

OPTIMIZATION OF WORKING CONDITIONS IN VOLTAMMETRIC SIMULTANEOUS DETERMINATION OF RETINYL ACETATE AND α -TOCOPHERYL ACETATE

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Abstract: This scientific study describes a comparison of various working electrode materials and organic solvents in simultaneous voltammetry determination of retinyl acetate (or retinyl palmitate) and α -tocopheryl acetate based on their anodic oxidation using square wave voltammetry. It has been found that the glassy carbon is the electrode material of choice providing the most favourable current responses and well developed peak(s) in 99.7% acetone with $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$. It can be assumed that anodic oxidation of these two analysts could be utilized to develop a simple and rapid simultaneous voltammetry method for determination in cosmetic products.

Keywords: retinyl acetate; α -tocopheryl acetate; glassy carbon electrode; square wave voltammetry, cosmetic products

INTRODUCTION

Retinyl acetate (vitamin A acetate; RAc) and α -tocopheryl (vitamin E acetate; α -TAc) have been already electrochemically studied for development of voltammetric methods focusing on their determination in cosmetic products (Atuma, Lindquist and Lundstroem, 1974; Wilson, Lin and Webster, 2006). It is worth of mentioning that these two common additives have not been simultaneously determined yet. Generally, both substances have strong antioxidant properties and naturally protect the presented fats against undesirable rancidity.

In previous electrochemical studies, it has been found that RAc provides three oxidation peaks (da Silva, et al., 2015). On the other hand, only one anodic peak was observed for α -TAc in non-aqueous solutions (Sýs, et al., 2016). For their simultaneous voltammetric determination, it is necessary to ensure that anodic peak potential (E_p^a) values of these three RAc oxidation peaks are sufficiently different from the E_p^a value of α -TAc.

Hence, their peaks have to be well separated. Generally, synthetic α -TAc represents the most common additive in cosmetic products compared to naturally occurring α -tocopheryl due to its higher chemical stability.

From chemical point of view, RAc, RPa and α -TAc can be classified as acetic-acid esters and the true vitamins (Figure 1). In 2014, Webster studied the electrochemical behaviour of several retinoids in CH_2Cl_2 containing $0.2 \text{ mol.L}^{-1} \text{ Bu}_4\text{NPF}_6$ using cyclic voltammetry at the glassy carbon electrode (GCE) confirming that retinal, retinoic acid, retinyl palmitate, and retinyl acetate are being oxidized in a similar way as retinol (Tan, Urbančok and Webster, 2014). Later, Polish scientists studied the electrochemical oxidation of retinyl propionate at PtE in acetonitrile containing $0.1 \text{ mol L}^{-1} [\text{Bu}_4\text{N}]\text{ClO}_4$. The comparison of FTIR spectra and the calculation of electron density have suggested them that electrochemical oxidation probably takes place on the C3 position of cyclohexene ring with participation of two electrons and one proton (Masek, Chrzescijanska and Zaborski, 2014).

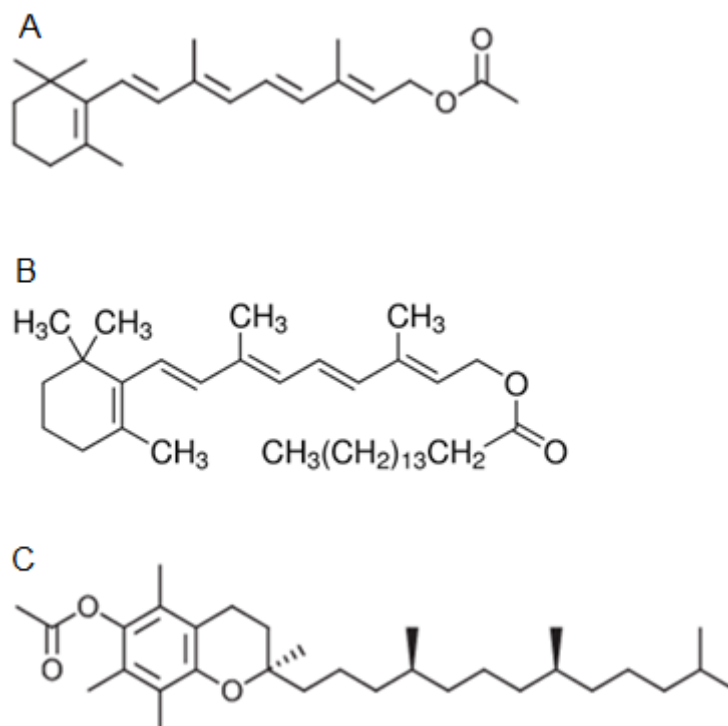


Figure 1 Chemical structure, of retinyl acetate (A), retinyl palmitate (B) and α -tocopheryl acetate (C).

