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CHARACTERIZATION OF α-TOCOPHEROL EXTRACTION INTO A SELECTED CARBON PASTE BINDER

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An extraction of α-tocopherol from different water-ethanolic mixtures into a commonly used paste binder was studied by ultraviolet spectrometry and anodic square wave voltammetry. It has been observed that distribution of the α-tocopherol between two immiscible phases is controlled by equilibrium constant that is significantly affected by the amount of organic solvent present in the aqueous phase. Moreover, it is evident that extraction of some biologically active compounds into the carbon paste could be used as the accumulation step within the extractive stripping voltammetric procedure to improve the sensitivity of their appropriate electroanalytical determination.

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Introduction

Compared to common variants of stripping voltammetry [1], the corresponding step without electrolytic accumulation is not controlled by electrolysis but, alternatively, by (i) adsorption of analyte onto the working electrode surface, which is the case of the adsorptive stripping voltammetry (AdSV [**2**]), (ii) extraction into the electrode bulk as in extractive stripping voltammetry (ExSV), or eventually, by (iii) specific reactions with chemically modified electrodes (CMEs [3]).

Just a few scientific papers have been focused on extraction of lipophilic analytes into the carbon paste binders [4] being the second major components of carbon paste electrodes (CPEs [5]); disintegration of carbon paste prepared from common graphite powders in the presence of organic solvents [6] and unsatisfactory reproducibility of measurements based on extraction and rather little knowledge about the interface electrochemistry belonging to major reasons [7].

Generally, carbon pastes can be defined as homogenized heterogeneous mixtures of different kinds of carbon powders and paste binders, the content of them must not exceed 30% (*w/w*) [8]. In electroanalysis, paraffin and silicone oil (SO) are still very popular paste binders. In combination with glassy carbon powder, the respective configurations, known as the so-called glassy carbon paste electrodes (GCPEs), represent unique sensors stable also in aqueous/organic solvent mixtures [6,9,10].

In this contribution, an extraction of tocopherols (α-TOH) into the SO from different aqueous-ethanolic mixtures was studied in details using ultraviolet spectrometry (UV) and compared with ExSV utilizing the square-wave voltammetric (SWV) mode as the electrochemical technique of choice.

The α-TOH is the most biologically active form of vitamin E offering a high electroactivity thank of its reduction capabilities [11]. Moreover, all tocopherols absorb UV irradiation because of the chromanol ring in their structure connected with long alkyl chain (see Fig. 1), which is also responsible for their lipophilicity [12].

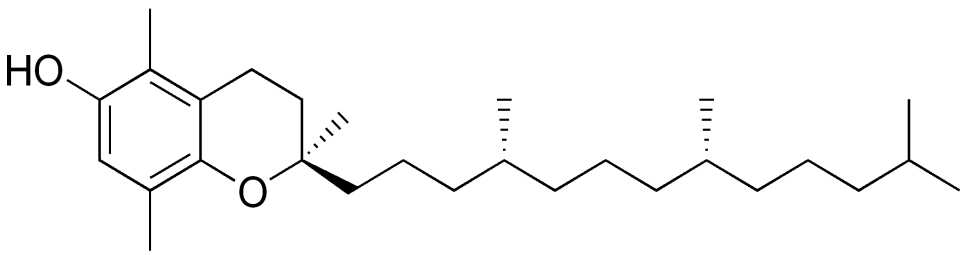

Fig. 1 Chemical structure of α-tocopherol

From a physical point of view, extraction of an analyte dissolved in water-organic mixture (*water phase*) into a lipophilic paste binder (*organic phase*) can

be described using the corresponding quantities described in the theoretical part which follows. However, the system presented herein cannot be considered as a typical example of the "*liquid-liquid extraction*" due to the presence of ethanol in aqueous phase. Thus, the effect of ethanol in water mixtures on the extraction efficiency of entrapping α-TOH into the paste binder used was studied. Because the amount of analyte deposited onto the carbon paste of a CPE is always controlled by extraction equilibria, it is clear that many electroactive analytes with lipophilic properties (such as vitamins [13], alkaloids [14], and steroids [15]) can be determined using ESV in combination with pulse voltammetric techniques [16].

