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Determination of *Trans*-Resveratrol Using Voltammetric and Amperometric methods at Carbon Fiber Rod Electrode and Carbon Paste Electrode

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A new method for the determination of *trans*-resveratrol employing voltammetry (direct current voltammetry (DCV), differential pulse voltammetry (DPV), adsorptive stripping differential pulse voltammetry (AdSDPV)) and flow injection analysis (FIA) with electrochemical detection (ED) at a carbon fiber rod electrode (CFRE) was developed and compared with the voltammetric determination using carbon paste electrode (CPE) and FIA with spectrophotometric detection (SPD). Optimum conditions were found to be Britton-Robinson (BR) buffer pH 2 – methanol (1:1, v/v) for voltammetry (except for AdSDPV where BR buffer pH 10 – methanol mixture, 95:5, v/v was used), and BR buffer pH 10 – methanol (1:1, v/v) medium with the detection potential +1.0 V or wavelength 306 nm for FIA. Practical applicability of the methods was tested on the determination of *trans*-resveratrol in Evelor pastilles. The results were in agreement with the results obtained using CPE and spectrophotometric detection and also with the content declared by the manufacturer.

Keywords: Carbon fiber rod electrode, Carbon paste electrode, Voltammetry, FIA, Resveratrol.

1. INTRODUCTION

As a chemical entity, resveratrol (3,5,4´-trihydroxystilbene) is known since 1940 when it was first isolated from the roots of white hellebore (*Veratrum grandiflorum*) and later from *Polygonum cupsidatum*, a medical plant [1]. Resveratrol is a natural compound belonging to the group of polyphenolic phytoalexins, which are produced by plants in response to exogenous stimuli like fungal infection, UV light, ozone exposition or mechanical damage [2]. Numerous animal studies have demonstrated that this polyphenol holds promise against numerous age-associated diseases [3] including cancer [4], diabetes [5], Alzheimer, cardiovascular and pulmonary [6] diseases. Other

beneficial health effects such as antioxidative [7], neuroprotective [8], phytoestrogenic and anti-inflammative [9] properties have also been reported. Resveratrol can be found in many plants, which are often a component of human diet, for example in wine grapes (also in red and white wine), peanuts, cabbage, beetroot, broccoli, blueberries, cranberries, buckwheat and many others. Resveratrol exists in two isomeric forms *trans*-resveratrol and *cis*-resveratrol. The *trans*-isomer is the more stable form. Both isomers can be present in varied amounts in plants, but the amount of *trans*-resveratrol usually predominates [10]. The determination of resveratrol in real matrices usually requires utilization of separation methods. Among currently used methods prevails HPLC with spectrophotometric [11], or MS [12] detection, GC-MS [13] or electrophoresis [14]. The presence of hydroxy groups in the molecule is the reason for its electrochemical oxidizability, and this allows us to utilize electrochemical detection. The oxidation process of resveratrol at glassy carbon electrode (GCE) is quite complex, pH dependent, and all its steps are irreversible. Resveratrol gives two oxidation peaks corresponding to its oxidizable moieties, a phenolic and a resorcinolic functional groups. Resveratrol strongly adsorbs on the electrode surface and the final oxidation products block the electrode surface [15]. The anodic voltammetric behavior of *trans*-resveratrol was also studied at carbon paste electrodes (CPE) [16], and utilized in the construction of various biosensors [17,18].

The well-known advantages of CPEs [19-22] are broad potential window, low background current, possibility of chemical or biological modification of the carbon paste composition and ease of renewal of working surface.

The carbon fiber rod electrodes (CFRE) are classified as composite electrodes and they consist of carbon fiber rod, which is made from cross-section of carbon fibers covered by epoxy resin. Utilization of carbon fiber rods is generally connected with industrial constructions requiring light but firm materials. The construction of the electrode from the carbon fiber rod is very simple, involving just the connection of an electrical contact to several centimeters long piece of the rod. Electrochemical properties of carbon fiber epoxy composites are known from a few previously published papers [23-25]. CFRE has also been used as electrochemical detector in HPLC [26]. CFREs are available at very low cost and at various shapes and sizes. It follows from our previous study of electrochemical properties of CFRE with various diameters [27] that the best ratio between signal and noise was at CFRE with the diameter of 2 mm.

