

The University of Pardubice
Faculty of Chemical Technology
Department of Analytical Chemistry

MSc. Granit Jashari

**Application of electroanalytical methods in food
and pharmaceutical analysis**

Supervisor: Prof. Dr. Ivan Švancara

Supervisor-consultant: Radovan Metelka, Ph.D.

Doctoral Thesis

Pardubice 2023

TITLE

Application of electroanalytical methods in food and pharmaceutical analysis

ANNOTATION

The main purpose of the research conducted within the frame of this doctoral thesis was to develop new electroanalytical approaches and methods applicable in food and pharmaceutical analysis. Due to the importance of the presence of lipophilic vitamins in food and pharmaceutical formulations, the simultaneous detection of lipophilic vitamins (A, E and K), and related compounds had been the primary goal of the dissertation. Since lipophilic analytes are usually occurring in complex matrices, the simplification of real samples treatment prior the analysis and elimination of toxic organic solvents in comparison with the previous methods have been the other benefits of this work. In addition, the development of low-cost and easy-to-use disposable sensors for the determination of selected biologically active compounds was yet another target of the whole research.

KEYWORDS

Lipophilic vitamins, foodstuffs, pharmaceuticals, voltammetry, electrodes and sensors

NÁZEV

Využití elektroanalytických metod v potravinářské a farmaceutické analýze

ANOTACE

Hlavním účelem výzkumu uskutečněného v rámci předkládané dizertační práce bylo vyvinout nové elektroanalytické přístupy a metody aplikovatelné v potravinářské a farmaceutické analýze. Vzhledem k důležitosti přítomnosti lipofilních vitaminů v potravinách a farmaceutických přípravcích byla hlavním cílem simultánní detekce těchto vitaminů (A, E a K) a příbuzných látek. Jelikož se tyto lipofilní látky obvykle vyskytují ve složitých matricích, dalším zlepšením v rámci této práce bylo zjednodušení zpracování reálných vzorků a eliminace použití toxických rozpouštědel ve srovnání s dosud publikovanými metodami. Navíc byly vyvíjeny levné a snadno použitelné jednorázové sensory pro stanovení vybraných biologicky aktivních látek.

KLÍČOVÁ SLOVA

Lipofilní vitaminy, potraviny, farmaceutika, voltametrie, elektrody a sensory

Table of Contents

ANNOTATION	2
KEYWORDS	2
Introduction	4
1. Results and discussion	6
1.1. Characterization of carbon paste electrode with various surfactants	6
1.2. Electrochemical determination of vitamin A and related compounds	8
1.2.1. Simultaneous determination of lipophilic vitamin esters	8
1.2.2. Extractive stripping voltammetry of lipophilic vitamins in cow's milk and cream	10
1.2.3. A new voltammetric approach for the determination of β -carotene	11
1.3. Simultaneous determination of vitamin E and K	13
1.4. Possibilities of simultaneous voltammetric determination of individual tocopherol isomers	15
1.5. Voltammetric approaches for the determination of selected biologically active compounds	17
1.5.1 Voltammetric determination of nitrites in meat products after reaction with ranitidine	17
1.5.2 Comparison of amperometric biosensor with portable Raman spectrometer determination of ethanol in alcoholic drinks	18
2. Conclusions	21
3. References	23
4. List of publications	26
5.1 Publications in impacted journals	26
5.2 Publications in other peer-reviewed journals	27
5.3 Conference proceedings	27
5.4 Oral presentations	28

Introduction

Biologically active compounds are essential for human body, neutralize free radicals that can cause damages. Thus, in the last decades there is an increased need to monitor them. [1-4]. Data from numerous scientific studies have shown the effect of these compounds on human health for the prevention of many diseases such as atherosclerosis, cardiovascular, metabolic, diabetes, rheumatism, inflammation, age-related eye diseases, and cancer [5-11]. To prevent such damage from harmful free radicals, it is recommended to maintain a healthy diet, with fruits and vegetables that are naturally rich in various antioxidants and phytochemicals such as polyphenols, flavonoids, oligosaccharides, and cyclic polyols including cyclitols [12-14]. Electroanalytical methods can provide very sensitive procedures for the determination of many compounds. They are also characterized by many other favourable characteristics, including relatively low-cost instrumentation, fast analysis times, no special sample handling required, miniaturized equipment, smaller reagents consumption, and the possibility to determine several analytes simultaneously [15-19].

The main purpose of this dissertation research was the development of new electroanalytical methods that can be used in food and pharmaceutical analysis. The focus was mainly on the development of new approaches for the simultaneous detection of lipophilic vitamins (A, E and K), vitamin D was not included in this study. Considering the physicochemical properties of lipophilic vitamins, which are not soluble in aqueous solvents, many parameters that play a key role, such as working electrode material (metal, carbonaceous, boron-doped diamond), different organic solvents, different ratios of organic solvents with water, the effect of anionic, cationic, and non-ionic surfactants, parameters of electrochemical methods, etc., have been investigated.

In previous work, the electroanalytical group in the Department of Analytical Chemistry, University of Pardubice, reported several electrochemical methods for the determination of lipophilic vitamins using square-wave adsorptive stripping voltammetry (SWAdV) on carbon-based electrodes. The methods mainly relied on two steps: the accumulation of substances in an organic solvent or in a mixture of organic solvent with water for a certain period, and then the electrochemical detection in a predominantly acidic buffer medium when using square-wave voltammetry (SWV) [16-21]. The respective methods developed shown results that were promising for the simultaneous determination of lipophilic vitamins. In the works presented in this thesis, the nonaqueous carbon paste electrode (CPE) was initially studied for the possibility of use in the analysis of lipophilic vitamins (**Publication**

1) [22]. Simultaneous determination of retinyl acetate (RAc) or palmitate (RPA), and alpha-tocopheryl acetate (α -TOAc) in cosmetic products has been achieved for the first time (**Publication 2**) [23]. Monitoring vitamin A as sum of retinoids and carotenoids (VA) in cow's milk and cream was possible without any need for sample treatment, where the extraction of analytes was done directly on glassy carbon paste electrode (GCPE) with subsequent electrochemical detection by SWV (**Publication 3**) [24]. A gold electrode (AuE) was used to detect β -Carotene (BCA) using nontoxic solvents in raw vegetables and pharmaceutical preparations (**Publication 4**) [25]. Simultaneous determination of vitamin E (VE) and vitamin K (VK) at the glassy carbon electrode (GCE) using SWAdSV in pharmaceutical preparations was also achieved (**Publication 5**) [26]. The electrochemical behaviour of alpha, gamma, and delta tocopherols (α -TOH, γ -TOH, and δ -TOH) was investigated to find out optimum working conditions for their simultaneous voltammetric detection. After thorough optimization of working conditions, their voltammetric determination has been possible using mathematical evaluation of current signals (**Publication 6**) [27].

The development of fast and low-cost methods for the determination of the selected biologically active substances was another goal of this doctoral thesis. Considering the large consumption of meat products and alcoholic beverages, two biologically active analytes, nitrites (used as additives in meat products) and ethanol (consumed by humans in alcoholic beverages), which have a major impact on human health, their monitoring is very important in the food industry. A new voltammetric method for the determination of nitrites in meat products has been developed. The cathodic reduction of 2-methyl-2H-furan-3-one at -0.210 V on GCE covered with a thin layer of electrochemically reduced graphene oxide (ERGO) and adsorbed sodium o-dodecylbenzenesulphonate (SDBS) surfactant was more preferred over the anodic oxidation of *N*-nitroso-dimethylamine at +0.8 V (**Publication7**) [28]. Immobilization of alcohol dehydrogenase into graphene-based composite material was utilized to construct a relatively simple bioanalytical device suitable for amperometric detection in the flow injection analysis (FIA) regime and applicable to the determination of ethanol in highly alcoholic drinks and some white wines (**Publication 8**) [29].

1. Results and discussion

All the experimental results of this dissertation were published, see in refs. [22-29], and the corresponding articles are available in appendices as Publications I to VII.

1.1. Characterization of carbon paste electrode with various surfactants

During this doctoral research, the main subject of interest was the development of electroanalytical methods based on pulse voltammetric techniques that can be used for the simultaneous detection of lipophilic vitamins. The selection of working electrode material has an important role in electrochemical methods. Various electrode materials including metal, carbon-based, and modified electrodes have been used for electroanalysis of lipophilic compounds. Carbon-based electrodes are the most widely used working electrodes for electrochemical sensing applications. They possess many advantages such as low cost, wide potential window, low background current, good stability, and it is very easy to modify them.