Theoretical

Generally, an extraction equilibrium (characterized by corresponding extraction constant, K_{ex}) is affected by other side-equilibria because of the presence of the different forms of an analyte in both immiscible phases. If the compounds of interest being able of participating in the side reactions are present in these phases, the analyte (A) may undergo the solvation, protonation, hydrolysis, as well as some complex-forming reactions. From an analytical point of view, it is necessary to define the distribution ratio of an analyte between the two immiscible phases, regardless of the actual representation in different forms [17]. Practically, this is usually defined as a distribution ratio (q_A) given by the following equation

$$q_A = \frac{(c_A)_o}{(c_A)_w} \tag{1}$$

where $(c_A)_o$ and $(c_A)_w$ denote the concentration of an analyte in either organic or aqueous phase, respectively.

Under favourable conditions, when the extraction is not being affected by the side-reactions, the distribution ratio corresponds to the distribution coefficient (Q_A). However, it is necessary to introduce the extraction step (E_A), expressing a percentage of the total number of moles of the extracted substance transferred from a given volume of the aqueous phase (V_w) into a volume of the organic phase (V_o). According to the previous definition, the relationship between the distribution ratio and the extraction step can be described by Eq. (2). In order to present the dependency of the extraction step on different ratios of water and organic phase volumes (V_w/V_o) graphically, the E_A values are plotted against logarithms of q_A values [18,19]

$$E_A(\%) = \frac{100(c_A)_o V_o}{(c_A)_o V_o + (c_A)_w V_w} = \frac{100 q_A}{q_A + V_w/V_o} \tag{2}$$

According to the Beer–Lambert–Bouguer law, it can be assumed that a difference of absorbances (A) is directly proportional to the amount of analyte extracted into the organic phase. Thus, the value of the distribution ratio can simply be calculated by Eq. (3), where A_0 is the analyte absorbance in the aqueous phase before the extraction step and A_i is a value denoting the decrease after the extraction of an analyte into the organic phase

$$q_A = \frac{(c_A)_o}{(c_A)_w} = \frac{A_0 - A_i}{A_i} \tag{3}$$

The content of silicone oil (SO) in carbon paste (or ethanol content in aqueous phase) is usually expressed as the volume fraction (φ), defining the ratio of a substance volume (V_A) with respect to the volume of the total mixture (V_{total}), which is a dimensionless quantity ranging from 0 to 1 and defined by the following equation

$$\varphi_A = \frac{V_A}{V_{total}} \tag{4}$$

Due to the geometry of the electrode holder used, the volume of organic phase (V_o) can be calculated as the volume of a cylinder,

$$V_o = \pi r^2 h \varphi_o \tag{5}$$

where r is the radius of the electrode surface and h is the height of the carbon paste column. Simply, the size can be found using a repetitive control of the respective voltammetric measurements (no secondary oxidation peak of α-TOH [11]) after renewing the electrode surface. Similarly, the weight (m) of carbon paste extruded from the holder can be used to calculate the V_o parameter (see Eq. 6) if the mass fraction (w) and the density (ρ) of the carbon paste binder chosen are known

$$V_o = \frac{mw}{\rho} \tag{6}$$

Experimental

Chemicals and Reagents

All chemicals were of analytical reagent grade and purchased from Sigma-Aldrich or Lach-Ner. Ultrapure water (ρ = 18.3 MΩ cm; Milli-Q system, Millipore) was used to prepare 0.1 mol dm^{-3} Britton–Robinson pH 7.0 buffer. A stock solution

of 0.01 mol dm^{-3} (+)-α-tocopherol (from vegetable oil; 1000 UI g^{-1}) was prepared by dissolving the appropriate amount of the substance in 99.5% ethanol .

Apparatus and Instrumentation

Spectrophotometric measurements. A Shimadzu UV-VIS spectrometer was used to realize spectrophotometric measurements in ultraviolet region when using 1-cm quartz cuvettes.
Voltammetric measurements. Typical three-electrode configuration was employed with GCPE (working), Ag/AgCl/3.0 mol dm^{-3} KCl (reference) and Pt wire (counter) electrodes. The corresponding SWV experiments were carried out using an electrochemical system AUTOLAB (model PGSTAT 101, Ecochemie / Metrohm) operated *via* the Nova 1.11 software (the same supplier).