The aim of this work was to develop new electrochemical method for the determination of *trans*-resveratrol at carbon fiber rod electrode (CFRE). We used batch voltammetric methods (differential pulse voltammetry (DPV), direct current voltammetry (DCV), cyclic voltammetry (CV), adsorptive stripping differential pulse voltammetry (AdSDPV)), and flow injection analysis (FIA) with amperometric detection. For comparison purposes, the obtained results were compared to the results of methods utilizing CPE and spectrometric detection. The practical applicability of the methods was demonstrated on the determination of *trans*-resveratrol at CFRE and CPE in pastilles of Evelor. To the best of our knowledge, CFREs have not yet been used as amperometric detector in FIA or for voltammetric determination of *trans*-resveratrol.

2. EXPERIMENTAL

2.1. Reagents and chemicals

The stock solution (1×10^{-3} mol L^{-1}) of *trans*-resveratrol (Sigma-Aldrich, USA) was prepared by dissolving the exact amount of the analyte in methanol (p.a., Lach-Ner, Czech Republic) and stored in dark at 4 °C. Britton-Robinson (BR) buffers were prepared from a solution containing 0.04 M phosphoric acid, 0.04 M acetic acid and 0.04 M boric acid, and an appropriate amount of aqueous 0.2 M sodium hydroxide solution. All the chemicals used were of analytical reagent grade and were purchased from Lachema, Czech Republic. Methanol was used for dissolving pastilles of Evelor resveratrol 50 mg (Medochemie Ltd., Cyprus). All aqueous solutions were prepared using deionized water obtained from MiliQ Plus system (Millipore, USA).

2.2. Carbon fiber rod electrode and carbon paste electrode

The carbon fiber rod electrodes (CFRE) were produced from carbon fiber rods (RCM Pelikan, Czech Republic) of 2 mm diameter for voltammetry and 3 mm diameter for amperometry and of 1 m length. The electric contact was made of a copper wire which was connected by a conducting paint EL-2 (Elchemco, Czech Republic) to the 5 cm long piece of carbon fiber rod. For fixing the electrode in the holder compatible with the used instrumentation, a laboratory parafilm M (Pechiney plastic packaging, USA) and a plastic tube were used. The surface of the CFRE was renewed mechanically by alumina polishing powder suspension (1 μm particle size, Bioanalytical Systems, Inc., USA).

Carbon paste contained 250 mg of spherical microparticles of glassy carbon with a diameter of 0.4–12 μm (Alpha Aesar, USA) and 90 μL of mineral oil (Fluka Biochemica, Switzerland). The working electrode body was made of teflon (made in-house) with a 3 mm inner diameter for voltammetry or 2 mm inner diameter for amperometry. The surface of the CPE was renewed mechanically by protruding the piston and smoothing the surface with wet filter paper.

2.3. Apparatus

For voltammetric measurements, a computerized voltammetric analyzer Eco-Tribo Polarograph with software PolarPro 4.0 (all Polaro Sensors, Czech Republic) was used. The three-electrode arrangement with Ag/AgCl (3M KCl) reference electrode RAE 113 (Monokrystaly, Czech Republic), auxiliary platinum wire electrode, and working CFRE or CPE with a 2 mm diameter was used.

The FIA system consisted of the high-pressure piston pump HPP 5001 (Laboratorní přístroje, Czech Republic), injection valve D with 20 μL sample loop, and spectrophotometric detector Sapphire 800 UV/VIS (both Ecom, Czech Republic), electrochemical detector CHI 802B (CH Instruments, USA) with three-electrode system consisting of Ag/AgCl (3M KCl) reference electrode RAE 113 (Monokrystaly, Czech Republic), auxiliary platinum wire electrode, and working CFRE or CPE with a 3 mm diameter. The amperometric detector working in a wall-jet configuration and employing electrochemical oxidation of phenolic hydroxy groups, was placed behind the UV/VIS detector

operating at 306 nm. The system was operated by Clarity 2.3.0 program (DataApex, Czech Republic) and CHI 6.26 program (CH Instruments, USA) working in the Windows XP system (Microsoft, USA).

An ultrasonic bath PS02000A (Powersonic, USA) was used for the dissolution of the analytes. The pH of the solutions was measured with a pH meter Jenway 4330 with a combined glass electrode (both Jenway, UK). The stability of the stock solution of *trans*-resveratrol was followed spectrophotometrically using a spectrophotometer Agilent 8453 (Agilent, USA). All experiments were carried out at a laboratory temperature.