Carbon paste electrodes made of mixture of carbon powder and pasting liquid (binder) represent one of major types of carbon-based electrodes. Considering that carbon pastes have some drawbacks that limit their use in certain experiments, the most mentioned problem is their low stability in many organic solvents, where generally used mixtures of carbon pastes undergo a rapid and usually total dissolution. To circumvent this unwanted behaviour, various modifications using different -binders and surfactants can increase the stability of carbon paste material. In case of surfactants, an explanation can be found in the specific molecular structure of surfactant containing both hydrophobic and hydrophilic groups. Surfactant molecules are dissolved via their hydrophobic “tails” in carbon paste binder that links together the individual carbon particles, whereas hydrophilic “heads” are directed onto the electrode surface, representing the interfacial layer.

Carbon pastes made of natural or synthetic graphite particles can absorb a higher ration of binder than a compact glassy carbon or carbon nanotubes powder. The relationship between the type of carbon material including five different CPEs and the amount of surfactant at constant mineral oil (MO) content (20% w/w) was investigated. Obtained results from CPEs prepared with chemically purified natural graphite powder and glassy carbon Sigradur are presented as dependence of the ohmic resistance on the content of SDS (w/w) and the types of CPEs containing 20% MO (see Fig. 1, Publication 1). The CPEs prepared from raw shungite (mineralized carbon) and multi-wall carbon nanotubes (MWCNTs) with the same amount of 20% MO and 10% SDS were unstable in tested organic solvent. Table 1, Publication 1., shows

electrochemical characterization of all prepared CPEs using cyclic voltammetry (CV) of ferrocenium/ferrocene redox couple measured in nonaqueous media. From the presented data it was found that the optimal composition of non-aqueous carbon paste depends on the type of carbon material used, and the amount and type of non-electroactive surfactant. Chemically purified natural graphite powder can accommodate the largest amount of SDS (20-50%), contrary to glassy carbon, spectroscopic graphite powder (RWB) carbon, and MWCNT (only 10% of SDS at constant content of 20% MO).

In order to select the most suitable surfactant, several types of surfactants such as anionic SDS, cationic cetylpyridinium chloride, benzethonium chloride, 1,3-didecyl-2-methylimidazolium chloride, cetyltrimethylammonium bromide, didodecyldimethylammonium bromide, amphoteric sodium lauroamphoacetate (SLAA), and neutral TritonTM X-100 were investigated and compared in baseline measurements. The results obtained using CV in pure MeCN with 0.1 mol L⁻¹ LiClO₄ showed that CPE modified with SDS provided no signal due to the electrochemically inert sulfonic group (Fig. 5, Publication 1). On the other hand, all types of cationic surfactants were inapplicable, because of their electroactivity in the interested potential range. TritonTM X-100 and sodium lauroamphoacetate were unstable in MeCN working media. SDS was confirmed as a suitable surfactant modifier with excellent stabilizing effect. Electrochemical studies for the redox pair ferrocenium/ferrocene obtained for CPEs with varying SDS amounts ranged from 20 to 40% (w/w) at a constant ratio with the binder (20% MO w/w) and the respective carbon constituent were compared in peak currents. Obtain results show almost comparable peak currents. From the experimental data it was found that the amount of surfactant in natural and synthetic graphite can be in higher content, than in the compact carbon materials, such as glassy carbon powder and carbon nanotubes (Fig. 4, Publication 1).

Several organic solvents such as MeCN, methanol (MeOH), tetrahydrofuran, acetone (ACE), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide, and isopropyl alcohol, all containing 0.1 mol L⁻¹ LiClO₄, were compared in baseline measurements at CPE made of 40% (w/w) MO and SDS using LSV. From the experimental data it was found that a significant increase of the background currents and shorter potential ranges were obtained for all tested solvents, except for MeCN (Fig. 6, Publication 1). The presence of water up to 10% does not cause damage in the morphological structure of the electrode surface, while the presence of water in a percentage greater than 1% causes an increase in background current. Moreover, a comparison of nonaqueous CPEs modified with SDS with a commercial GCE has been carried out using CV in pure MeCN, when the electrochemical behaviour of 500 μmol L⁻¹ α-TOH,

eugenol, and dopamine was examined. Comparable results of peak height and overall shape of the respective current responses to those obtained at the GCE were obtained Figure 1 (Fig. 1).

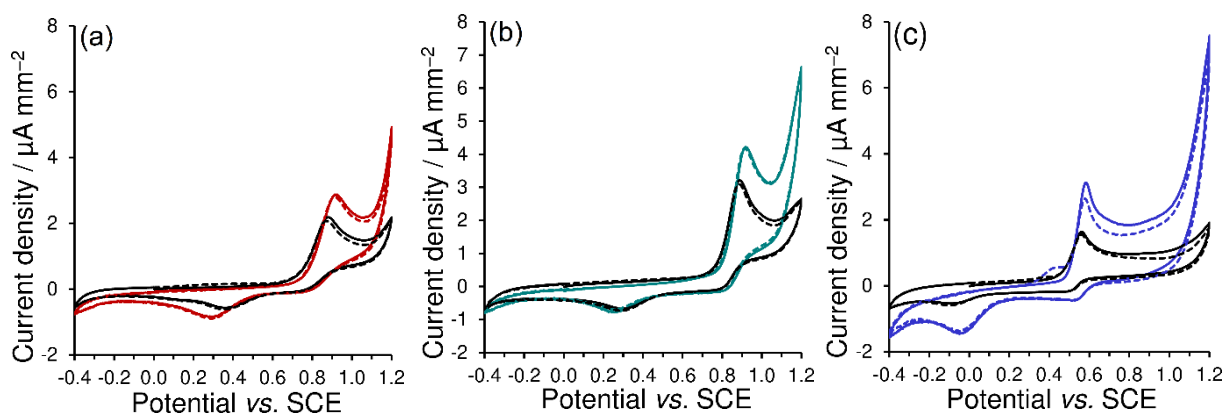


Figure 1. Repetitive cyclic voltammograms (two cycles) $500 \mu\text{mol L}^{-1}$ dopamine (a), eugenol (b) and α -tocopherol (c) recorded on CPE containing 40% (w/w) MO and modified with 40% (w/w) SDS (coloured) and GCE (black lines) in MeCN with $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ at $E_{\text{step}} = 2.5 \text{ mV}$ and $\nu = 100 \text{ mV s}^{-1}$, where dotted lines represent the second repetitions.

From this detailed study, it has been demonstrated that the optimal composition of the nonaqueous carbon paste depends mainly on the type of carbon material, and amount of non-electroactive surfactant, whereas the type of organic binder is less important. MeCN as optimal working medium offered a wider potential range with low background current over other organic solvents. The comparisons of the surfactant-modified CPE with the GCE on the model redox pairs and selected biological compounds have proved that the CPE/SDS could find its application in nonaqueous electrochemistry, especially for nonpolar electroactive organic compounds. Moreover, nonaqueous carbon paste electrode can offer interesting application in food and pharmaceutical analysis.

1.2. Electrochemical determination of vitamin A and related compounds

1.2.1. Simultaneous determination of lipophilic vitamin esters

Starting from separate studies for all-*trans* retinol [20] and α -TOAc [21,30-32] and considering that these compounds are present together in cosmetic products, a completely new voltammetric approach for simultaneous determination of RAc or RPa and α -TOAc is presented in this thesis. Firstly, the electrochemical behaviour of RAc, RPa and α -TOAc was studied at GCE using CV in pure ACE. From the obtained data it was observed that RAc and RPa provided single oxidation peak at +0.85 V, accompanied with some other broad and overlapped signals at more positive potentials. On the other hand, α -TOAc gave only a single oxidation peak at

+1.4 V. The distance between the oxidation peaks indicates the possibility for a simultaneous determination of these analytes. In addition, the effect of scan rate on the electrode behaviour of 500 $\mu\text{mol L}^{-1}$ of all analytes was investigated. The relationships obtained by plotting the I_p against square root of scan rate revealed that the oxidation of RAc and RPa are diffusion-controlled processes unlike the oxidation of α -TOAc, which is governed by the adsorption.

Two metal-based electrodes, gold (AuE) and platinum electrode (PtE), and GCE were compared for the simultaneous detection of 50 $\mu\text{mol L}^{-1}$ RAc and α -TOAc in pure MeCN. From the results it was shown that the electrode material had not any significant effect on the peak potential. The current response for RAc at all tested electrodes was nearly identical, whereas α -TOAc provided twice higher response at GCE. To select the most suitable working medium, several organic solvents, such as methanol, isopropyl alcohol, acetonitrile, acetone, and *N,N*-dimethylformamide, retinyl acetate and α -tocopheryl acetate were compared in terms of peak potential position and current response (Table 1). No effect on the peak position and higher peak current was obtained in acetone, therefore it was chosen as an optimum.

