets having a typical planar configuration **(Zhu et al., 2010)**. Overall, it can be stated that the surface is characterized by higher homogeneity than in the previous case of MWCNTs. It is thus not suprising that more satisfactory reproducibility of adsorption will be assumed.

Glassy carbon paste electrode

From the physical point of view, glassy carbon paste can be defined as a dispersion of conductive glassy carbon beads in lipophilic paste binder which is moreover non-coductive and viscous **(Švancara et al., 1996)**.

Generally, an electric contact between graphite sheets of graphite powder in a CPE (not shown SEM) is better than between beads of glassy carbon in a GCPE because significant part of each particle of glassy carbon is covered by thin layer of the paste binder. For this reason, glassy carbon pastes are suitable (stable) electrode materials for electrochemical measurements in aqueous organic mixtures **(Dejmkova et al., 2012; Sýs et al., 2016)**.

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. Consequently, there are not any changes in structure after each restoration and polishing of electrode surface which results in satisfactory reproducibility.

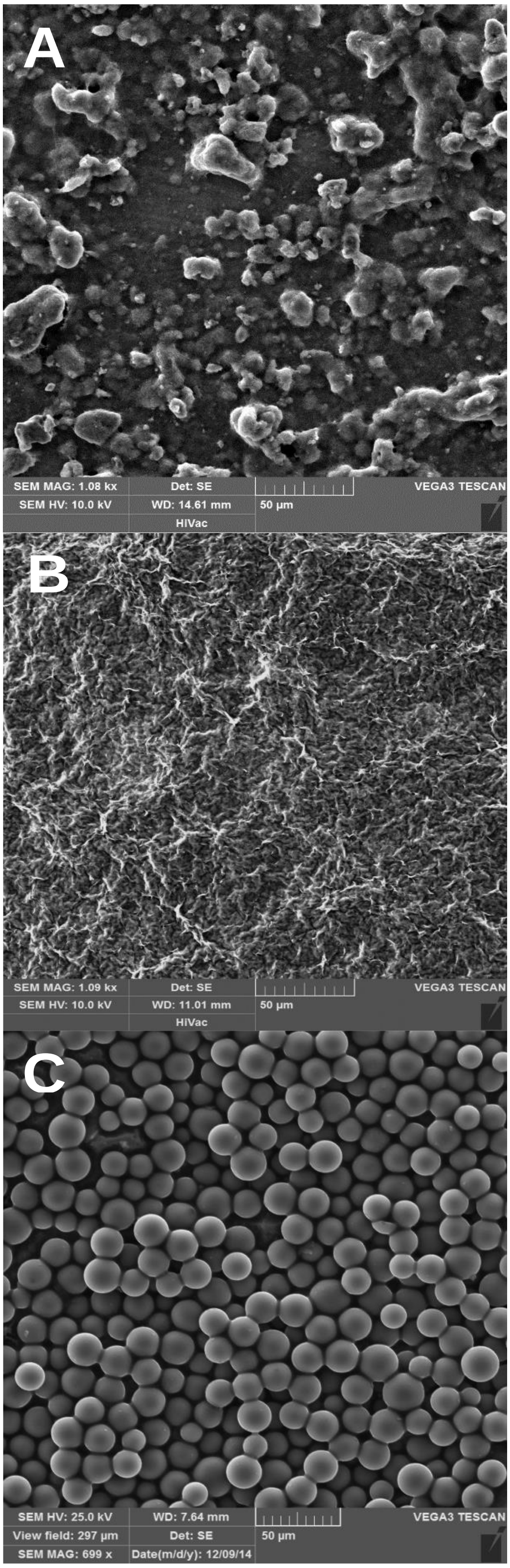

Figure 1 SEM images of CPE/MWCNTs (A), CPE/Graphene (B) and GCPE (C) surface structures. CPE/MWCNTs electrode.

