Effect of Pasting Liquids on the Extraction of Vitamin K_1

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Abstract
Atactic polypropylene, petroleum jelly, paraffin oil, paraffin wax, and several types of silicone oils were used for preparation of the respective glassy carbon paste electrodes (GCPEs) selected thanks to their stability in an aqueous-acetonitrile mixture. Each GCPE contained always 20% (w/w) of the pasting liquid chosen. In order to select the most suitable one, electronalytical performance of the individual pasting liquids for extracting 100 μmol L^{-1} phylloquinone (vitamin K_1) as a model compound was studied with the aid of the sequent square wave voltammetry and when using a speed of stirring 400 min^{-1} for 5 min (i.e. open circuit conditions). From experimental data obtained in these tests, it has been concluded that a viscous silicone oil of the MW 8000 type can be selected as the most suitable component.

Key words: Extraction, Glassy carbon paste electrode;Square wave voltammetry; Pasting liquid, Phylloquinone.

Introduction
Glassy carbon paste electrodes (GCPE) were defined as a special kind of the composite working electrodes by Švancara et al. in 1996 [1]. These heterogeneous sensors can be simply prepared by mixing of glassy carbon with selected paste liquid and by subsequent packing of resulting mixtures into the cavity of electrode holders. Unlike conventional carbon paste electrodes, GCPEs are stable at measurements in different aqueous-organic mixtures due to the repulsive effect of spherical shape of glassy carbon particles [1,2].
Theoretically, many nonpolar biologically active compounds (lipophilic vitamins [3], alkaloids [4], drugs [5], and steroid hormones [6]) can be selectively pre-concentrated by their extraction into the present paste liquid from an aqueous mixture and then, electrochemically detected using some sensitive voltammetric technique. This unique approach can be considered to be a simplified definition of extractive stripping voltammetry (ExSV).
However, it should be note that the ExSV does not represent a typical stripping method like anodic stripping voltammetry based on electrochemical deposition of heavy metals [7] because some residua of the electrode reaction products always remain extracted (entrapped) in the paste liquid. Their removal is feasible only mechanically by extruding of sufficient amount of paste and subsequent smoothing using a dry filter paper [8].
In this contribution, a model extraction of phylloquinone into different paste liquids (paraffin oil; PO, petroleum jelly; PJ, paraffin wax; PW, atactic polypropylene; AP, and several silicone oils; SO) from 60% acetonitrile (ACN) was performed to select the most suitable of them. For demonstration, chemical structures of respective paste liquids are shown in Fig. 1.
PO can be characterized as viscous liquid containg different alkanes of 15–30 carbon atoms to in the chain. PJ is a semi-solid mixture of hydrocarbons with number of carbon higher than 25. PW contain predominantly straight-chain hydrocarbons with an average chain length of 20

to 30 carbon atoms. Unlike previously mentioned pasting liquids, low molecular weight (3.0×10^2 to 1.2×10^5 g mol^{-1}) AP and SO belong to long chain polymers.

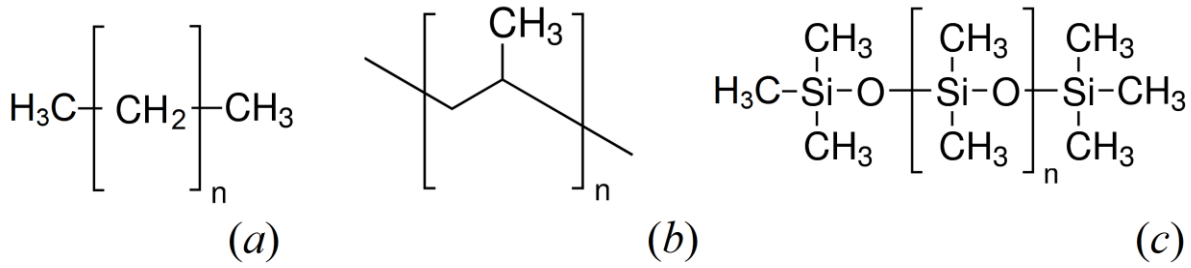

Fig. 1. Chemical structures of some commonly used pasting liquids; namely: paraffin oil, petroleum jelly, and paraffin wax (all under symbol *a*); polypropylene (*b*); and silicone oil (*c*).