Preparation of the Working Electrode

Glassy carbon paste was prepared by thorough hand-mixing of graphite glassy carbon powder (Sigradur-G, HTW Maitingen, Germany) with 20 % (*w/w*) MV 8000 silicone oil (Lučební závody Kolín, the Czech Republic) in ceramic mortar for approx. 30 min. The resulting homogenized paste was packed into the cavity of the Teflon® piston-driven electrode holder with an end-hole of 3 mm in diameter [20]. Due to the extraction of analyte into the carbon paste bulk, the surface of GCPE had to be renewed with the subsequent smoothing using a dry filter paper.

Procedures

Ultraviolet spectrometry. These measurements were done only for the 1:1 and 2:1 V_w/V_o ratios. The appropriate volume of 50 μmol dm^{-3} α-TOH in aqueous-ethanolic mixtures with different amounts of ethanol was shaken together with the corresponding volume of silicone oil in small vials at 25 °C for 60 min. Afterwards, the aqueous-ethanolic mixture was put into the cuvette and absorption spectra measured in the range from 200 to 340 nm. All spectrophotometric measurements were made minimally in five replicates. Absorbance values of absorption maximum (290) nm were used for the calculations indicated above (Eq. 3).
Extractive Stripping Voltammetry (ESV). The GCPE was immersed into 50 μmol dm^{-3} α-TOH in aqueous-ethanolic mixtures without applying any voltage and at a speed of magnetic stirrer 400 min^{-1} at 25 °C for 240 s. Subsequently, the

SWV experiment performed in 0.1 mol dm^{-3} Britton–Robinson buffer (pH 7.0) was chosen for anodic oxidation of extracted α-TOH in the potential range from 0 to +0.5 V, at a potential step (E_{step}) of 0.01 V, potential amplitude (E_{ampl}) 0.025 V and frequency (f) 2 Hz.

Results and Discussion

In Fig. 2, bold line represents a typical UV-spectrum of α-TOH in pure ethanol without any extraction step (blank). It was observed that the different amount of ethanol in V_w did not cause any noticeable changes of α-TOH absorbance. Thus, the UV spectrometry has proven to be almost ideal to monitor the ethanol effect on extraction step of α-TOH into the SO phase. After extraction (V_w/V_o = 1) for 60 s, a significant decrease of absorbance at 290 nm was observed, which is demonstrated by solid, dashed, and dotted lines in Fig. 2. Moreover, the respective absorbance values varied with the ethanol content changed.

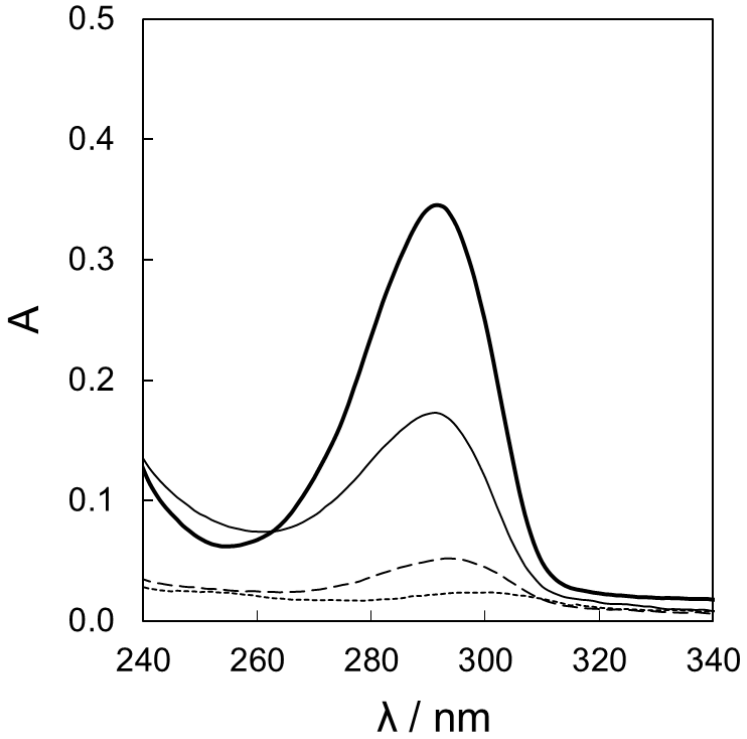

Fig. 2 Absorption spectra of 50 μmol dm^{-3} α-TOH in 99.5% ethanol without previous extraction into silicone oil (bold line), after its extraction from 80% (solid line), 70% (dashed line) and 60% (dotted line) ethanol into silicone oil for 60 min (V_w/V_o = 1)