Electrochemical behaviour of retinol

Retinol adsorbed on GCE surface in 0.1 mol.L^{-1} acetate (pH 4.5) buffer provides two peaks at anodic potential (E_p^a) 0.680 and 1.075 V similarly like at the GCE in methanolic (95%) 0.05 mol.L^{-1} acetate (pH 5) buffer solution **(Wring et al., 1988)**.

Surprisingly at other tested electrodes, the retinol provides three oxidation peaks as following: E_{p1}^{a} = 0.750 V; E_{p2}^{a} = 0.970 V; E_{p3}^{a} = 1.330 V at CPE/MWCNTs, E_{p1}^{a} = 0.725 V; E_{p2}^{a} = 1.090 V; E_{p3}^{a} = 1.240 V at CPE/Graphene and E_{p1}^{a} = 0.670 V; E_{p2}^{a} = 1.080 V; E_{p3}^{a} = 1.230 V at GCPE. In comparison with litearture **(Atuma et al., 1975)**, this phenomenon was also obtained by voltammetric measurements of a retinyl palmitate at GCE in a mixture of two organic solvents.

In all these cases, it was found that the most sensitive peak current response (I_p) was always observed for the first anodic peak (Figure 2). In this paper, values of I_p are expresed as current density (j) due to different surface area of working electrodes.

Optimization of the working conditions

From physical point of view, adsorption efficiency is predominantly determined by specific surface area of used nanomaterials. In contrast with the previous case, extraction efficiency is determined by an extraction equilibrium constant (K) affected by many factors such as selection of paste binder, selection of organic solvent miscible with water, their volumetric ratio (φ), their polarity represented by appropriate dielectric constants (ε_r), time of accumulation, speed of stirring, temperature, presence of salts etc.

From the previous paragraph it is evident that the overall optimization depends on finding optimal working conditions. In contrast with conventional stripping voltammetry where an analyte accumulation is controlled by electrolysis, AdSV together with ExSV represent to optimize time-consuming analytical methods.

Effect of carbon nanomaterials amount

In contrast with CNs functionalized by different kinds of functional groups **(McAllister, et al, 2007)**, unmodified

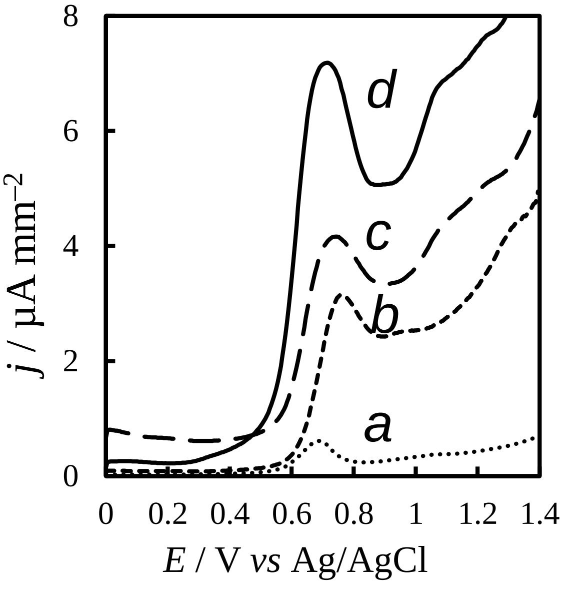

Figure 2 Voltametric responses of 100 µmol.L^{-1} retinol adsorbed on surfaces of GCE (a), CPE/MWCNTs (b), CPE/Graphene (c) and extracted into GCPE with 15% SO (d).

MWCNTs and single layer graphene can be classified as suitable electrode materials for accumulation of fat-soluble vitamins by the adsorption due to their high lipophilicity.

During optimization of appropriate amount of MWCNTs and graphene immobilized on CPE surface, it was found that a presence of these CNs did not have any effect on shifting of $E_{\mathrm{p1}}{}^{\mathrm{a}}$ which could be theoretically caused by their catalytic actions.