2.4. Procedure

Solutions for voltammetry were prepared by mixing 5 mL of methanolic solution containing the respective amount of the *trans*-resveratrol with 5 mL of BR buffer of chosen pH (for FIA, BR buffer was ten times diluted by deionized water) in volumetric flask and filling to the 10 mL mark by a mixture of methanol and BR buffer of chosen pH (1:1, v/v).

The solution for measurement of real sample of Evelor was prepared by dissolving 1 pastille of Evelor in 100 mL of methanol, then 0.25 mL of this solution was mixed with 4.75 mL of methanolic solution containing the addition of 0; 0.2; 0.3 and 0.5 ml of standard solution of the *trans*-resveratrol ($c = 1 \times 10^{-3}$ mol L^{-1}), mixed with 5 mL of BR buffer of optimum pH and filled to 10 mL with a solution of methanol and BR buffer of optimum pH (1:1, v/v). All the solutions were filtered before being used for analyses.

Calibration dependences were measured in triplicate and evaluated by a linear regression method. The limit of detection (L_D) for the flow method was calculated as the amount of *trans*-resveratrol, which gave the signal three times higher than the background noise (S/N). For batch methods the limit of detection was calculated as the amount of *trans*-resveratrol, which gave three times higher signal than the standard deviation (SD) of the lowest measured resveratrol concentration of the calibration dependence.

3. RESULTS AND DISCUSSION

3.1. Voltammetric methods

The influence of the BR buffer pH in the range from 2 to 12 on voltammetric behavior of resveratrol was investigated using DPV, DCV and CV in the anodic potential range at CFRE and CPE. Both electrodes exhibited similar behavior. DP voltammograms of 1×10^{-4} mol L^{-1} *trans*-resveratrol at CFRE (Figure 1A) and CPE (Figure 1B) show the shift of the analyte peak potential to the less positive values with increasing pH, which can be explained by easier oxidation of dissociated hydroxy group reflecting the involvement of the protons in the reaction mechanisms. BR buffer pH 2 – methanol (1:1, v/v) was chosen as the optimum medium for all voltammetric techniques at both electrodes, because in this medium the voltammograms were highest and best developed.

