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OPTIMIZATION OF VITAMIN K_1 ADSORPTION ONTO SURFACE OF GLASSY CARBON ELECTRODE

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Abstract

Conditions for adsorption of phylloquinone (also known as vitamin K_1) onto surface of solid glassy carbon electrode from an aqueous-acetonitrile mixture were optimized to develop a sensitive electroanalytical method based on adsorptive stripping voltammetry. Whole procedure was focused on finding optimum working conditions such as selection of suitable material of accumulation cell, content of the organic solvent used, accumulation time, and stirring rate of magnetic stir bar. The reproducibility of the adsorption was expressed as the arithmetic mean of the relative standard deviations obtained in the individual optimization steps, namely 6.14 % (calculated from five repeated peak heights).

Souhrn

Za účelem vývoje citlivé elektroanalytické metody založené na adsorptivní rozpouštěcí voltametrii byly optimalizovány podmínky pro adsorpci fylochinonu (známého též jako vitamín K_1) na povrch pevné elektrody ze skelného uhlíku. Celková optimalizace spočívala v nalezení optimálních pracovních podmínek zahrnujících výběr vhodného materiálu akumulační cely, obsah použitého organického rozpouštědla, dobu akumulace a rychlost otáček magnetického míchadla. Reprodukovatelnost adsorpce byla vyjádřena jako průměr relativních standartních odchylek získaných při opakování jednotlivých optimalizačních kroků, a sice 6.14 % (vypočteno pro pět opakovaných měření výšek píků).