Oddly enough, it was observed that any presence of tested CNs caused statistically significant increasing of retinol current density due their high specific surface area. The highest value of the current density was observed at electrodes having ~5.7 µg CNs immobilized on 1.0 mm^{-2} electrode surface. It is reason why 20 µL dispersion of these CNs (2 $\mathrm{mg.mL}^{-1}$) onto electrode surface with diameter 3 mm was used.

Effect of paste binder amount

Fundamentally, it is considered that selection of the optimum paste binder depends on an analyte polarity. One rule to remember is “like dissolves like”, which means that ionic or polar solutes (which have a charge or slight charge) will dissolve in similarly polar solvents.

Based on comparison of dielectric constants of common paste binders (paraffin oil (PO); 4.6 – 4.8; paraffin wax (PW); 2.1 – 2.5; polypropylene (PP); 1.5; silicone oil (SO); 2.2 – 2.9 and vaseline (VA); 2.2 – 2.9), it seems that GCPE prepared from mentioned PP should be the best choice but it is not, unfortunately. An explanation can be find in different electrochemical properties of unmodified carbon pastes **(Švancara and Schachl, 1999)**.

According to previous experience **(Sýs et al., 2016)**, silicone oil (SO) known as a traditional paste binder in preparation of GCPE was selected although more detailed study should be done. From Figure 3, it is clear that current density does not increase generally with the increasing of SO content in the GCPE. But, the highest values were obtained at GCPEs with 10% and 15% content of present paste binder.

Experimentally, it was found that the highest tolerated content of some paste binder is about 30% **(Mikysek et al, 2009)**. Thus, it should be clear that any increasing of this content by extraction of retinol can cause significant deterioration of electrochemical characteristics, especially a substantial increase of ohmic resistance (R).

Effect of organic solvent amount

Selection of suitable organic solvent soluble in water and its content can dramatically affect the final efficiency of an accumulation step. An explanation why an ACN was chosen is relatively simple. In comparison with other eventual organic solvents, the ACN is totaly miscible with water and it has high boiling point 82 °C. Basically, it is necessary to prevent the evaporation of used organic solvent and thus to have constant working conditions during accumulation. During searching of optimum content of the ACN to determine retinol at GCE and GCPE it was found that 25% ACN content should be used to achieve optimum working conditions (Figure 4).

In this part of contribution, it is necessary to mentioned that always the the highest current response was obtained at 25% ACN, whether any content of silicone oil in GCPE and any amount of MWCNTs or single layer graphene onto CPE surfaces was used. Theoretically, optimum amount of any organic solvent is defined by equilibrium distribution of certain analyte between two immiscible phases; working electrode and aqeous-organic mixture **(Sýs and Vytřas, 2016)**.

Moreover, it was observed that any content of this organic solvent does not have any significant effect on negative increasing of background current response for solid electrode materials such as GCE and CPE covered by thin layer of carbon nanomaterials (not shown). However, it is necessary to remind that previous statement does not valid for carbon paste electrodes due to solubility of paste binders in organic solvents. The background current at GCPE with 15% SO increased with increasing of acetonitrile content.

Speed of stirring influence

The speed of stirring significantly affects the rate of retinol transport to an electrode surface where this analyte is adsorbed or can be further extracted into interior of the

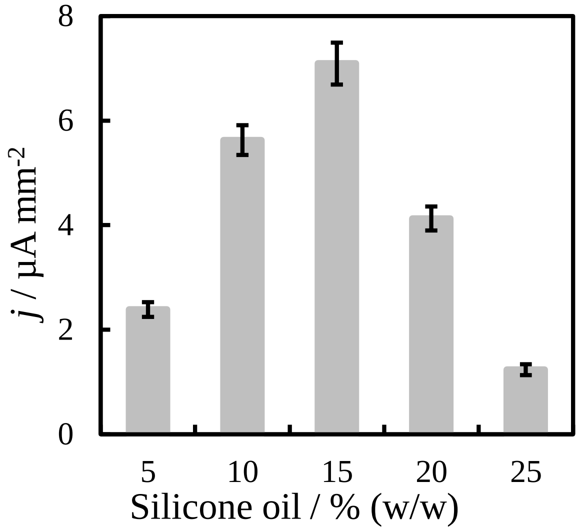

Figure 3 Dependence of retinol current density on content of paste binder.