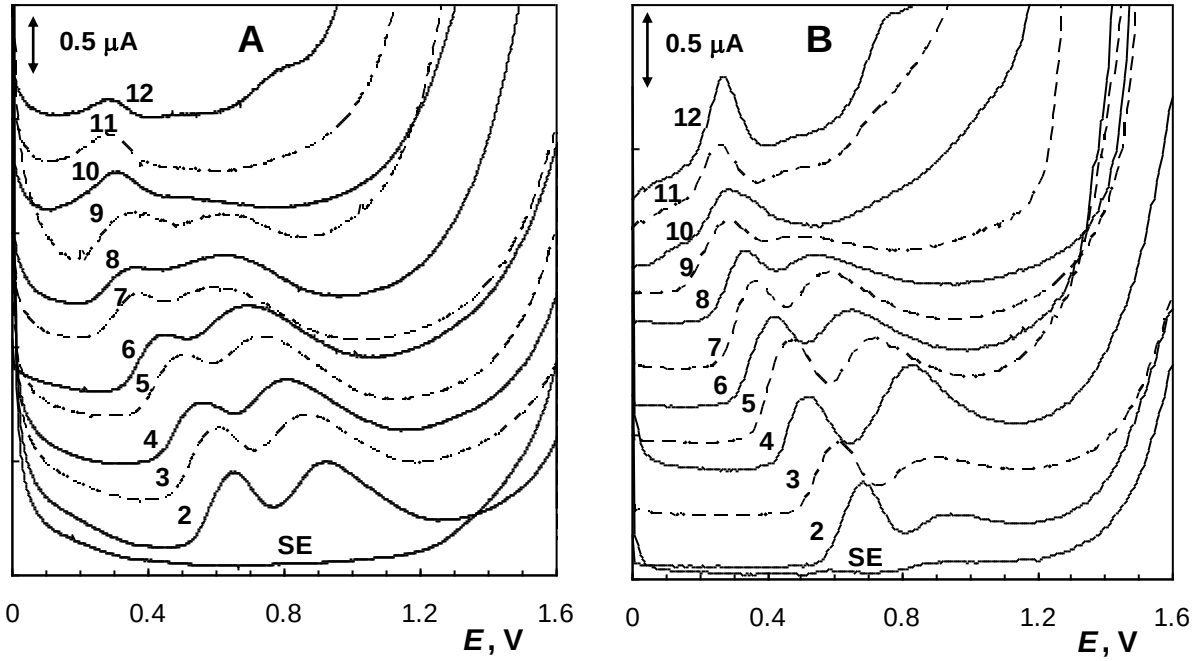

Figure 1. The anodic voltammograms of *trans*-resveratrol ($c = 1 \times 10^{-4}$ mol L^{-1}) measured by DPV at CFRE (A) or CPE (B) in BR buffer and methanol medium (1:1, v/v). Buffer pH corresponds to the number of the curve, supporting electrolyte BR buffer pH 2 with methanol (1:1, v/v) is shown (SE). Scan rate 20 mV s^{-1}, pulse width 100 ms, and pulse height 50 mV.

Table 1. The parameters of the calibration dependences of *trans*-resveratrol for voltammetric (DCV, DPV, CV, AdSDPV) detection at CPE and CFRE, supporting electrolyte BR buffer pH 2 – methanol (1:1, v/v); BR buffer pH 10 – methanol (95:5, v/v) was used for AdSDPV.

Method	Concentration range mol L^{-1}	Slope mA mol^{-1} L	Intercept nA	$R^{2\,a}$	$L_D{}^b$ mol L^{-1}
CPE (2 mm)					
DPV	8×10^{-7}–1×10^{-4}	5.9	17.9	0.9976	8.9×10^{-7}
DCV	6×10^{-7}–1×10^{-4}	6.8	21.1	0.9957	7.5×10^{-7}
CV	1×10^{-6}–1×10^{-4}	13.2	15.5	0.9989	9.7×10^{-7}
CFRE (2 mm)					
DPV	8×10^{-7}–1×10^{-4}	5.7	14.7	0.9974	8.2×10^{-7}
DCV	6×10^{-7}–1×10^{-4}	6.7	41.0	0.9946	7.6×10^{-7}
CV	6×10^{-7}–1×10^{-4}	16.4	127.3	0.9962	7.3×10^{-7}
AdSDPV	1×10^{-7}–1×10^{-4}	76.2	9.4	0.9983	2.2×10^{-7}

a. R^2, coefficient of determination
b. L_D, limit of detection

The cyclic voltammograms of *trans*-resveratrol ($c = 1 \times 10^{-4}$ mol L^{-1}) at both electrodes were recorded in optimum medium in the potential range from 0 to +1.5 V at increasing scan rates from 2 to 1000 mV s^{-1}. The oxidation process of *trans*-resveratrol is not accompanied by a reduction wave in a

reverse scan, which indicates that the oxidation reaction is totally irreversible at both electrodes. A direct proportionality was observed for the dependence of peak current on the square root of the scan rate, thus documenting the diffusion-controlled process at both electrodes.

The calibration curves were measured under the optimum conditions (BR buffer pH 2 and methanol, 1:1, v/v) in the concentration range from 6×10^{-7} to 1×10^{-4} mol L^{-1} at both electrodes. They are linear within the whole studied concentration range, with similar detection limits ranging from 7.3 to 9.7×10^{-7} mol L^{-1}. The obtained results are summarized in Table 1.

The possibility of increasing the sensitivity of the determination by adsorptive accumulation of the analyte on the surface of CFRE or CPE was also investigated. The influence of accumulation potential (E_{acc}) on the peak current of 8×10^{-6} mol L^{-1} *trans*-resveratrol was measured for potentials from 0 to 0.6 V, with the accumulation time (t_{acc}) from 30 s to 10 min in BR buffer (pH 2, 7, 10 and 12), always in a mixture with methanol (95:5, v/v). It follows from these results that accumulation of *trans*-resveratrol at CPE in all tested media is not significant, but results at CFRE are different (see Figure 2). The strongest accumulation of the analyte at CFRE was found in BR buffer pH 10 – methanol (95:5, v/v) at the accumulation potential 100 mV for accumulation time 10 min. Under these conditions, the calibration dependence using adsorptive stripping differential pulse voltammetry (AdSDPV) in the concentration range from 1×10^{-7} to 1×10^{-5} mol L^{-1} at CFRE (see Table 1 and Figure 3) was measured. The calculated limit of detection was 2.2×10^{-7} mol L^{-1}.