The α -TAc, known as synthetic analogue of α -tocopheryl (Sýs, et al., 2017), lacks a free hydroxy group in its molecular structure which can be involved in the electrochemical reaction (da Silva, et al., 2015). Moreover, the ester functional group is not electron-rich enough for anodic oxidation as the delocalized electron system of benzene ring. It should be remembered that all tocopherols are oxidized by radical mechanisms in non-aqueous media (Wilson, Lin and Webster, 2006).

These compounds are present as additives in cosmetic products, and, so far, analytical chemists had focused on the separate determination of these two analytes. In the present study, we optimized electrode materials, various working media, and instrumental parameters of the voltammetric technique chosen for simultaneous determination of retinyl acetate (or retinyl palmitate) and alpha-tocopheryl acetate in selected cosmetic products.

MATERIAL AND METHODOLOGY

Analytical standards of $\geq 90\%$ retinyl acetate (RAc), 99.9 % retinyl palmitate (RPa), 96% α -tocopheryl acetate (α -TAc) and 99.99 % LiClO₄ were purchased from Sigma-Aldrich. Selected organic solvents, such as 99.9% acetonitrile (ACN), 99.9 % methanol (MeOH), 99.8 % dimethylformamide (DMF), 99.5 % isopropanol (iPrOH), and 99.7% acetone were obtained from Lach-Ner (Neratovice, Czech Republic). An ultrapure water with resistivity lower than 18.3 M Ω cm was prepared in Milli-Q purification unit (Merck Millipore, Burlington; USA).

All electrochemical measurements were performed with conventional three-electrode set-up consisting one of the working electrodes tested (glassy carbon electrode, GCE, $d=3\text{mm}$; gold electrode; gold electrode, AuE, $d=3\text{mm}$; and Pt-electrode, PtE, $d=2\text{mm}$; all being purchased from Metrohm, Czech Republic), together with the saturated calomel electrode (SCE) as the reference and Pt-counter electrode. This configuration was completed by a bridge filled with 0.1 mol L⁻¹ LiClO₄ and connected to a potentiostat PGSTAT101 operated via the Nova software (version 1.11; both from Metrohm, Czech Republic). Square-wave voltammetry (SWV) of simultaneous retinyl acetate and α -tocopheryl acetate at the chosen working electrodes were

used under the following conditions: applied potential: from 0 to +1.6 V, $E_{\text{step}} = 5$ mV, potential of amplitude (E_{ampl}) 140 mV, and a scan rate, $\nu = 50$ mV·s⁻¹. All measurements were made at least in five replicates. If not stated otherwise, all changes in the experimental conditions are shown in the legends of the corresponding figures in the further sections.

RESULTS AND DISCUSSION

Retinyl acetate (RAc) and α -tocopheryl acetate (α -TAc) can be classified as acetic acid esters and essential vitamins. At glassy carbon electrode (GCE), it was found that retinal, retinoic acid, retinyl palmitate, and retinyl acetate are oxidized in similar way as retinol (Masek, Chrzescijanska and Zaborski, 2014; Tan, Urbančok and Webster, 2014). α -TAc, known as synthetic analogue of α -tocopheryl, lacks a free hydroxyl group in its molecular structure which can be involved in the electrochemical reaction (Wilson, Lin and Webster, 2006; da Silva, et al., 2015; Sýs, et al., 2017).