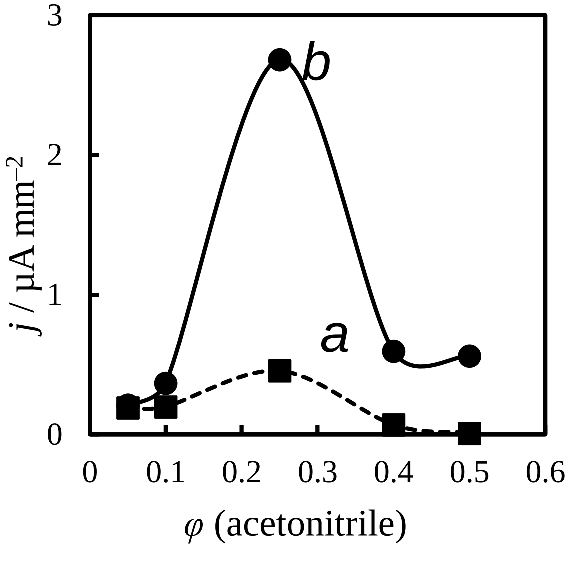

Figure 4 Dependency of current density obtained at solid GCE (a) and GCPE with 20% SO (b) on different ACN content used during accumulation step.

electrode material. In addition, the current density of voltammetric oxidation of the retinol is a linear function of its accumulated amount during adsorption or extraction. In this study, it was observed that values of speed of stirring higher than 400 rpm did not have any significant effect on the increasing of final current density. The value 400 rpm therefore was chosen as optimum.

Effect of accumulation time

The optimum time is defined as the time required to reach an equilibrium of retinol distributed between nonpolar electrode surface and used aqueous-organic mixture (“*adsorption*“) or also between nonpolar paste binder and previously mentioned mixture (“*extraction*“). Accumulation time is therefore the parameter which has fundamental effect on the sensitivity of studied variants of stripping voltammetry.

In both cases, it is possible to say with certainty that any significant increasing of the current density was not visible at values of the accumulation time higher than 15 min. However, it is important to state that 10 min is sufficient time to achieve the equilibrium for ExSV at GCPE with 15% SO content due to high relative standard deviation (RSD) about 8% which was calculated for 5 repetitions. In this case, values of retinol current density were statistically the same for 10 and 15 min of accumulation due to relatively high value of RSD. Thus, value 10 min was selected as optimum accumulation time.

Selection of suitable supporting electrolyte

Various supporting electrolytes such as 0.1 mol.L^{-1} phosphate (pH 7.05) buffer, 0.1 mol.L^{-1} acetate (pH 4.5) buffer, 0.1 mol.L^{-1} KCl solution (pH 6.2), solutions of diluted inorganic acids like 0.05 mol.L^{-1} H_2SO_4 (pH 1.13), 0.1 mol.L^{-1} HCl (pH 1.02), 0.1 mol.L^{-1} HNO_3 (pH 1.05) and alkaline solutions of 0.2 mol.L^{-1} KOH (pH 13.4) or 0.1 mol.L^{-1} NaOH (pH 12.3) were tested to achieve the high sensitivity of final electrochemical methods. It was found that the best recognition of individual anodic peaks at potentials 0.395, 0.635 and 0.850 V was obtained at solid GCE using NaOH solution. Almost identical peak oxidation current responses of retinol were observed at 0.690 V (solid GCE) in solutions of strong inorganic acids. Corresponding peak potentials differed minimally (±50 mV) according to types of used electrodes.