Table 1. Comparison of anodic peak potential and current response in various solvents at the GCE.

Solvents	Retinyl acetate		α -Tocopheryl acetate	
	E_p^a (V)	I_p^a (μA)	E_p^a (V)	I_p^a (μA)
Acetonitrile	0.811	1.046	1.294	2.356
Acetone	0.871	2.534	1.380	1.657
Methanol	0.806	1.825	1.309	1.131
Dimethylformamide	0.836	1.369	1.279	0.516
Isopropanol	0.851	1.305	1.399	0.274

Values are given as arithmetic mean of five repetitions.

The influence of the content of water (0, 10, 20, 30, 40, and 50 % v/v) in ACE on RAc and α -TOAc detection was studied. In presence of water, the oxidation peak of RAc has decreased contrary to α -TOAc. After all optimization steps, the proper procedure is based on direct anodic oxidation of RAc or RPa and α -TOAc at GCE in pure ACE containing 0.1 mol L^{-1} LiClO_4 . Two linear ranges for RAc in intervals from 3.1 to 140 $\mu\text{mol L}^{-1}$ and from 140 to 400 $\mu\text{mol L}^{-1}$, one linear range 2.8–180 $\mu\text{mol L}^{-1}$ for RPa, and one linear range 5.3–400 $\mu\text{mol L}^{-1}$ for α -TOAc were attained. Detection limits (LOD) of 0.9 $\mu\text{mol L}^{-1}$ RAc (or 0.8 $\mu\text{mol L}^{-1}$ RPa) and of 1.6 $\mu\text{mol L}^{-1}$ α -TOAc were calculated. The repeatability for level of significance $\alpha = 0.05$ is presented as the relative standard deviation (RSD) and values of 2.27 % and 2.19 % were calculated for ten replicates of a model mixture of 50 $\mu\text{mol L}^{-1}$ RAc and α -TOAc,

respectively. Acceptable recoveries in range 97.8–106.1% were achieved. The developed method was applied in three types of cosmetic products such as hand cream, refreshing cleaning body milk, and face. The samples were dissolved directly in the working medium without any treatment. The results of all analyses and the calculated amounts are presented in Table 2, Publication 2. The voltammograms of sample face cream, and hand cream analysis are shown in Fig. 2.

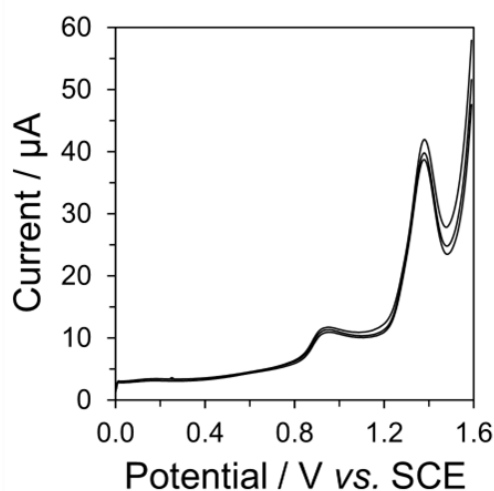


Figure 2. Voltammograms of sample face cream δ hand cream. SWV at GCE in 99.7% acetone containing $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$, $E_{\text{step}} = 10 \text{ mV}$, $E_{\text{ampl}} = 40 \text{ mV}$ and $f = 30 \text{ Hz}$.

We can conclude that the main advantages achieved were the simplicity of preparation of cosmetic samples, using less hazardous ACE as the working medium, elimination of steps like saponification and extraction/re-extraction into organic solvent which are quite complex, and the possibility of monitoring RAc (or RPa) and α -TOAc simultaneously.

1.2.2. Extractive stripping voltammetry of lipophilic vitamins in cow's milk and cream

Milk and all its products are considered as very complex sample for analysis and often require time-consuming sample preparation. Several steps, such as alkaline hydrolysis, liquid-liquid extraction, filtration, and evaporation of the used solvent, are usually performed before the analysis. This research offers a simple voltammetric approach for monitoring VA as a sum of retinoids and carotenoids in cow's milk and cream product without sample preparation steps. The method is based on two steps: the first step is the direct extraction of analytes from the milk into a pasting liquid (nonpolar binder) of GCPE and, after transferring the working electrode to the electrochemical cell, subsequent electrochemical detection by SWV in 0.1 mol L^{-1} Britton-Robinson buffer with pH 4.5 in second step.

Since the procedure for extracting lipophilic vitamins from cow's milk usually involves an alkaline hydrolysis step, this step could be eliminated by extraction into electrode material of GCPE. Five GCPEs containing always 20% (w/w) portion of the organic binder, differing only in the type of used organic binder (APP, paraffin oil, PW, SO, and vaseline), were investigated to find the most suitable composite for the extraction of lipophilic vitamins. The silicon oil as an organic binder provided better extraction efficiency of lipophilic vitamins into GCPEs from a sample of cow's milk (3.5% of fat) for 10 minutes of accumulation time and stirring rate of 400 rpm (Table 1, Publication 3).

The extraction equilibrium has been achieved after 10 minutes; longer period did not cause any change in peak response (Fig. 2, Publication 3). The stirring rate faster than 300 rpm did not have any impact on the final peak current signal, therefore it was chosen as optimum for subsequent experiments. From the obtained results, it was observed that milk fat globules (MFG) mainly contain VA (carotenoids and retinoids), especially all-*trans*-retinol, which can serve as an important marker of fat content. However, peaks of individual compounds cannot be distinguished due to the significant broadening and intensive overlapping of the oxidation signals (Fig. 3, Publication 3). Analysis of several samples of milk and cream with different percentages of fat revealed that the voltammetric signal was higher in samples with the lowest percentage of fat, even though it is assumed that samples with higher amounts of fat have a higher content of lipophilic vitamins. This can be explained by the fact that the lipophilic vitamins in the samples are evenly distributed between the pasting liquid, and milk fat during extraction ('liquid-liquid') and these vitamins are more isolated in the creams (12-31% fat) than in milk (0.5-3.5% fat) due to the much higher fat content (Fig. 5, Publication 3). The advantage of this method is attributed to the simplicity of sample preparation without the need for a complicated step. However, the direct extraction of lipophilic vitamins (mainly VA) can be used only for the semi-quantitative determination of milk fat at this stage of development.

1.2.3. A new voltammetric approach for the determination of β -carotene

The aim of the research was to propose a new voltammetric approach for the determination of BCA in foodstuffs and pharmaceutical supplements. The focus of the work was to avoid hazard organic solvents and to simplify sample preparation. Various organic solvents, such as MeCN, mixture of MeCN and toluene (1:1), mixture of MeCN and methyl tert-butyl ether (1:1), and pure ACE, all containing $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$, were tested. BCA provided the highest oxidation current at GCE using CV in the mixture of MeCN and toluene

(Fig. 1, Publication 4). Nevertheless, ACE was preferred due to its high extraction efficiency for foodstuffs being a less hazardous solvent. Two metal-based electrodes (AuE and PtE), and GCE were compared in voltammetric measurement of $200 \mu\text{mol L}^{-1}$ BCA in pure ACE with 0.1 mol L^{-1} LiClO_4 using SWV. GCE provided almost twice broader anodic peak at $+0.499 \text{ V}$ in comparison with metallic electrodes (Fig. 3).

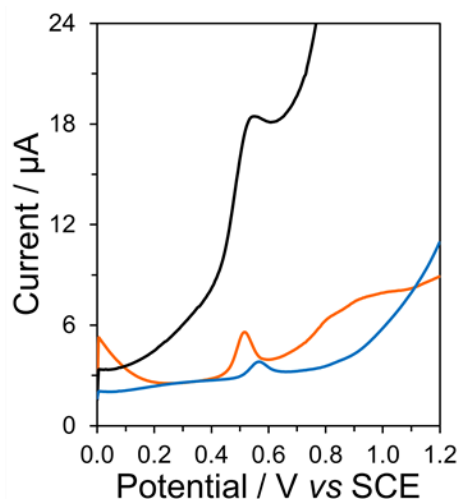


Figure 3. Comparison of square-wave voltammetric records for $200 \mu\text{mol L}^{-1}$ BCA obtained at GCE (black), AuE (orange), and PtE (blue line) in pure acetone.

In addition, a decrease in the peak of interest with the increasing number of consecutive measurements ($\text{RSD} = 12.73\%$) was observed for GCE, unlike in the case of AuE ($\text{RSD} = 1.33\%$). The analytical performance of the new method for the voltammetric determination of BCA in the optimized conditions has been tested. The method is based on the oxidation of BCA at AuE in ACE as an organic working medium with the possibility to perform analysis at the same electrode surface. Compared to previously reported methods, a wider linear range within $5\text{--}600 \mu\text{mol L}^{-1}$ BCA and LOD of $1.6 \mu\text{mol L}^{-1}$ were obtained (Table 1, Publication 4). Accuracy was calculated from the analysis of a model sample and a recovery of 95.4% (error less than 5%) was attained.