Platinum electrode (PtE), gold electrode (AuE), and GCE were investigated in simultaneous voltammetric determination of RAc (or retinyl palmitate; RPa) and α -TAc in organic medium. The effect of electrode materials ascertained in our initial experiments the pathway of the anodic peak potentials and anodic peak current response (I_p^a) is surveyed in Table 1. From the results given, it seems that the peak separation is not affected by the electrode material of choice. Yields of RAc and α -TAc electrode reactions at GCE gave rise to higher current responses and well distinguished peaks.

Table 1 Comparison of anodic peak potential and current response at various working electrodes.

Electrode	Retinyl acetate		Tocopheryl acetate	
	E_p^a (V)	I_p^a (μ A)	E_p^a (V)	I_p^a (μ A)
GCE	0.811	1.046	1.294	2.356
AuE	0.831	1.148	1.334	0.881
PtE	0.831	1.078	1.299	1.370

Values are given as arithmetic mean of five repetitions.

In this study, it was necessary to select the optimum working medium. Acetonitrile (ACN), acetone, methanol (MeOH), dimethylformamide (DMF), isopropanol (iPrOH) containing always 0.1 mol L⁻¹ LiClO₄ were tested to select the most suitable organic solvent. Figure 2 shows the effect of organic solvents on RAc and α -TAc anodic peak potentials and peak current responses obtained at GCE. Based on demonstrated values, it can be stated that ACN and acetone can be eventually utilized for working medium preparation. However, acetone was preferred for calibration measurements and analysis of samples due to easier dissolution of cosmetic products in sample solutions.

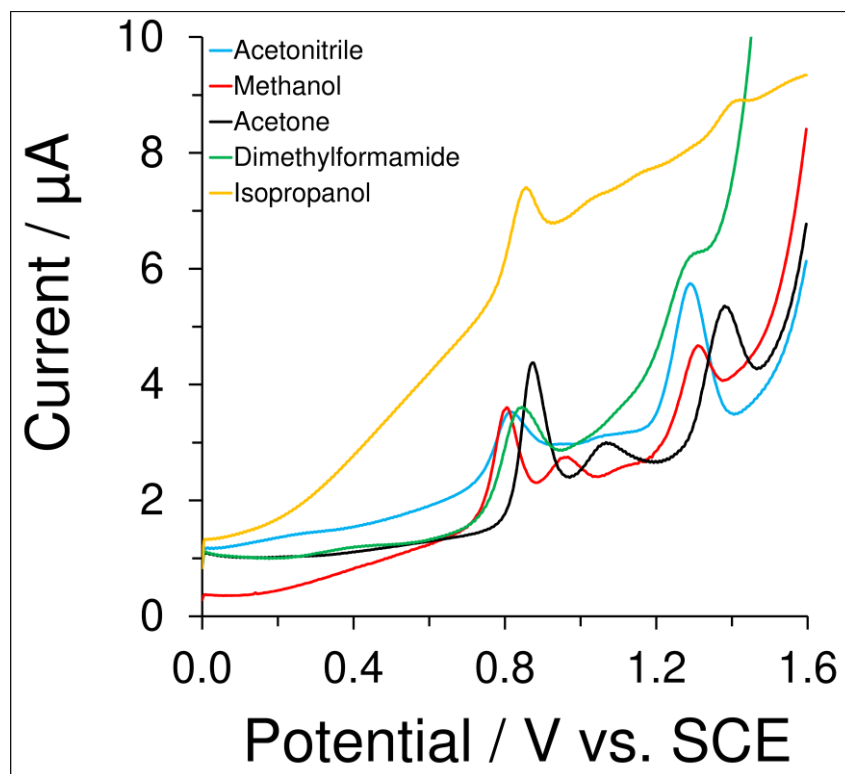


Figure 2 Square-wave voltammograms of $50 \mu\text{mol L}^{-1}$ retinyl acetate and α -tocopheryl acetate at GCE in different organic medium containing always $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ at potential step 5 mV , frequency 20 Hz and potential amplitude 25 mV .

Moreover, the effect of water present in organic medium was studied, if any content of water is presented (10 to 50 %), the anodic oxidation peak of retinyl acetate was significantly decreased; on the other hand, the signal of α -tocopheryl acetate increased, as well the background current in presence of water increased as well.