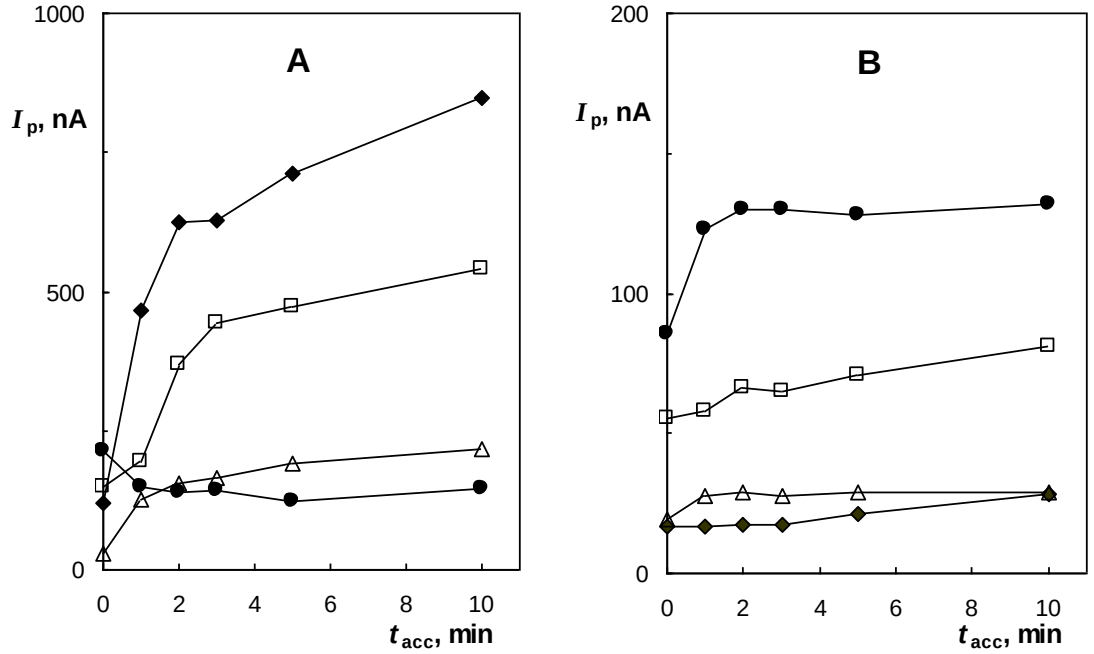

Figure 2. The dependence of peak current of *trans*-resveratrol ($c = 8 \times 10^{-6}$ mol L^{-1}) at CFRE (**A**) or CPE (**B**) on accumulation time at selected accumulation potential (at CFRE: 0.4 V (●); 0.3 V (□); 0.1 V (△; ◆), at CPE: 0.4 V (●); 0.2 V (□; △); 0.1 V (◆)), measured by adsorptive stripping differential pulse voltammetry in BR buffer pH 2 (●); 7 (□); 10 (△) or 12 (◆) with methanol (95:5, v/v). Scan rate 20 mV s^{-1}, pulse width 100 ms, and pulse height 50 mV.