Similar observations were noted in case of the V_w/V_o = 2 ratio. The highest values of extraction efficiency were obtained in 60% ethanol for both ratios applied. An appropriate relationship between the extraction step (E) and logarithm of distribution ratio (q) is illustrated in Fig. 3. It was found that distribution of

α-TOH between the two immiscible phases depended entirely on the V_w/V_o ratio. Thus, it could be assumed that the extraction of all lipophilic analytes into the carbon paste binder chosen is controlled by the same principles. However, one has to take into account the amount of organic phase contained in the carbon paste mixture which is relatively low.

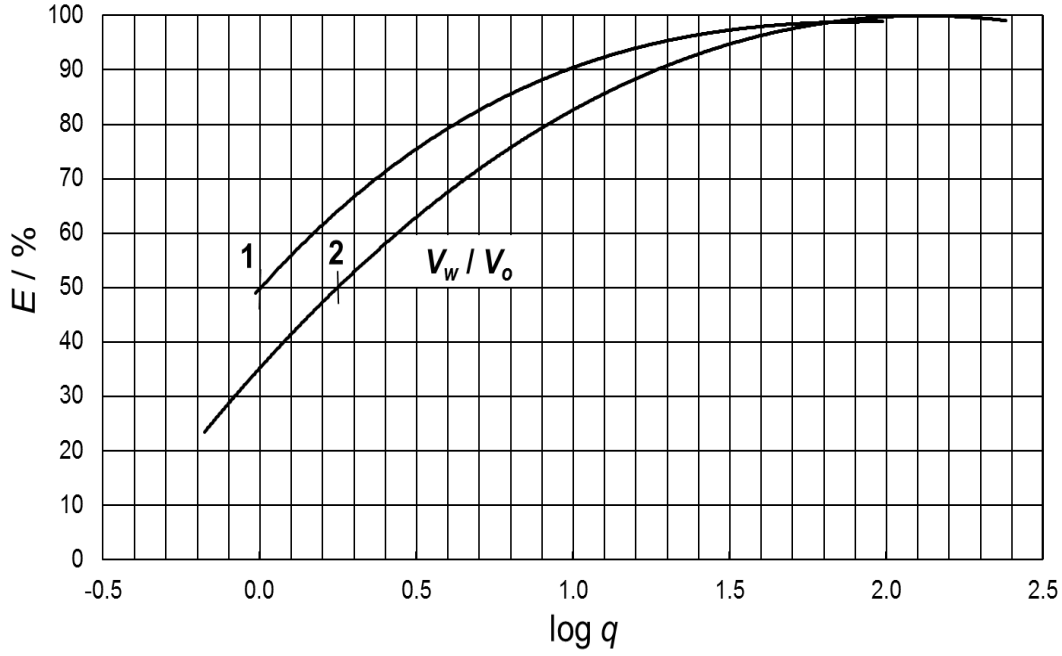

Fig. 3 Dependence of extraction step on the logarithm of distribution ratio for different V_w/V_o ratios

During AdSV of α-TOH, it was observed that approximately 4 mm of paste column (h) had to be pushed out from the electrode holder to remove the remains of extracted analyte.

The V_o of about 3.9 ± 0.1 mm^3 SO in the glassy carbon paste was calculated using arithmetic mean ($\bar{x}$) for five measurements (n = 5) of the carbon paste weight (m), mass fraction (w) and the density of SO (ρ). Value up to 2 500 of the volume ratio (V_w/V_o) could be deduced because α-TOH was always extracted into glassy carbon paste from a volume of 10 cm^3 water-ethanolic mixtures. Due to a very high V_w/V_o value, it is clear that just a negligible part of the total amount dissolved in V_w was transferred into V_o. For that reason, it is clean that the analyte concentration in the aqueous phase remained practically constant and that such accumulation step in ESV could be repeated many times.