Firstly, it was necessary to find optimum working parameters of SWV such potential step, potential amplitude and frequency. Generally, potential step and frequency affect the peak properties (peak height and shape) because they have a significant effect on final scan rate. In this study, it was observed that values of 10 mV potential step, 40 mV potential amplitude and 30 Hz frequency are optimum to achieve maximum sensitivity, presented as slopes of RAc and α -TAc calibration curves.

Generally in simultaneous voltammetric determination, it should be taken in account that RAc and α -TAc compete for a reactive spot on the working electrode. Thus, it was important to find out if individual analytes interact with each other within the scope of calibration measurements, when one had concentration $10\text{-}100 \mu\text{mol L}^{-1}$ and other had a constant concentration of $50 \mu\text{mol L}^{-1}$. Furthermore, it was found to have no effect on currents or peak potentials, when the concentration of both analytes was simultaneously increased, which could be used for analytical purposes (Figure 3). The precision of repeated measurements was calculated for ten replicate measurements of $50 \mu\text{mol L}^{-1}$ RAc and α -TAc in mixture and the obtained responses evaluated. The values of relative standard deviation (RSD), 2.27% and 2.19% , were calculated and the precision desired has been achieved for a common level of significance ($\alpha = 0.05$).

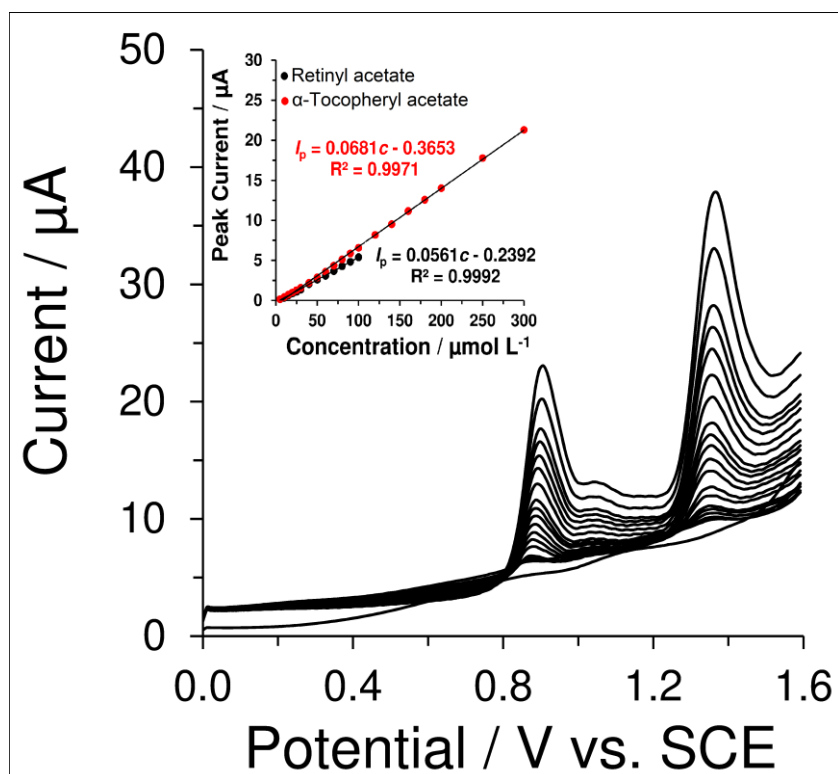


Figure 3 Voltammograms and corresponding calibration curves of 10-300 $\mu\text{mol L}^{-1}$ of α -tocopheryl acetate and 10-100 $\mu\text{mol L}^{-1}$ of retinyl acetate whose content simultaneously increased. SWV at GCE in 99.7% Acetone containing 0.1 mol L^{-1} LiClO_4 , $E_{\text{step}} = 10$ mV, $E_{\text{amp}} = 40$ mV and $f = 30$ Hz.

CONCLUSION

The presented voltammetric method based on direct anodic oxidation of retinyl acetate and α -tocopheryl acetate at the glassy carbon electrode in the supporting electrolyte containing 99.7 % acetone and 0.1 mol L^{-1} LiClO_4 is not still completed. It means that other subsequent measurements will be proposed, especially interference study, sample (cosmetic products) preparation for analysis, validation of developed voltammetric method etc.

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