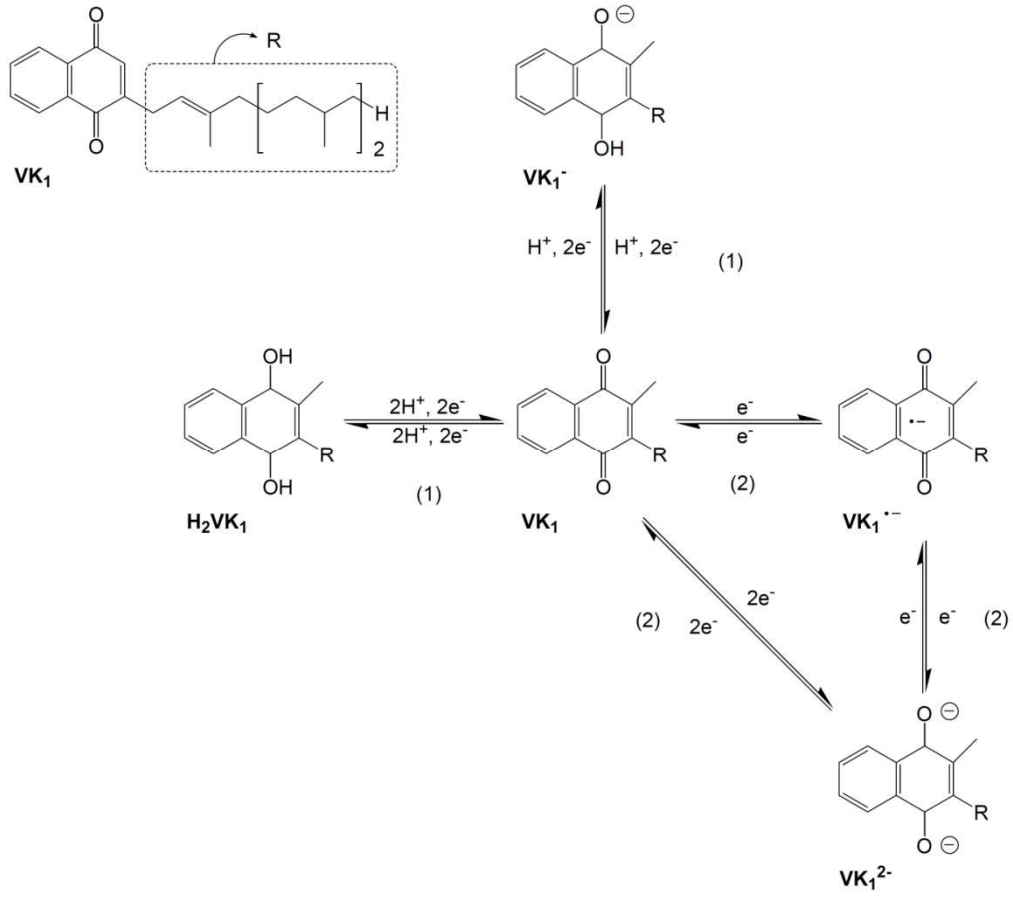
1. Introduction

Generally, vitamin K includes a group of three fat-soluble quinones, such as phylloquinone (VK_1; vegetable origin), menaquinone (VK_2; bacterial origin) and menadione (VK_3; synthetic) [1]. These compounds are essential for the function of several proteins involved in blood coagulation. A deficiency of vitamin K results in the decreased blood levels of prothrombin and clotting factors with subsequent hemorrhagic tendencies [2]. Due to this, the determination of phylloquinone level in the endogenous plasma may be of clinical importance.

From chemical point of view, the phylloquinone is a polycyclic aromatic ketone with long alkyl chain responsible for its lipophilicity [1]. Unlike all other lipophilic vitamins, only those of the K series cannot be electrochemically oxidized in usual aqueous electrolytes [3] because they usually occur in their oxidized forms as quinones [4]. Ideally reversible electrochemical system (quinone/hydroquinone) is used in the simultaneous determination of the lipophilic vitamins.

First, it is necessary to reduce the phylloquinone to phyllohydroquinone (H_2VK_1) using a constant negative voltage (as shown in Scheme 1, two protons and two electrons participate on this redox reaction). After that, the H_2VK_1 can electrochemically be oxidized together with other adsorbed lipophilic vitamins (naturally occurring in their reduction forms) using the anodic voltammetric technique [5].

It should be mentioned that the electrochemical mechanism of phylloquinone shown in the scheme is quite simplified. Mostly, there is a sequence of one-electron radical reactions which precede protonation and hydration of the molecule. These reactions were included into the electrochemical square-scheme mechanisms presented in details by Webster [6].



Scheme 1. Electrochemical behaviour of phylloquinone in the various well-buffered electrolytes (1) and unbuffered electrolytes (2) [3].

In this paper, optimization of the VK_1 adsorption onto surface of glassy carbon electrode (GCE) from an aqueous-acetonitrile mixture is presented. It represents one of several key steps in development of sensitive electroanalytical method based on adsorptive stripping voltammetry (AdSV). The AdSV is similar to anodic stripping voltammetry (ASV) but unlike that, the preconcentration step is controlled by adsorption instead by electrolysis. And just for a series of vitamin K, it is a preferred method [4,5,7]. Subsequently, in our case, electrochemical detection of VK_1 was performed by square wave voltammetry (SWV). The purpose of this study was to note that current analytical methods for determination of concentration level of VK_1 in the human plasma do not dispense without liquid chromatography with fluorometric or mass spectrometry detection [8,9]. In the presence, all electroanalytical methods remain academic matters, although they provide comparable sensitivity [10,11].

2. Experimental

2.1. Chemicals and reagents

Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone), n-hexane (95%), and acetonitrile (ACN) of HPLC purity (99.8%) were purchased from Sigma Aldrich (Prague, Czech Republic). Acetic acid (99.8%), phosphoric acid (85%), and boric acid necessary for preparation of 0.1 mol L^{-1} Britton-Robinson buffer (pH 4.0) as supporting electrolyte were from Lach-Ner (Neratovice, Czech Republic). Deionized water with electric resistivity ~18.3 MΩ cm (Milli-Q system, Millipore) was used to prepare the supporting electrolyte as well as aqueous-acetonitrile mixtures. All other chemicals were used with analytical grade purity.

2.2. Instrumentation

All electrochemical measurements were carried out in a 50 mL glass cell at 25 °C. A conventional three-electrode system was used, consisting of a solid GCE (working; with diameter of 2 mm) from Metrohm (Prague, Czech Republic), Ag/AgCl/3.0 M KCl (reference) and platinum wire (auxiliary) electrodes, connected to potentiostat Autolab PGSTAT101 (Metrohm) compatible with software Nova version 1.11. was used.