From analytical point of view, the highest current response of the first anodic peak was observed for 0.1 mol.L^{-1} acetate (pH 4.5) buffer, and because of this, it was chosen as the optimum. By the way, direct voltammetric determination of retinol was previously performed in this acetate buffer **(Wring et al., 1988; Filik et al., 2006)**. For demonstration, voltammograms of retinol obtained at solid GCE recorded in different selected supporting electrolytes are shown in Figure 5.

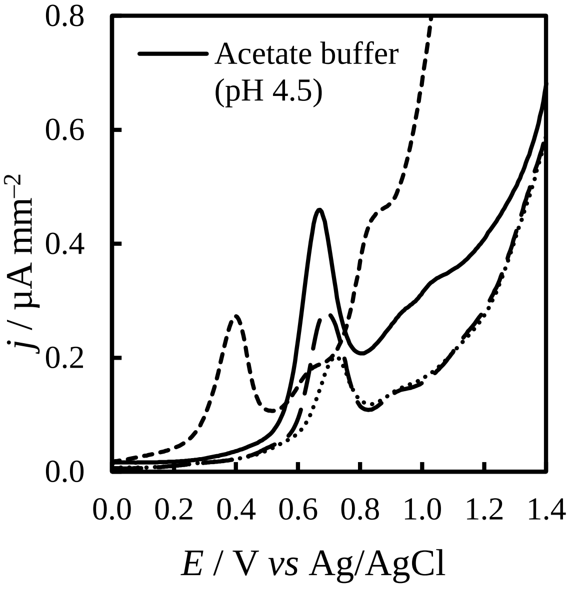

Figure 5 Voltammograms of 100 µmol.L^{-1} retinol adsorbed from 25% ACN on solid GCE surface at 400 rpm for 10 min and subsequently electrochemically oxidized in different strictly aqueous electrolytes.

Comparison of AdSV with ExSV in retinol determination

Sensitivity of non-conventional voltammetric methods

Almost three times lower values of detection limits (LOD) were obtained at CPEs covered by thin layer of CNs than at solid GCE (see Table 1). Moreover, it is necessary to state that also linear range was three times prolonged at these modified working electrodes. Based on this finding, it can be concluded that an insignificant progress in the sensitivity of the AdSV was achieved using carbon nanomaterials.

Unlike solid GCE, relatively high values of background current were observed at GCPE, CPE/MWCNTs and CPE/Graphene. It is caused probably by presence of ACN at accumulation step. This phenomenon resulted in significantly high intercept values of calibration curves and therefore corresponding LODs cannot have lower values, unfortunately.

From all tested non-conventional electrochemical methods, the highest sensitivity in retinol determination was observed using ExSV at GCPE. However, it was determined that linearity of calibration curve was ranged only from 1.0 to 40 µmol.L^{-1} retinol. In comparison with the ExSV, linear ranges of appropriate calibration curves obtained at CPE/MWCNTs and CPE/Graphene were found from 10 to 100 µmol.L^{-1} retinol. For demonstration, calibration curve together with corresponding anodic voltammograms of retinol measured at the GCPE is shown in Figure 6.

Table 1 also shows interesting comparison of non-conventional voltammetric methods based on ex-situ accumulation step with other already developed voltammetric methods in retinol determination. Surprisingly, AdSV at different working electrodes and ExSV at GCPE presented in this contribution have significantly better analytical parameters than others.

Due to satisfactory sensitivity of ExSV, it can be predicted that electrochemical methods based on stripping voltammetry with synergistic type of accumulation could be alternative analytical method to reverse high-performance liquid chromatography **(Hite, 2003)** in determination of retinoids which are commonly used as food additives.

Table 1 Comparison of non-conventional voltammetric methods.