Experimental

Chemicals

Phylloquinone (viscous liquid) and acetonitrile (ACN) of HPLC purity (99.8%) were purchased from Sigma Aldrich. Paraffin wax (PW), petroleum jelly (PJ) from the same company, silicone oils (SO) types MW 8000, 1000, 500, 350, and 200 from Lučební závody (Kolín, Czech Republic), Paraffin oil (PO) from Merck and atactic polypropylene (AP) from Spolana (Neratovice, Czech Republic) were used for the GCPEs preparation. Hydrochloric acid (35 %) and potassium chloride for preparation of supporting electrolyte were from Lach-Ner (Neratovice, Czech Republic). Deionized water with electric resistivity >18.3 MΩ·cm (Milli-Q system, Millipore, USA) was used for preparing all used solutions.

Preparation of the glassy carbon paste electrodes

GCPEs were prepared by mixing of 100 mg glassy carbon powder Sigradur-G (distribution of particle size 5 – 20 μm) from HTW Hochtemperatur-Werkstoffe GmbH (Maintingen, Germany) and 25 mg corresponding pasting liquid (20%; w/w) in ceramic mortar for 20 min. The resultant homogeneous pastes were pressed into the cavities (with the same diameters of 3 mm) of piston-like electrode holders (Ø = 3 mm). It must be noted that the height of column in the cavity must be less than 2 cm due to difficult extrusion of rather tough glassy carbon paste, especially in case of very viscous PW.

Freshly made paste electrodes should not be employed generally in any experiments due to their initially unstable electrochemical behavior caused by incomplete homogenization, typical for electrodes containg the SO [9]. For this reason, it is recommended to leave all types of GCPEs at the laboratory conditions for one day minimally. Only after this self-homogenization process, GCPEs are ready to be used for experimentaion in ExSV.

Apparatus

All electrochemical measurements were carried out at conventional three-electrode system consisting the GCPE of choice (working), Ag/AgCl/3M KCl with salt bridge (reference) and a Pt-wire (auxiliary) connected together to a potentiostat (model "EmStat"; PalmSens BV, Houten, The Netherlands) compatible with "PSTrace 3.0 software" from the same manufacturer.

Procedure

ExSV was performed in two separate steps. At first, its extraction into chosen paste liquid was done by immersing of appropriate GCPE in 60% ACN containing 100 μmol.L^{-1} vitamin K_1 at

400 min^{-1} for 5 min. After that, square wave voltammetric (SWV) scan was performed in the 0.01 mol L^{-1} HCl and 0.1 mol L^{-1} KCl from -0.4 to +0.8 V at deposition potential (E_{dep}) -0.4 V, deposition time (t_{dep}) 120 s, equilibrium time (t_{eq}) 5 s, potential step (E_{step}) 5 mV, potential of amplitude (E_{ampl}) 25 mV and frequency (f) 10 Hz. Each experiment was repeated at least five times.

Theoretical considerations

Generally, vitamin K includes a group of three fat-soluble quinones, such as phylloquinone (K_1; vegetable origin), menaquinone (K_2; bacterial origin) and menadione (K_3; synthetic)[10]. 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 [11]. Due to this, the determination of phylloquinone level in the endogenous plasma may be of clinical importance.

Electrochemical behaviour of phylloquinone in aqueous-organic mixture is shown in Fig. 2. This is consistent with the reduction via two electrons to form the corresponding phyllohydroquinone during the cathodic scan and re-oxidation to the phylloquinone within the anodic scanning [12].

It is evident that all vitamins K represent reversible redox couples. Therefore, it is possible to electrochemically reduce the phylloquinone by applying some negative E_{dep} and afterwards, to be oxidized together with other fat-soluble vitamins by anodic voltammetric technique [3].