Analysis of several raw vegetables and pharmaceutical capsules was conducted using the newly developed approach. The results were in good agreement with those acquired by UV-Vis spectrophotometry reference method (Table 2, Publication 4). Several advantages were achieved compared to already reported methods. Real samples were directly extracted using acetone as a solvent, which is not as toxic as the chlorinated hydrocarbons used before. Analytical performance of proposed method provides wider linear range as well as a lower detection limit than already published approaches. In addition, the same procedure can be applied to assay both food and pharmaceutical samples.

1.3. Simultaneous determination of vitamin E and K

In two earlier studies it was shown that it is possible to utilize SWAdSV and GCE with good results in the determination of lipophilic vitamins [17], and vitamin K₁ [19]. By combining these two investigations, a simple and fast procedure has been developed for the simultaneous determination of VK and VE in food and pharmaceutical samples using SWAdSV.

The working methodology was based on three steps: the first step was the accumulation of lipophilic vitamins in open circuit on the nonpolar surface of the GCE in an aqueous-organic mixture, followed by the application of a negative potential for a certain period for electrochemical reduction of phyloquinone (VK₁) to phylohydroquinone (H₂VK₁), and finally sequential electrochemical oxidation of reduced compounds providing current signals according to their various redox potentials. The work on this study is described in four main sections: the optimization of adsorption of vitamins, optimization of their voltammetric detection, analytical performance of the developed voltammetric method and analysis of food supplements.

The adsorption of the analytes to the working electrode is affected by several factors, such as type of organic solvent, accumulation time, stirring rate and ionic strength of the solution. Since VE and VK₁ are fat-soluble compounds, they are soluble in polar organic solvents and their aqueous mixtures. The effect of MeCN content (30 to 70% v/v) on the current signal response for both analytes was investigated using stirring rate of 300 rpm and accumulation time of 5 minutes. It was observed that MeCN content higher than 50% did not have any effect on the peak current response, therefore the mixture MeCN-water in ratio 1:1 was taken as an optimum (Fig. 4A). In addition, absence and presence of 0.0001, 0.001, 0.01, and 0.1 mol L⁻¹ KCl in 50% MeCN was investigated. No significant increase in peak heights was found (Fig. 4B). Based on the obtained results, 0.1 mol L⁻¹ KCl was used in further experiments to lower the ohmic resistance of the solution. The stirring rate affects the adsorption of the analytes to the working electrode, thus different speeds (100, 200, 300, 400, and 500 rpm) were tested. Setting the stirring rate faster higher than 400 rpm did not cause any significant increase in peak current responses, therefore this value was taken as optimum. Basically, the adsorption of the nonpolar analytes onto a nonpolar solid substrate (working electrode) is an equilibrium process. Hence, the accumulation time is one of the main parameters to be optimized in order to achieve a sufficient adsorption of the analyte. The accumulation time from 30 s to 900 s was investigated and the results shown that the adsorption

of the analytes intensified up to 300 s, afterwards no significant increase in peaks heights was observed. The electrode surface became probably already saturated at higher accumulation times, therefore the value of 300 s was considered as an optimum (Fig.1, Publication 5).

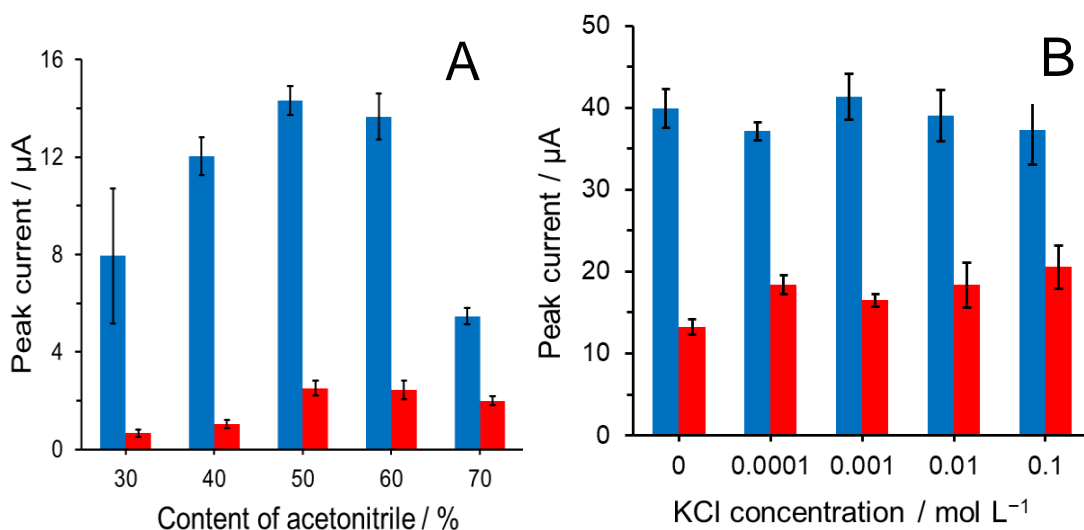


Figure 4. Dependence of anodic peak current on different content of acetonitrile (30 to 70 % v/v of 50 µmol L⁻¹ vitamin K₁ (red) and α-tocopherol (blue), adsorbed at 300 rpm for 300 s. Voltammetric detection was carried out in 0.01 mol L⁻¹ HNO₃ containing 0.1 mol L⁻¹ KCl (pH 2.08) at $E_{\text{dep}} = -0.1$ V, $t_{\text{dep}} = 60$ s, $E_{\text{step}} = 5$ mV, $E_{\text{ampl}} = 25$ mV and $f = 20$ Hz (A). The effect of ionic strength on peak current response in 50 % acetonitrile content of 50 µmol L⁻¹ vitamin K₁ (red) and α-tocopherol (blue), adsorbed at 300 rpm for 300 s. Voltammetric detection was carried out in 0.01-mol L⁻¹ HNO₃ containing different content of KCl (pH 2.08) at $E_{\text{dep}} = -0.1$ V, $t_{\text{dep}} = 60$ s, $E_{\text{step}} = 5$ mV, $E_{\text{ampl}} = 25$ mV and $f = 20$ Hz (B).

Two linear ranges were obtained, the short linear ranges for VK₁ and α-TOH determination were relatively short, 77–1000 nmol L⁻¹ for VK₁ and 29–1000 nmol L⁻¹ for α-TOH, with detection limits (LOD) of 25 and 10 nmol L⁻¹, respectively. Additionally, linear ranges for higher concentrations were found: 1.0–7.0 µmol L⁻¹ VK₁ and 1.0–10 µmol L⁻¹ α-TOH described by regression equations $I_p (\mu\text{A}) = 1.964 c (\mu\text{mol L}^{-1}) - 1.046$ with $R^2 = 0.9997$ and $I_p (\mu\text{A}) = 3.416 c (\mu\text{mol L}^{-1}) + 1.4105$ with $R^2 = 0.9973$, respectively. Due to low value of intercept, standard addition method can be used for quantitative analysis. The precision was taken as recovery of measurements for eight replicates and the RSD values of 4.7% and 6.6% for VK₁ and α-TOH, respectively, were achieved. Two food supplements were analysed, and the results obtained by developed voltammetric method were in good agreement with HPLC (Table 2, Publication 5). The main advantages of the method are the possibility of simultaneous determination of VK₁ and α-TOH, simple preparation for samples (especially in case of

pharmaceutical sample, which can be directly dissolved in working media), and minimal interference of accompanying substances.

1.4. Possibilities of simultaneous voltammetric determination of individual tocopherol isomers

During the research of simultaneous determination of VK and VE in food supplements, it was observed that in the obtained voltammograms of food supplement sample there several signals indicating the presence of forms of VE. These three overlapping peaks were attributed to the anodic oxidation of α -TOH, γ -TOH, and δ -TOH at +0.464 V, +0.539 V, and +0.604 V, respectively. Based on this finding, a detailed study on the possibility of simultaneous determination of three forms of VE have been carried out.

Various working electrode materials, presence of water in organic solvent, and the effect of non-ionic, cationic, and anionic surfactants were tested. AuE, PtE, GCE, boron doped diamond electrode, pyrolytic graphite electrode, and GCE/MWCNTs were tested for the simultaneous voltammetric detection of 50 $\mu\text{mol L}^{-1}$ tocopherol forms in pure MeCN containing 0.1 mol L^{-1} LiClO₄ (Fig. 2, Publication 6). Only GCE provided the widest peak separations with peak potentials at +0.645 V for α -TOH, +0.752 V for γ -TOH, and +0.857 V for δ -TOH. The reason of such observation is most probably due to slower charge transfer at GCE comparing to metal-based electrodes (AuE and PtE) and other carbon electrodes, where peaks were strongly overlapped. Hence, GCE was chosen as working electrode for further experiments.