Sensor	Method	Linear range (mol.L^{-1})	LOD (mol.L^{-1})	References
GCE	DPV	–	4.7 × 10^{-5}	**(Wang, 2000)**
TFME	DPV	–	8.8 × 10^{-6}	**(Wang, 2000)**
TFLE	DPV	–	4.7 × 10^{-6}	**(Wang, 2000)**
PtE	LSV	8.2 × 10^{-5} to 1.1 × 10^{-3}	–	**(Budnikov et al., 2004)**
GCE	LSV	9.7 × 10^{-5} to 2.3 × 10^{-3}	4.8 × 10^{-5}	**(Budnikov et al., 2005)**
GCE	CV	2.9 × 10^{-5} to 9.8 × 10^{-4}	1.5 × 10^{-7}	**(Ziyatdinova et al., 2010)**
GE/MWCNTs	CV	5.0 × 10^{-5} to 1.5 × 10^{-3}	4.0 × 10^{-5}	**(Ziyatdinova et al., 2012)**
GCE/MWCNTs	SWV	5.0 × 10^{-6} to 2.0 × 10^{-4}	8.0 × 10^{-7}	**(Filik et al., 2016)**
GCE	AdSV	1.0 × 10^{-5} to 4.0 × 10^{-5}	3.5 × 10^{-6}	This paper
GCPE	ExSV	1.3 × 10^{-6} to 5.0 × 10^{-5}	4.5 × 10^{-7}	This paper
CPE/MWCNTs	AdSV	5.0 × 10^{-6} to 1.0 × 10^{-4}	1.3 × 10^{-6}	This paper
CPE/Graphene	AdSV	4.0 × 10^{-6} to 1.2 × 10^{-4}	1.0 × 10^{-6}	This paper

Note: CV; cyclic voltammetry, DPV; differential pulse voltammetry, GE/MWCNTs; multi-walled carbon nanotubes modified graphite electrode, LOD; limit of detection, LSV; linear sweep voltammetry, PtE; platinum disk electrode, SWV; square wave voltammetry, TFLE; thin-film lead deposited GCE, TFME and thin-film mercury deposited GCE.