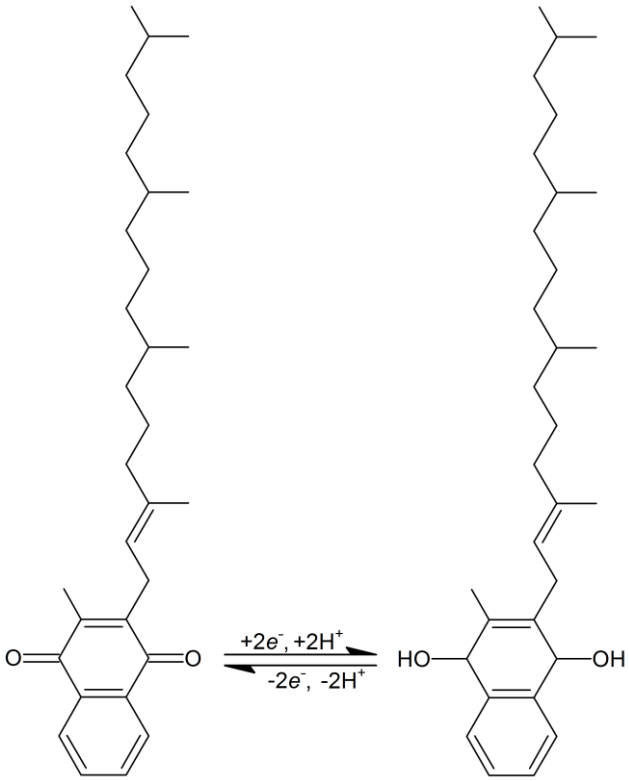

Fig. 2. Simplified electrochemical behavior of phylloquinone extracted into GCPE in aqueous supporting electrolyte.

Results and discussion

In 0.01 mol L^{-1} HCl with 0.1 mol L^{-1} KCl (pH ~2.0), phylloquinone provided only one relatively broad and symmetric peak from 0 to +0.6 V at GCPEs containing PJ, PO, PW and SO. On the other hand, asymmetrical peak from +0.1 to +0.4 V was obtained at GCPE with AP (not shown). The highest anodic current signals of phylloquinone were obtained at GCPEs containing PO, PW, and SO (see Table I). However, almost four-fold background current responses (~5 μA) were observed for GCPEs based on PW and PO. An explanation can be find in similar chemical structures of pasting liquids.

Viscous SO type MW 8000 was chosen as the most suitable pasting liquid for extraction of phylloquinone from its aqueous-acetonitrile mixture. From chemical point of view, the SO is a mixture of long linear chains of polydimethylsiloxane (PDMS) and type MW represents the mean value of molecular weight. This polymeric organosilicon compound is predominantly used as the solid sorbent in microextraction methods for isolation of nonpolar organic compounds [13,14]. Moreover, it has been found experimentally that electrochemical properties of GCPEs containing different SOs (especially significant as the increase of background

current and deteriorated reproducibility; RSD >15%) are getting worse with the decreasing molecular weight.

Table I.
Comparison of electrochemical performance of GCPEs tested for voltammetric detection of phylloquinone.

Electrode	Pasting liquid	R (Ω)	E_p (V)	I_{back} (μA)	I_p (μA)	RSD (%)
GC/AP	Atactic polypropylene	12.8	0.330	0.59	1.026	6.4
GC/PJ	Petroleum jelly	5.7	0.310	0.43	7.581	9.2
GC/PO	Paraffin oil	10.2	0.232	3.18	10.976	9.4
GC/PW	Paraffin wax	4.1	0.188	5.02	11.087	8.2
GC/SO	Silicone oil (type MV 8000)	6.3	0.233	0.72	11.223	7.0

R – ohmic resistance, E_p - peak potential, I_{back} - background current, I_p - peak current, RSD - relative standard deviation of peak current for five replicates (n = 5).

Conclusion

This study can be understood as an initial step in the overall optimization procedure being inevitable for proposing a new electroanalytical method for the determination of vitamin K in various foodstuffs and clinically significant samples. Herein, it is important to emphasise that vitamin K is a group of several fat-soluble quinones with almost identical electrochemical properties. For this reason, vitamin K should be expressed as a quantity equivalent to phylloquinone, representing the most biologically active form.

Acknowledgments

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