Since tocopherols are soluble in polar organic solvents, DMF, propylene carbonate, ethanol (EtOH), and MeCN were examined to find the most compatible working medium. Two low current response and broad anodic peaks of the three tocopherol forms were observed in DMF and propylene carbonate, on the other hand, three overlapping peaks with higher current signal were obtained in EtOH and MeCN (Fig. 3A, Publication 6). Considering that three well-defined voltammetric signals from individual tocopherol forms were obtained only in MeCN, the mixture of MeCN with water in ratio (90:10 % v/v) was further studied. The presence of water caused a shift of anodic peaks to more negative potentials and a significant decrease of peak currents (Fig. 3B, Publication 6). In addition, the effect of different contents (0.1, 0.01, and 0.001 mol L^{-1}) of nonionic (Triton X-100), anionic (SDS), and cationic (cetylpyridinium chloride and cetyltrimethylammonium bromide) surfactants on peaks separation was investigated. No improvement on the overlapped peak signals was observed (Fig. 5).

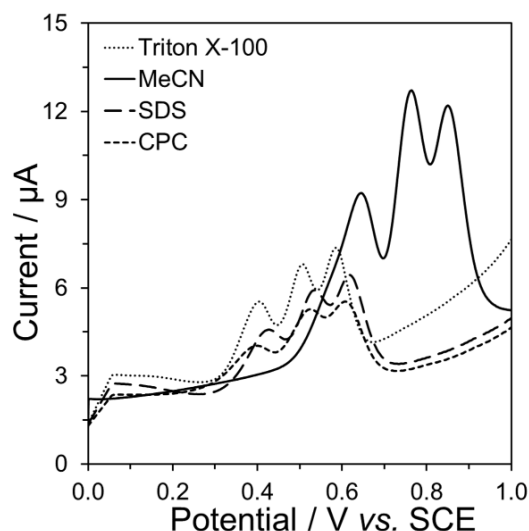


Figure 5. Voltammograms of $50 \mu\text{mol L}^{-1}$ α -TOH, γ -TOH and δ -TOH at GCE in pure MeCN containing 0.1 mol L^{-1} LiClO_4 and 0.001 mol L^{-1} CPC, SDS or Triton X-100 at $E_{\text{step}} = 1 \text{ mV}$, $E_{\text{ampl}} = 25 \text{ mV}$ and $f = 20 \text{ Hz}$.

The peak height and width increased with a higher concentration of analyte. Hence, it was necessary to find out if the concentration ratio can distort the recognition of individual tocopherols. The obtained voltammograms showed that overlapping of γ -TOH and δ -TOH anodic peaks is evident if one of these forms has a higher concentration than the other, while α -TOH and δ -TOH peaks increased with a higher concentration of both forms. Overlapping was suppressed in case when concentrations of all three forms were mutually increased (Fig. 4, Publication 6).

Furthermore, a comparison of four different methods for evaluation of current signals, obtained during the voltammetric analysis of tocopherols, has been carried out. The precision, defined as the result of variability found for 10 repeated measurements, was calculated for the tocopherols mixture ($50 \mu\text{mol L}^{-1}$ of each form) and presented as RSD. RSD values lower than 5% were obtained for evaluation using linear baseline, zero base, and deconvolution of signals, contrary to polynomial baseline evaluation with RSD over 5%. The accuracy was presented as recovery values in percentage. Acceptable accuracy values ranging from 72% to 119% were attained only for the deconvolution method, (see Table 3, Publication 6). However, it is important to note that evaluation of voltammetric signals is not possible without the use of the mathematical deconvolution of overlapping peaks. The current electrochemical study includes detailed and relevant information for the possible development of a direct voltammetric method for the simultaneous determination of α -TOH, γ -TOH, and δ -TOH in foodstuffs or pharmaceutical preparations.

1.5. Voltammetric approaches for the determination of selected biologically active compounds

1.5.1 Voltammetric determination of nitrites in meat products after reaction with ranitidine

In this research, a completely new voltammetric method is proposed for the first time based on the cathodic reduction of the product 2-methyl-2H-furan-3-one, after a specific reaction of nitrites with ranitidine (RAN) in an acidic environment [33,34]. GCPE covered with a thin film of electrochemically reduced graphene oxide and adsorbed SDBS was used for the determination of nitrite ions in 0.1 mol L^{-1} Britton-Robinson buffer at pH 2. Graphene oxide was electrochemically immobilized on GCPE using CV (20 cycles) in 0.1 mol L^{-1} phosphate buffer. Unmodified GCPE, GCPE modified with graphene oxide, graphene oxide and surfactant SDS, and graphene oxide and surfactant SDBS were compared. It was evident that the reduction peak at the GCPE modified with graphene oxide and SDBS provided higher current peak response, probably due to an electrostatic interaction between methylfuran cation and sulphate anion from anionic surfactant (Fig. 2, Publication 7). Surfactant content in $10 \text{ }\mu\text{L}$ volume applied on the surface of GCPE/ERGO/SDBS was optimized using different concentration (0.1 , 0.5 , 1.0 , 1.5 , and 2.0 mmol L^{-1}). Concentration higher than 1.0 mmol L^{-1} did not influence the current peak reduction, shown in Fig. 6. Thus, the concentration of 1.0 mmol L^{-1} was taken as optimum.

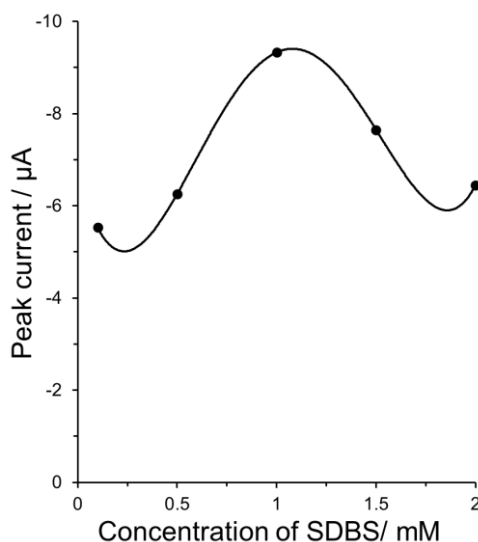


Figure 6. Effect of surfactant concentration (0.1 , 0.5 , 1.0 , 1.5 , and 2.0 mmol L^{-1}) on peak current obtained for mixture of $320 \text{ }\mu\text{mol L}^{-1}$ ranitidine with $250 \text{ }\mu\text{mol L}^{-1}$ nitrite after reaction time of 60 s in 0.1 mol L^{-1} BRB of pH 2.

The pH of working media solution had a key role on the conversion of nitrite ion to nitrosonium cation which reacts with ranitidine. Because the conversion occurs in acidic media,

the pH could affect the current response of the cathodic peak. The obtained results shown that the maximum reduction current signal was at the pH 2. In higher pH values, the response was dramatically decreased and over pH 5 no signal was observed (Fig. 3A, Publication 7). The product (nitrosamines) of the reaction of RAN with nitrites in acidic medium was used for the determination of nitrites. Hence, the concentration of RAN within a range from 80 $\mu\text{mol L}^{-1}$ to 540 $\mu\text{mol L}^{-1}$ at constant content of nitrites of 250 $\mu\text{mol L}^{-1}$ in 0.1 mol L^{-1} BRB pH 2 was optimized. A linear dependence of current response was found up to 320 $\mu\text{mol L}^{-1}$ RAN. Consequently, the reaction time was optimized. After two minutes, the constant value of the reduction peak current has been achieved (Fig. 3B, Publication 7).

In the optimized conditions, two linear ranges from 6.2 $\mu\text{mol L}^{-1}$ to 125 $\mu\text{mol L}^{-1}$ and from 150 $\mu\text{mol L}^{-1}$ to 300 $\mu\text{mol L}^{-1}$ nitrites were found, characterized by R^2 of 0.9991 and 0.9963, respectively, with a detection limit of 1.89 $\mu\text{mol L}^{-1}$ nitrites. Repeatability of analysis of the proposed voltammetric method was determined as RSD of 3.8% using ten repeated measurements. Satisfactory accuracy (difference between determined and true analyte content) characterized with a recovery value of 95.4% has been found. Compared to the methods mentioned above, one of the advantages is the elimination of multiple interferences because the previous voltammetric methods were based on anodic oxidation in contrast to the cathodic reduction ($E_p = -0.2$ V) used in this method, where interference of accompanying substances is not expected. The developed method has been successfully applied in the quantification of nitrites in some meat products such as beef sausages, chicken sausages, lunchmeat, and smoked meat where the results have been satisfactory and comparable to reference methods using Griess Reagent Kit (G-7921) (Table 2, Publication 7).