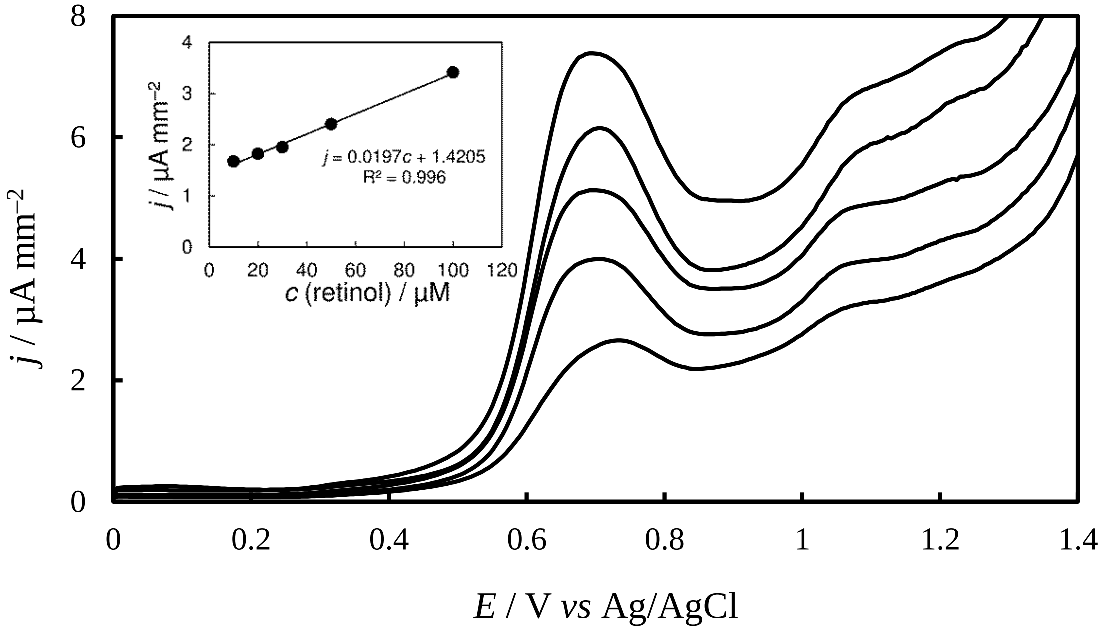

Figure 6 Voltammograms with corresponding calibration curve of retinol obtained at GCPE with 15% content of silicone oil.

Table 2 Reproducibility of accumulation steps for sensors applied in the AdSV and ExSV.

Sensor	E_p^a (V)	j (µA.mm^{-2})	j_{RSD} (%)
solid GCE	0.680	0.379	7.5
GCPE	0.670	4.516	8.2
CPE/MWCNTs	0.750	3.085	6.3
CPE/Graphene	0.725	3.447	4.9

Note: E_p^a; anodic peak potential, j; current density and RSD; relative standard deviation.

Especially, retinyl palmitate, known as synthetic vitamin A, is usually added to fortified a low fat milk more nutritious than they would be without the addition of vitamins that were removed.

The seasonal variation of retinol in cow´s farm milk can be also monitored by ExSV (LOD = 0.128 mg.L^{-1}) because retinol content is about 1.0 mg per 1 L milk **(Hodulová et al., 2015)**. Moreover, it seems that retinoids such as retinyl acetate and already mentioned retinyl palmitate could be also determined in cosmetic products **(Ziyatdinova et al., 2010)**.

Reproducibility of retinol accumulation

Reproducibility of retinol accumulation by adsorption on GCE, CPE/MWCNTs and CPE/Graphene surfaces or extraction into the SO of GCPE was calculated for 5 repetitions. These experiments were performed always using freshly polished GCE, newly prepared CPEs covered by thin layer of CNs, renewed GCPE, standard solution of retinol as well. At laboratory conditions, the accumulation was performed by immersing of selected working electrode (open circuit) into 10 mL of 100 μmol.L^{-1} retinol solution containing 25% ACN stirred at 400 rpm for 10 min.

From values shown in Table 2, it is evident that satisfactory reproducibility (RSD ≤5%) was obtained only at the GCE/Graphene. This finding can be explained by a homogeneous structure of graphene layer. Fortunately, values of RSD for other electrodes were not calculated higher than 9%.

CONCLUSION

Nowadays, all lipophilic vitamins and their synthetic analogs are determined dominantly by HPLC in foodstuffs. The reason is a possibility of their separation in within one analysis. However, it is necessary to state that this kind of analysis includes a lot of time consuming steps such as hydrolysis of lipophilic sample matrix, extraction of vitamins into an organic solvent and own separation.

In comparison to HPLC, ExSV at GCPE represents a simple analytical tool applicable in determination of lipophilic vitamins because these analytes can be directly extracted from sample matrixes or simple sample preparation is assumed. This non-conventional stripping voltammatry usually is not able to detect each form of certain vitamins (individual retinoids, tocopherols, cholecalciferols and phylloquinones) due to their close peak potentials and significant width of peaks. Anyway, individual groups of these vitamins could be easily determined as weight equivalents of their most biologically active forms.

Despite high specific surface area of MWCNTs or single layer graphene, it should be concluded that any used variant of AdSV is not such sensitive as ExSV at GCPE. Additionally, restoration of electrode surfaces with the immobilized carbon nanomaterials is practically impossible after each accumulation step. An improvement in the sensitivity and reproducibility of ExSV could be found in using GCPE containing the optimal portion of graphene which has higher specific surface area than MWCNTs. It is necessary to state that from scientific point of view, this possibility has not been tested yet.

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