2.3. Preparation of the glassy carbon electrode

Due to the fact that both forms (VK_1/ H_2VK_1 redox couple) remain adsorbed on the surface of the GCE, it was necessary to renovate the electrode surface by rinsing with pure

hexane and subsequently by polishing on a furry pad with presence of wet alumina powder (particle size 1.0 μm) for 30 s. After subsequent rinsing of the surface by distilled water, the GCE was ready for new electrochemical experiment.

2.4. Procedure

An adsorption of VK_1 onto GCE surface started when this electrode was immersed into 10 mL of 200 μM VK_1 solution with a content of ACN at selected value of stirring rate for certain time. After that, the enriched GCE together with other electrodes was used at anodic SWV performed in 0.1 mol L^{-1} Britton-Robinson buffer (pH 4.0) with following parameters: potential window, from –0.6 to +0.4 V, deposition potential (E_{dep}); –0.6 V, deposition time (t_{dep}); 120 s, potential step (E_{step}); 5 mV, potential of amplitude (E_{ampl}); 25 mV, and frequency (f); 20 Hz.

3. Results and discussion

3.1. Selection of accumulation cell material

It is generally known that all lipids may additionally be adsorbed on the walls of the accumulation cells. Target VK_1 contained in these fats (analytes) can therefore be severely detained. This negative phenomenon is usually caused by nonpolar interactions and reflected by significant decreasing of current response. For confirmation, two different types of accumulation cells were compared. In contrast with conventional glass cell, about 20% decrease in current signal was observed when a plastic accumulation cell was used.

3.2. Effect of the acetonitrile content

All lipophilic vitamins are insoluble in pure water, this is why they are also known as fat-soluble vitamins. On the other hand, they are completely soluble in nonpolar organic solvents, several polar organic solvents, as well as in aqueous mixtures of these polar organic solvents. From the possible choice of water-soluble solvents, acetonitrile (ACN) was selected in this study due to its satisfied volatility (boiling point 85 °C) and polarity index (5.8). The ACN content in its aqueous mixtures was expressed as volume fraction (φ) defined as ratio of organic solvent volume (V_{org}) to total volume (V_{total}).

According to Fig. 1 (voltammogram *d*), the highest efficiency of aVK_1 adsorption onto surface of GCE was obtained at $\varphi = 0.6$ ($I_p = 3.93 \pm 0.10$ μA at $E_p = +0.05$ V). This volume fraction has therefore been chosen as optimum. For comparison, it can be recalled that the fraction $\varphi = 0.5$ was found to be optimum for α-tocopherol (vitamin E) in our recent

study [12]. This can be explained by different lipophilicity of these two vitamins. In general, the polarity decreases with increasing of the side alkyl chain. Since the VK_1 alkyl chain is significantly longer than that of α-tocopherol (see Fig. 2), a higher content of ACN was desirable.