1.5.2 Comparison of amperometric biosensor with portable Raman spectrometer determination of ethanol in alcoholic drinks

In this work, an amperometric biosensor and a Mira-DS handheld Raman spectrometer were compared in determining the amount of EtOH in various alcoholic beverages such as rum, vodka, homemade plum brandy, white and red wines, plus two different types of beer. Both analytical approaches have been optimized and compared in the real samples analyses. Three different modifications of screen-printed carbon electrode (SPCE) were tested for higher current yields during NADH anodic oxidation. SPCEs covered by thin layer of reduced graphene oxide, MWCNTs or carbon ink with always 5% RhO_2 (w/w) has been compared in the electrochemical response of NAD^+/NADH redox couple. Higher peak at a relatively low potential of +0.307 V was obtained at SPCE modified with reduced graphene.

Two different procedures for fabrication of amperometric ADH biosensors have been compared to choose the most suitable one. Firstly, the consecutive layers of the respective components (RGO, RhO₂, ADH, NAD⁺, GTA, and Nafion[®]; in this order), and second as a composite of all components prepared as follows: in a vial, portions of 1.0 mg reduced single-layer graphene oxide, 50 µg rhodium dioxide, 8.75 mg alcohol dehydrogenase, 1.75 µg β-nicotinamide adenine dinucleotide sodium salt, 125 µL 1% glutaraldehyde and 375 µL 1% Nafion[®] were mixed and homogenized using an ultrasonic bath for 30 minutes. After two hours, a volume of 10 µL of the prepared dispersion was applied onto the surface of screen-printed carbon electrode and left for drying in the room temperature for 1 hour. Amperometric responses of 40% EtOH (v/v) revealed that the fabrication of the biosensor using the composite procedure shown a stable peak current signal (Fig. 7).

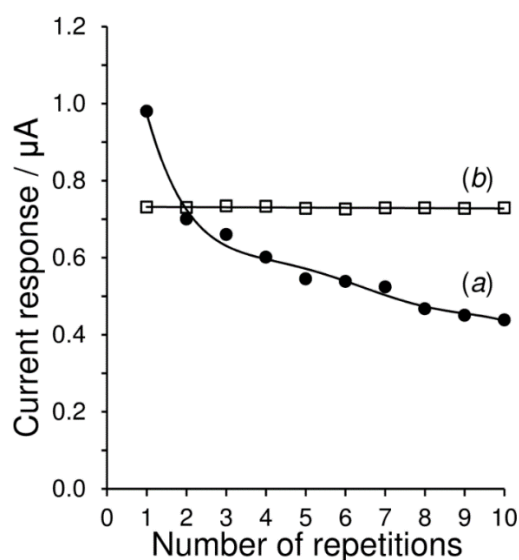


Figure 7. The comparison between ADH biosensor built up in successive layers (a) and ADH biosensor with a composite layer (b) in amperometric response stability of 40% EtOH (v/v). Experimental conditions: injection volume 100 µL, flow rate 1.6 mL min⁻¹ of 0.1 mol L⁻¹ PB (pH 8.5) and applied potential 0.2 V.

The analytical performance of developed biosensor was studied. However, no significant improvement in the sensitivity comparing to other ADH-based electrochemical biosensors has been achieved. Nevertheless, all analytical parameters indicate that the biosensor can be used in analysis of highly alcoholic drinks. Two linear ranges of 0.25-10% and 10-50% EtOH (v/v) have been obtained and no memory effect was observed (Fig. 2, Publication 8). If the developed biosensor is stored in a freezer at -21 °C, its lifetime would be minimally 7 days.

The effect of possible interfering substances, such as isopropyl alcohol, n-propanol, ethyl acetate, and MeOH, in amperometric detection with electrochemical biosensor was

investigated. It was observed that all tested compounds provided a false positive current signal upon injection (Fig. 4, Publication 8). The low selectivity of the proposed biosensor can be found in low substrate specificity of the enzyme used. On the other hand, it was shown that the ADH-based sensing can be applied in the analysis with high content of EtOH, where the presence of interfering substances is minimal. In case of the Raman spectrometer, it can identify and determine EtOH in the presence of the above-mentioned interfering substances without any problems.

Three real samples of highly alcoholic spirits (home-made plum brandy, commercially vodka, and white rum), were chosen for analysis. The results of determinations in real samples were fully comparable to the stated content of EtOH in vodka and rum (Table 2, Publication 8). However, both analytical methods encountered limits in the determination of EtOH in red wine and darker beers. There is still a need for further improvements in order to achieve better selectivity of the electrochemical biosensor. The comparison between the developed amperometric biosensor and Mira-DS portable Raman spectrometer showed that both tools cannot be used universally, due to some limitations in analysis of samples with complex matrices.

2. Conclusions

In this doctoral thesis, several new electrochemical approaches for the simultaneous determination of lipophilic vitamins in foodstuffs, pharmaceutical preparations, and cosmetic products have been presented. In the first research, the detailed data about the preparation and the possibilities of (bulk-)modified carbon paste electrodes with surfactants were of special interest. It was found that nonaqueous carbon paste electrode depends on the type of carbon material and the amount of non-electroactive surfactant.

A new, simple, and rapid electroanalytical method for simultaneous determination of retinyl acetate (or retinyl palmitate) and alpha-tocopheryl acetate in cosmetic products was proposed and developed for the first time. The method is based on direct anodic oxidation of analytes at glassy carbon electrode in acetone containing LiClO₄. Complicated steps for samples preparation (saponification and extraction into organic solvent), were avoided due to direct dissolution of samples in the supporting electrolyte, unlike in HPLC. It offers satisfactory detection capabilities for routine analysis of cosmetic products and related samples. The results of several samples analysed by the developed method shown statistically identical values. In the future the electroanalytical approach could potentially replace the time-consuming chromatographic methods.

Extractive stripping voltammetry of vitamins at a glassy carbon paste electrode was proposed for the analysis of cow's milk and cream. The procedure is based on two steps: the extractive accumulation of milk fat globules into a glassy carbon paste electrode containing lipophilic binder and transfer of the electrode to electrochemical cell for subsequent detection by square-wave voltammetry in Britton-Robinson buffer (pH 4). The obtained results show that the vitamin A (carotenoids and retinoids), especially all-*trans*-retinol, dominates the milk fat globules. However, the results suggest that direct extraction of lipophilic vitamins (mainly all-*trans*-retinol) from continuously stirred milk and cream samples, and subsequent voltammetric detection could only be used for semi-quantitative determination of milk fat, whereas the individual forms were not possible to distinguish due to overlapping of signals.

A novel approach for the voltammetric determination of β -carotene has been presented. The method is based on anodic oxidation of β -carotene in pure acetone containing LiClO₄ at gold electrode using square-wave voltammetry. Compared to the already reported methods, several improvements were attained due to a new sample preparation using acetone (solvent not toxic like previously used chlorinated hydrocarbons) providing wider linear range and lower limit of detection. Moreover, the same method is suitable in food and pharmaceutical analysis.

The results from analysis of raw carrots, sweet potatoes, and nutritional capsules were in a good agreement with the reference spectrophotometric assay.

The simultaneous voltammetric determination of vitamin E and K in food supplements at glassy carbon electrode using square-wave adsorptive stripping voltammetry was developed. The presented method was suitable for determining the sum of vitamin E (all tocopherol forms) and vitamin K (phyloquinone). The procedure was based on ex situ adsorptive accumulation of the vitamins onto surface of glassy carbon electrode in acetonitrile-water (ratio 1:1 v/v), followed by the transfer of the working electrode to the electrochemical cell. However, the main advantages of the method are based on the ex-situ accumulation, where the sample preparation consists of the dissolving the sample direct in the accumulation medium, which minimizes interference. Two food supplements were analysed, and the obtained results were comparable with reference HPLC method.

Possibilities for simultaneous voltammetric detection of tocopherols was investigated in detail, and the optimum parameters were found. In the comparison with the reported scientific papers, the simultaneous voltammetric detection of tocopherols was simplified and well-defined peaks of tocopherols were obtained at GCE in pure MeCN using SWV with potential step of 1 mV. However, the analysis of voltammetric signals is not possible without a deconvolution process for the overlapped signals. Thus, the present electrochemical study includes appropriate information and instructions for the development of the voltammetric approach in simultaneous determination of α -TOH, γ -TOH and δ -TOH in foodstuffs.