The anodic current response of the α-TOH linearly increased if the extraction time was lower than 5 min (not shown). Otherwise, for extraction requiring time higher than 12 min, the peak current response was almost constant, about 11.7 ± 0.6 μA. It is evident that such a time duration is needed in order to attain the corresponding extraction of α-TOH from 60% ethanol to SO present in

the carbon paste. Figure 4 shows typical anodic voltammograms of α-TOH obtained after its extraction into the paste from different water-ethanolic mixtures.

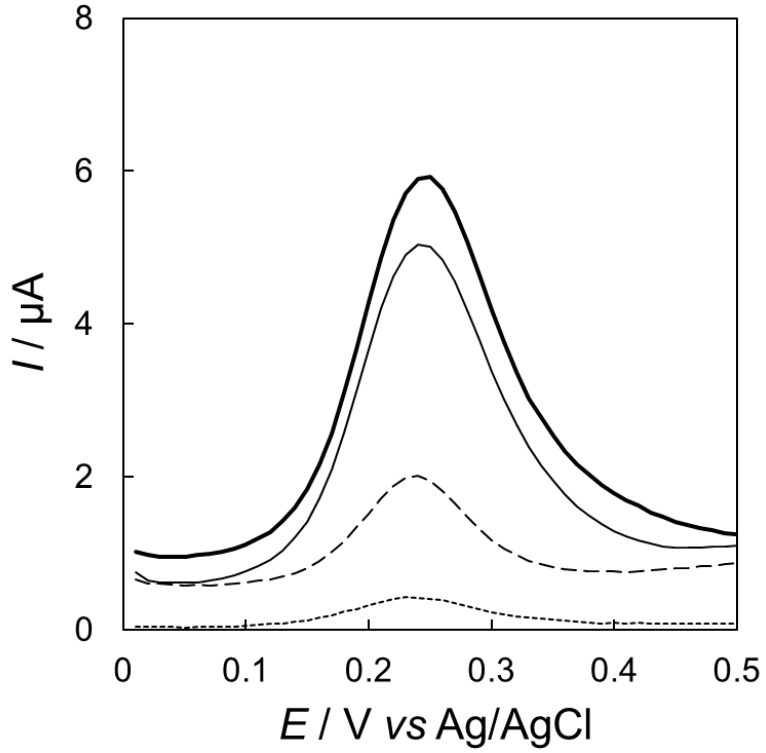

Fig.4 SWV of 50 μmol dm^{-3} α-TOH extracted into GCPE (20% SO) from 60% (bold line), 70% (solid line), 40% (dashed line) and 20% (dotted line) ethanol at speed of stirring 400 min^{-1} for 4 min was performed in 0.1 mol dm^{-3} Britton–Robinson (pH 7.0) buffer at E_{step} = 0.01 V, E_{ampl} = 0.025 V and f = 2 Hz

Evidently, comparable results were found by UV-spectrometric measurements. Under certain conditions, the extraction step is directly proportional to the anodic current response because two electrons and one proton participate in the electrode oxidation of one molecule of the α-TOH extracted [13]. Using the AdSV procedure, unfortunately, the distribution ratio of studied lipophilic vitamin could not be determined because the oxidation peak current response (I_p) of the α-TOH in aqueous phase was not comparable with responses obtained after extraction; probably because of different kinetics of the corresponding electrode reactions. It seems that direct anodic oxidation of α-TOH in aqueous-organic mixtures at solid electrode materials is usually controlled by diffusion [21]. However, some passivation / saturation of carbon-based electrode surfaces should be expected. Thus, the anodic oxidation of the α-TOH soluble in carbon paste and performed in purely aqueous electrolyte is a typical example of interface electrochemistry [7] because the electrode surface represents the interface between the aqueous supporting electrolyte and lipophilic carbon paste binder.

Conclusion

Based on the study presented here, it can be concluded that extraction of electroactive compounds with lipophilic properties into a selected paste binder is applicable to isolate such substances from the sample matrix. It seems that analytes characterized by high lipophilicity — especially, lipophilic vitamins — cannot be extracted into the paste binder if a sufficiently high content of an organic solvent (soluble in aqueous phase) is not ensured. In contrast to this, alkaloids such as cannabinoids, capsaicinoids, etc., could be isolated *ex-situ* from the sample matrix with a very low content of the organic solvent and subsequently determined in pure aqueous electrolyte.

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