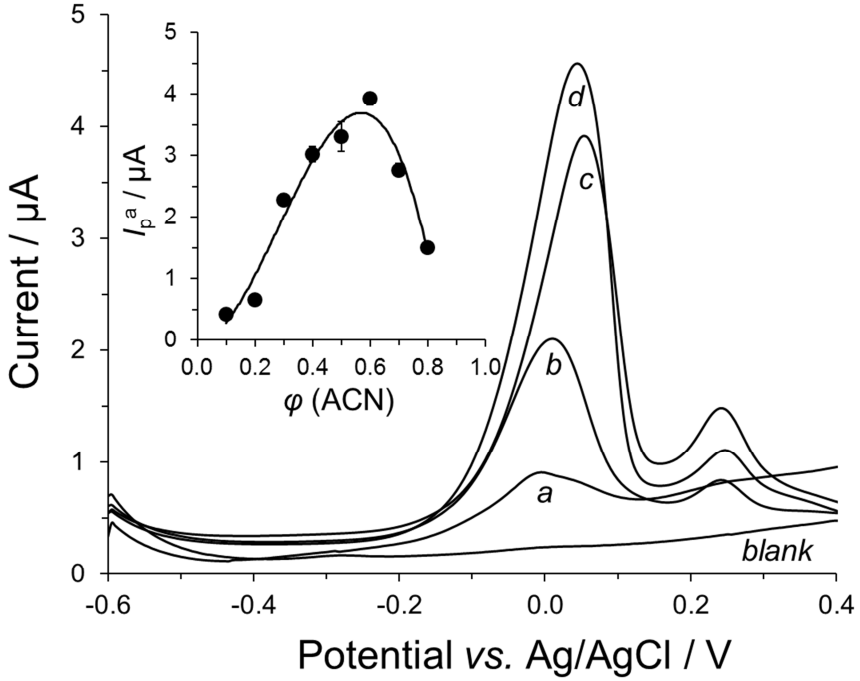


Figure 1. Dependence of VK_1 anodic peak current on volume fraction of ACN: *a*, 0.1; *b*, 0.4; *d*, 0.6; *c*, 0.8. VK_1 (200 mol L^{-1}) adsorbed onto surface of GCE at 400 min^{-1} for 5 min, and subsequently detected in 0.1 mol L^{-1} Britton-Robinson buffer (pH 4.0) at E_{dep} = –0.6 V, t_{dep} = 120 s, E_{step} = 5 mV, E_{ampl} = 25 mV, and f = 20 Hz.

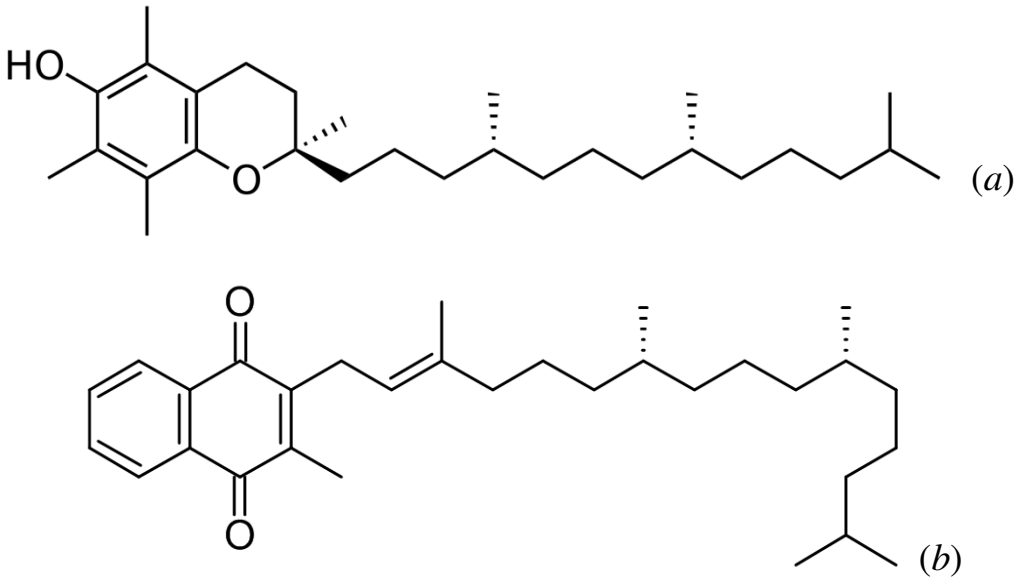


Figure 2. Chemical structure of α-tocopherol (*a*) and phylloquinone (*b*).

3.3. Effect of accumulation time

Principally, it can be stated that the optimum value of accumulation time is defined as the time period required reaching equilibrium of certain analyte distributed between nonpolar electrode surface and used aqueous-organic mixture [13]. Resulting shape of the graph (see Fig. 3), which describes peak current dependence on corresponding accumulation time, is then expressed by a typical saturation curve (adsorption equilibrium isotherm). The adsorption equilibrium seemed to be achieved after 12 minutes, as no significantly higher peak current values were observed for longer periods. As a result, twelve-minute time was chosen as optimum.

Additionally, it is also important to note that in the range of 1 to 10 minutes, approximately linear dependence of the peak current on accumulation time was observed.