Monitoring the content of nitrites in meat products was another subject in focus. The approach is based on cathodic reduction of electroactive product formed after chemical reaction of ranitidine and nitrosonium ion, originated from nitrites. The indirect determination of nitrites at glassy carbon paste electrode covered with a thin film of electrochemically reduced graphene oxide with presence of adsorbed dodecyl benzene sulfonate in Britton-Robinson buffer (pH 2) was successfully carried out. The analysis of several meat products shown statistically comparable results with commercially available spectrophotometric assay (Griess Reagent Kit G-7921). Prospects seem to be in the working electrode proposed, which could simply be converted to a planar configuration and used as disposable screen-printed sensor.

The immobilization of alcohol dehydrogenase into graphene-based composite was used to set up a simple bioanalytical device suitable for amperometric detection of ethanol in highly alcoholic spirits and white wines using flow injection analysis. However, there is still a need for further improvements in order to achieve better selectivity and simplify the preparation steps of biosensor.

3. References

1. Ball G. F. M. (2004). *Vitamins: Their Role in the Human Body*, Blackwell Publishing, Oxford.
2. Gonnet M., Lethuaut L., Boury F. (2010). New trends in encapsulation of liposoluble vitamins. *Journal of Controlled Release*, 146, 276–290.
3. Webster R. D. (2012). Voltammetry of the liposoluble vitamins (A, D, E and K) in organic solvents. *The Chemical Record*, 12, 188–200.
4. Ahmad S. R., Gokulakrishnan P., Giriprasad R., Yattoo M. A. (2015). Fruit-based natural antioxidants in meat and meat products: a review. *Critical Reviews in Food Science and Nutrition*, 55, 1503–1513.
5. Wolf G. (2002). The experimental induction of vitamin A deficiency in humans. *The Journal of Nutrition*, 132, 1805–1811.
6. Rajakumar K. (2003). Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. *Pediatrics*, 112, 132–135.
7. Niki E., Yamamoto Y., Komuro E., Sato K. (1991). Membrane damage due to lipid oxidation. *The American Journal of Clinical Nutrition*, 53, 201S–205S.
8. Simons K., Ikonen E. (1997). Functional rafts in cell membranes. *Nature*, 387, 569–572.
9. Browne D., McGuinness B., Woodside J. V., McKay G. J. (2019). Vitamin E and Alzheimer’s disease: What do we know so far?. *Clinical Interventions in Aging*, 14, 1303–1317.
10. Bajaj R., Karthikeyan G., Sinha N., Lokhandwala Y., Rao D., Kaushik S. K., Jain S. L., Narayanan S., Seth A., Satyamurthi I. et al. (2012). CSI consensus statement on prosthetic valve follow up. *Indian Heart Journal*, 64, S3–S11.
11. Girolami A., Ferrari S., Cosi E., Girolami B., Lombardi A. M. (2017). Congenital prothrombin defects: They are not only associated with bleeding but also with thrombosis: A new classification is needed. *Hematology*, 23, 105–110.
12. Parazzini F. (2014). Resveratrol, inositol, vitamin D and K in the prevention of cardiovascular and osteoporotic risk: a novel approach in peri – and postmenopause. *Minerva Ginecologica*, 66, 513–518
13. Celentano C., Matarrelli B., Mattei P. A., Pavone G., Vitacolonna E., Liberati M. (2016). Myo-inositol supplementation to prevent gestational diabetes mellitus. *Current Diabetes Reports*, 16, 30

14. Sun Y., Jiang L., Wei D. (2013). Partial characterization, in vitro antioxidant and antiproliferative activities of patatin purified from potato fruit juice. *Food & Function*, 4, 1502.
15. Radi A. (1999). Electroanalysis of melatonin using castor oil–graphite paste electrode. *Analytical Communications*, 36, 43-44.
16. Sýs M., Švecová B., Švancara I., Metelka R. (2017). Determination of vitamin E in margarines and edible oils using square wave anodic stripping voltammetry with a glassy carbon paste electrode. *Food Chemistry*, 229, 621–627.
17. Sýs M., Žabčíková S., Červenka L., Vytrás K. (2016). Adsorptive stripping voltammetry in lipophilic vitamins determination. *Potravinarstvo Slovak Journal of Food Sciences*, 10, 260–264.
18. Sýs M., Žabčíková S., Červenka L., Vytrás K. (2017). Comparison of adsorptive with extractive stripping voltammetry in electrochemical determination of retinol. *Potravinarstvo Slovak Journal of Food Sciences*, 11, 96–105.
19. Sýs M., Jashari G., Švecová B., Arbneshi T., Metelka R. (2018). Determination of vitamin K1 using square wave adsorptive stripping voltammetry at solid glassy carbon electrode. *Journal of Electroanalytical Chemistry*, 821, 10–15.
20. Žabčíková S., Mikysek T., Červenka L., Sýs M. (2018). Electrochemical study and determination of all-trans-retinol at carbon paste electrode modified by a surfactant. *Food Technology and Biotechnology*, 56, 337–343.
21. Žabčíková S., Nallbani A., Sýs M., Mikysek T., Červenka L. (2018). Square wave voltammetry at carbon paste electrode modified with surfactant for alpha tocopheryl acetate determination in cosmetics. *Potravinarstvo Slovak Journal of Food Sciences*, 12, 135–142.
22. Jashari G., Švancara I., Sýs M. (2022). Characterisation of carbon paste electrodes bulk-modified with surfactants for measurements in nonaqueous media. *Electrochimica Acta*, 410, 140047.
23. Jashari G., Musliu A., Sýs M., Arbneshi T., Mikysek T., Švancara I., Metelka R. (2021). Simultaneous determination of lipophilic vitamin esters using square-wave voltammetry at the glassy carbon electrode. *Electroanalysis*, 33, 537-542.
24. Jashari G., Frühbauerová M., Sýs M., Červenka L. (2020). Extractive stripping voltammetry at a glassy carbon paste electrode for analysis of cow's milk and cream. *Potravinarstvo Slovak Journal of Food Sciences*, 14, 202-207.

25. Jashari G., Muriqi S., Arbneshi T., Metelka R., Švancara I., Sýs M. (2021). A new voltammetric approach for the determination of β -carotene in vegetables and pharmaceutical capsules using a gold electrode. *Talanta*, 227, 122088.
26. Kastrati K., Jashari G., Sýs M., Švecová B., Arbneshi T., Metelka R., Bílková Z., Korecká L. (2020). Simultaneous determination of vitamin e and vitamin k in food supplements using adsorptive stripping square-wave voltammetry at glassy carbon electrode. *Applied Sciences*, 10, 4759.
27. Jashari G., Kastrati G., Korecká L., Metelka R., Sýs M., Ashrafi A. M. (2021). Electrochemical behaviour of tocopherols: Possibilities of their simultaneous voltammetric detection. *Applied Sciences*, 11, 8095.
28. Berisha L., Maloku A., Haliti M., Jashari G., Ukmata U., Sýs. (2020). Voltammetric determination of nitrites in meat products after reaction with ranitidine producing 2-methylfuran cation. *Microchemical Journal*, 159, 105403.
29. Jashari G., Švancara I., Sýs M. (2020). Determination of ethanol in alcoholic drinks: Flow injection analysis with amperometric detection versus portable Raman spectrometer. *Electroanalysis*, 32, 1949-1956.
30. Michalkiewicz S., Pryciak M., Malyszko J., Oszczudlowski J. (2004). Voltammetric determination of α -tocopheryl acetate in pharmaceutical dosage forms. *Electroanalysis*, 16, 961–965.
31. Mikheeva E. V., Anisimova L. S. (2007). Voltammetric determination of vitamin E (α -Tocopherol acetate) in multicomponent vitaminized mixtures. *Journal of Analytical Chemistry*, 62, 373–376.
32. Hassan E. M., Khamis E. F., El-Kimary E. I., Barary M. A. (2008). Development of a differential pulse voltammetric method for the determination of Silymarin/Vitamin E acetate mixture in pharmaceuticals. *Talanta*, 74, 773–778.
33. Lee S. B., Lin C. Y., Gill P. M. W., Webster R. D. (2005). Transformation of α -Tocopherol (Vitamin E) and related chromanol model compounds into their phenoxonium ions by chemical oxidation with the nitrosonium cation. *The Journal of Organic Chemistry*, 70, 10466–10473.
34. Wilson G. J., Lin C. Y., R. D. Webster R. D. (2006). Significant differences in the electrochemical behavior of the α -, β -, γ -, and δ -Tocopherols (Vitamin E). *The Journal of Physical Chemistry B*, 110, 11540–11548.

4. List of publications

5.1 Publications in impacted journals

1. Jashari, G., Švancara, I., Sýs M. Characterisation of carbon paste electrodes bulk-modified with surfactants for measurements in nonaqueous media. *Electrochimica Acta*, 410, (2022), 140047.
2. Jashari, G., Kastrati, G., Korecká, L., Metelka, R., Sýs, M., Ashrafi, M. A. Electrochemical behaviour of tocopherols: possibilities of their simultaneous voltammetric detection. *Applied Sciences*, 11, (2021), 8095.
3. Jashari, G., Muriqi, S., Arbnesi, T., Metelka, R., Švancara, I., Sýs, M. A new voltammetric approach for the determination of β -carotene in vegetables and pharmaceutical capsules using a gold electrode. *Talanta*, 227, (2021), 122088.
4. Sýs, M., Mukherjee, A., Jashari, G., Adam, V., Ashrafi, M. A., Novák, M., Richtera, L. Bis(2,2'- bipyridil)Copper(II) Chloride Complex: Tyrosinase Biomimetic Catalyst or Redox Mediator?. *Materials*, 14, (2021), 113.
5. Jashari, G., Musliu, A., Sýs, M., Arbnesi, T., Mikysek, T., Švancara, I., Metelka, R. Simultaneous determination of lipophilic vitamin esters using square-wave voltammetry at the glassy carbon electrode. *Electroanalysis*, 33, (2021), 537-542.
6. Maloku, A., Berisha, L., Jashari, G., Arbnesi, T., Kalcher, K. Enhancement Effect of Cetyltrimethylammonium Bromide on Electrochemical Determination of Chlorophenols using a Carbon Paste Electrode. *Journal of Analytical Chemistry*, 75, (2020), 358-365.
7. Jashari, G., Švancara, I., Sýs, M. Determination of ethanol in alcoholic drinks: Flow Injection analysis with amperometric detection versus portable Raman spectrometer. *Electroanalysis*, 32, (2020), 1949-1956.
8. Berisha, L., Arsim, M., Majlinda, H., Jashari, G., Ardian, U., Sýs, M. Voltammetric determination of nitrites in meat products after reaction with ranitidine producing 2-methylfuran cation. *Microchemical Journal*, 159, (2020), 105403.
9. Kastrati, G., Jashari, G., Sýs, M., Švecová, B., Arbnesi, T., Metelka, R., Bílková, Z., Korecká, L. Simultaneous determination of vitamin E and vitamin K in food supplements using adsorptive stripping square-wave voltammetry at glassy carbon electrode. *Applied Science*, 10, (2020), 4759.

5.2 Publications in other peer-reviewed journals

1. Berisha, L., Shabani, E., Maloku, A., Jashari, G., Arbnesi, T. Electrochemical determination of dopamine and uric acid in blood serum using anionic surfactants at carbon paste electrodes. *Malaysian Journal of Analytical Sciences*, 24, (2020), 97-106.
2. Jashari, G., Fröhbauerová, M., Sýs, M., Červenka, L. Extractive stripping voltammetry at a glassy carbon paste electrode for analysis cow's milk and cream. *Potravinarstvo*, 14, (2020), 202-207.

5.3 Conference proceedings

1. Kastrati, G., Jashari, G., Sýs, M., Švecová, B., Tahir, A., Metelka, R., Bílková, Z., Korecká, L. Simultaneous adsorptive stripping square-wave voltammetric determination of vitamin E and vitamin K in food supplements. In XL. Moderní elektrochemické metody : sborník přednášek. Ústí nad Labem: BEST Servis, 2021. s. 122-125 s. ISBN 978-80-905221-8-3.
2. Jashari, G., Sýs, M., Mikysek, T., Metelka, R., Švancara, I. Electrochemical behaviour of β -Carotene at different working electrodes. In XL. Moderní elektrochemické metody : sborník přednášek. Ústí nad Labem: BEST Servis, 2021. s. 98-101 s. ISBN 978-80-905221-8-3.
3. Jashari, G., Musliu, A., Sýs, M., Arbnesi, T., Metelka, R., Mikysek, T., Švancara, I. Optimization of working conditions in voltammetric simultaneous determination of retinyl acetate and α -tocopheryl acetate. In Bezpečnosť a kontrola potravín : zborník prác z XVII. vedeckej konferencie s medzinárodnou účasťou online. Nitra: Slovenská poľnohospodárska univerzita v Nitre, 2020. s. 99-104 s. ISBN 978-80-552-2168-7.
4. Jashari, G., Sýs, M., Metelka, R., Švancara, I. Possibilities of simultaneous voltammetric detection of tocopherols in non-aqueous media. In XXXIX. Moderní elektrochemické metody : sborník přednášek. Ústí nad Labem: BEST Servis, 2019. s. 117-120 s. ISBN 978-80-905221-7-6.
5. Jashari, G., Sýs, M., Metelka, R., Švancara, I. Optimization of simultaneous voltammetric detection of tocopherols at glassy carbon electrode in organic solvents. In Monitorování cizorodých látek a hodnocení rizik v životním prostředí I.. Pardubice: Univerzita Pardubice, 2019. s. 67-74 s. ISBN 978-80-7560-234-3.
6. Nawar, A., Jashari, G., Sýs, M., Švancara, I. Electrochemical behaviour of phyloquinone (vitamin K1) at a carbon paste electrode modified with sodium dodecyl sulfate. In LII.

Seminář o tenzidech a detergentech. Pardubice: Univerzita Pardubice, 2019. s. 55-60 s. ISBN 978-80-7560-257-2.

7. Jashari, G., Sýs, M., Metelka, R., Švancara, I. Comparison of different electrode materials and organic solvents in simultaneous determination of retinyl acetate and tocopheryl acetate. In LII. Seminář o tenzidech a detergentech. Pardubice: Univerzita Pardubice, 2019. s. 49-54 s. ISBN 978-80-7560-257-2.

5.4 Oral presentations

1. Jashari G., Sýs M., Mikysek T., Metelka R., Švancara. Electrochemical behaviour of β -Carotene at different working electrodes, XL. Moderní elektrochemické metody, 8. – 12. November 2021 Jetřichovice u Děčína, Czech Republic.
2. Jashari G., Musliu A., Sýs M., Arbnesi T., Metelka R., Mikysek T., Švancara I. Optimization of working conditions in voltammetric simultaneous determination of retinyl acetate and α -tocopheryl acetate, XVIII. Vedecká konferencia s medzinárodnou účasťou, Piešťany, Slovakia, 22. – 24. September 2021.
3. Jashari G., Muriqi S., Sýs M., Arbnesi T., Švancara I., Metelka R. Optimization of working conditions in voltammetric detection of β -carotene, 3rd Cross-Border Seminar on Electroanalytical Chemistry, Virtual Seminar, 8. - 9. April 2021
4. Jashari G., Sýs M., Metelka R., Švancara I. Simultaneous voltammetric determination of tocopherols in non-aqueous media, 15th Karel Vytřas Seminar/Workshop on Sensing in Electroanalysis, Pardubice, Czech Republic, 12. - 16. November 2019
5. Jashari G., Sýs M., Metelka R., Švancara I. Comparison of different electrode materials and organic solvents in simultaneous determination of retinyl acetate and tocopheryl acetate, LII. Seminar O tenzidech a detergentech, Kraskov, Czech Republic, 6. - 8. November 2019, 49-54, ISBN 978-80-7560-257-2
6. Jashari G., Sýs M., Metelka R., Švancara I. Possibilities of simultaneous voltammetric detection of tocopherols in non-aqueous media, 26th Young Investigators' Seminar on Analytical Chemistry, Pardubice, Czech Republic, June 24-27, 2019, 17, ISBN 978-80-7560-224-4.
7. Jashari G., Sýs M., Metelka R., Ivan Švancara. Possibilities of simultaneous voltammetric detection of tocopherols in non-aqueous media, In: Moderní elektrochemické metody XXXIX: Jetřichovice, Czech Republic, 20. - 24. May 2019, 117-120, ISBN 978-80-905221-7-6.

8. Jashari G., Sýs M., Metelka R., Švancara I. Optimization of simultaneous voltammetric detection of tocopherols at glassy carbon electrode in organic solvents, *Monitorování cizorodých látek a hodnocení rizik v životním prostředí 1.* : Kojetín, Czech Republic, 10. – 12. April 2019, str. 67-74, ISBN 978-80-7560-234-3.
9. Jashari G., Sýs M., Švecova B., Arbnesi T., Metelka R. Determination of vitamin K1 using square wave adsorptive stripping voltammetry at solid glassy carbon electrode, 14th Seminar/Workshop on SENSING IN ELECTROANALYSIS, Pardubice, Czech Republic, November 13-17, 2018.