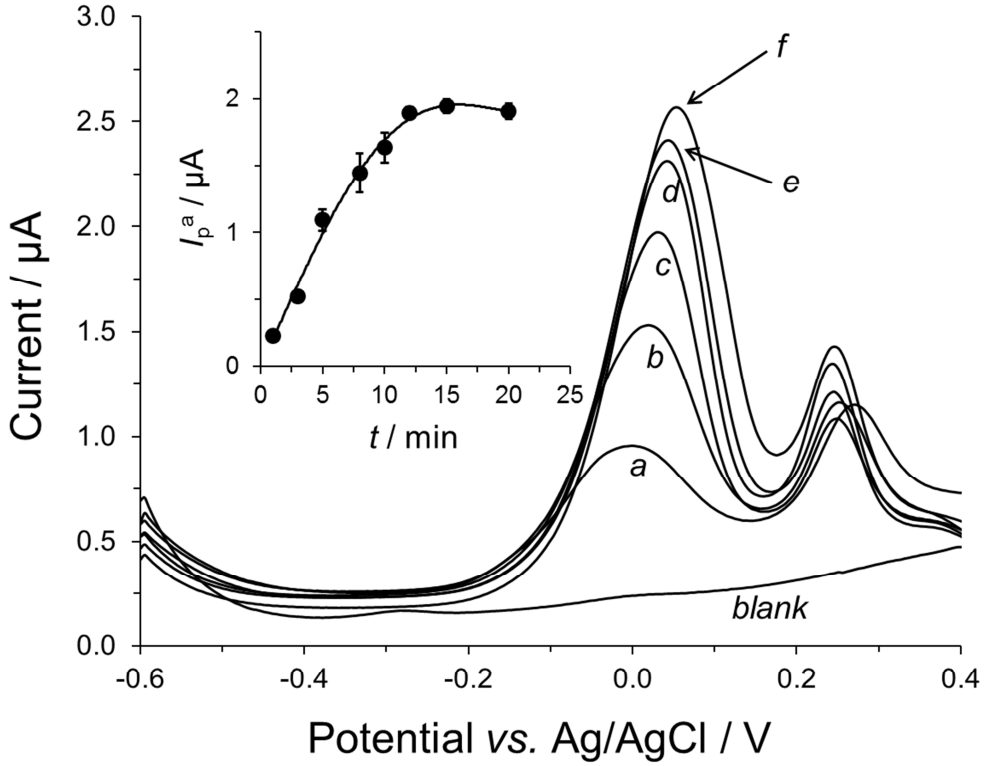


Figure 3. Dependence of VK_1 anodic peak current on different accumulation time: *a*, 2; *b*, 4; *c*, 8; *d*, 10; *e*, 12; *f*, 15 min. VK_1 (200 mol L^{-1}) adsorbed onto surface of GCE from 30% ACN at 400 min^{-1}, subsequently detected in 0.1 mol L^{-1} Britton-Robinson buffer (pH 4.0) at E_{dep} = –0.6 V, t_{dep} = 120 s, E_{step} = 5 mV, E_{ampl} = 25 mV, and f = 20 Hz.

It could be expected that the linear range of the final AdSV can be extended to the expense of sensitivity using a shorter accumulation time than the optimum. The following research will surely address this issue.

3.4. Effect of stirring rate

The speed of stirring (ω, expressed in min^{-1}) significantly influences the rate of VK_1 transport to the surface of the electrode on which the analyte is adsorbed. In this study, it was observed that the stirring speeds of the magnetic stirrer used (1.2 x 0.3 cm) higher than 400 min^{-1} did not have any significant effect on increasing the final current response (see Fig. 4). Therefore, this value of 400 min^{-1} was chosen as optimum.

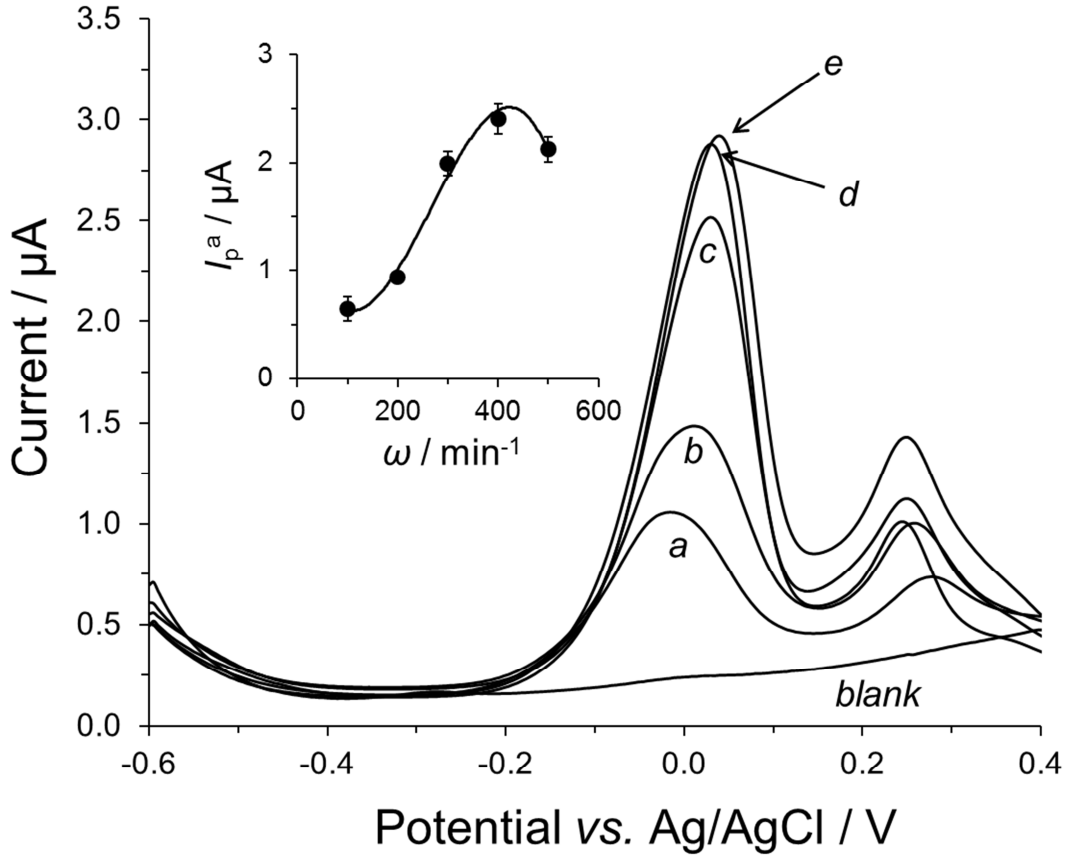


Figure 4. Dependence of VK_1 anodic peak current on different speed of stirring: *a*, 100; *b*, 200; *c*, 300; *d*, 400; *e*, 500 min^{-1}. VK_1 (200 mol L^{-1}) adsorbed onto surface of GCE from 30% ACN for 5 min, subsequently detected in 0.1 mol L^{-1} Britton-Robinson buffer (pH 4.0) at E_{dep} = –0.6 V, t_{dep} = 120 s, E_{step} = 5 mV, E_{ampl} = 25 mV, and f = 20 Hz.

3.5. Reproducibility of vitamin K adsorption

Reproducibility of VK_1 adsorption onto surface of GCE was expressed as an arithmetic mean ($\bar{x}$) of relative standard deviations (RSD) of the repeatabilities for individual steps of optimization. Each experiment was repeated at least five times ($n = 5$) and peak heights were used for the calculation. As a result, a value of 6.14 % was calculated, slightly higher than the critical value of 5 % RSD assumed to be optimum for the significance level $\alpha = 0.05$.

4. Conclusion

The results obtained suggest that optimal conditions for adsorption of VK_1 onto the GCE surface are as follow: acetonitrile content of 60%, accumulation time of 12 min and stirring speed of 400 min^{-1}. However, this study is not yet closed, further experiments will be desirable. During these, a search for optimal supportive electrolyte should be needed and anodic SWV parameters should be optimized to achieve the highest sensitivity of the final AdSV procedure.

Acknowledgment

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