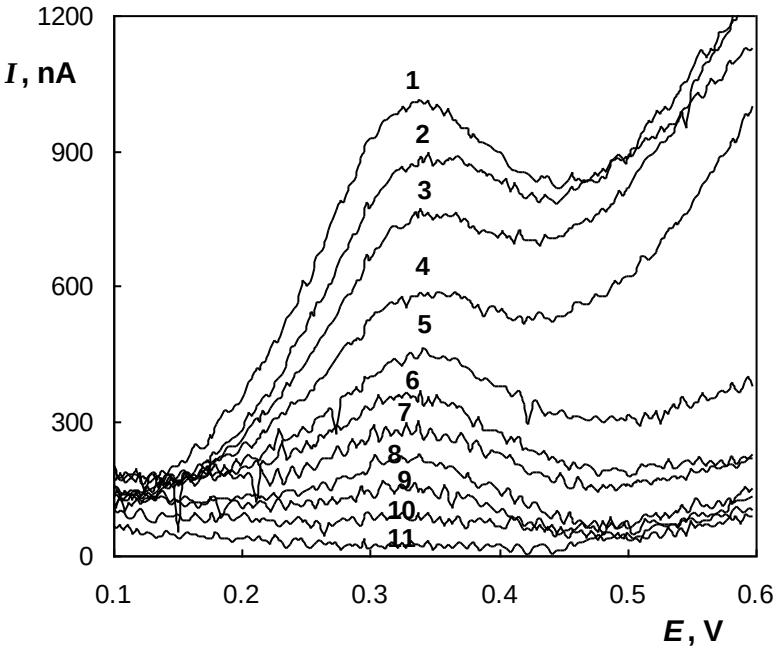

Figure 3. The AdSDPV voltammograms of 10 (**1**); 8 (**2**); 6 (**3**); 4 (**4**); 2 (**5**); 1 (**6**); 0.8 (**7**); 0.6 (**8**); 0.4 (**9**); 0.2 (**10**); 0.1 (**11**) × 10^{-6} mol L^{-1} *trans*-resveratrol (curves not smoothed) measured at CFRE in BR buffer pH 10 with methanol (95:5, v/v), accumulation time 10 min, accumulation potential 100 mV. Scan rate 20 mV s^{-1}, pulse width 100 ms, and pulse height 50 mV.

3.2. Flow injection analysis with electrochemical detection

For increasing the speed of analysis and automation purposes, new method for the determination of *trans*-resveratrol by FIA at CFRE was developed. To find the optimum pH of mobile phase and working potential, the hydrodynamic voltammograms of *trans*-resveratrol were measured in FIA arrangement in the potential range from +0.1 to 1.6 V at CFRE and CPE (both with 3 mm diameter), while pH of aqueous part of mobile phase was adjusted to the values of 2, 4, 6, 8, 10, and 12, respectively. The highest and best developed peak was found in BR buffer pH 10 – methanol (1:1, v/v) medium at the working potential +1.0 V at both CFRE and CPE.

Influence of the flow rate on peak heights was investigated in the range from 1 to 7 mL min^{-1}. With increasing flow rate, the peak height was increasing at CFRE and CPE in the whole range. UV/VIS detector peak height was increasing up to flow rate 3 mL min^{-1} and then it was almost constant. On the other hand, the peak area was decreasing with increasing the flow rate at all detectors. Also mechanical damage of paste surface of CPE due to the increasing flow rate above 4 mL min^{-1} was observed. For that reasons, the flow rate was kept on 3 mL min^{-1}.

The stability of the electrode response was tested by 10 repeated injections of 1 × 10^{-4} mol L^{-1} solution of *trans*-resveratrol. The relative standard deviations of the peak height were 4.5 % at CFRE, 5.4 % at CPE and 2.3 % at UV/VIS detector. The drift at all three detectors was negligible.

The concentration dependences were measured under the optimum conditions in the concentration range from 8 × 10^{-8} to 1 × 10^{-4} mol L^{-1} at both electrodes and compared with UV/VIS

detection (Table 2). To verify the linearity of calibration curves, their logarithmic forms were constructed (log I_p vs. log c) and the results show that all the measured calibration curves for both electrodes are linear (slopes of the logarithmic calibration curves are close to one). The limits of detection (L_D) for FIA with electrochemical detection were 9.5×10^{-8} mol L^{-1} for CFRE, 5.2×10^{-7} mol L^{-1} for CPE, and 8.3×10^{-8} mol L^{-1} for UV/VIS spectrophotometric detection.

Table 2. The parameters of the calibration dependences of *trans*-resveratrol for FIA determination with spectrophotometric (λ = 306 nm) and amperometric (E_{DET} = +1.0 V) detection at CPE and CFRE measured in the concentration range $8 \times 10^{-8} - 1 \times 10^{-4}$ mol L^{-1} , supporting electrolyte BR buffer pH 10 – methanol (1:1, v/v) medium, flow rate 3 mL min^{-1}, injected 20 μL.

Spectrometric detection at wavelength 306 nm

Evaluated	Slope AU (resp. AU s) mol^{-1} L	Intercept mAU (resp. mAU s)	R^2 [a]	Linearity[b]	L_D[c] mol L^{-1}
peak height	2556.9	0.65	0.9993	0.95	8.3×10^{-8}
peak area	6067.5	0.75	0.9987	1.01	

Amperometric detection at potential +0.1 V

Evaluated	Slope mA (resp. mC) mol^{-1} L	Intercept nA (resp. nC)	R^2 [a]	Linearity[b]	L_D[c] mol L^{-1}
CPE (3 mm)					
peak height	21.0	17.3	0.9980	1.00	5.2×10^{-7}
peak area	0.071	-0.0008	0.9988	1.17	
CFRE (3 mm)					
peak height	11.8	0.80	0.9993	0.95	9.5×10^{-8}
peak area	0.038	-0.0013	0.9934	0.99	

a. R^2, coefficient of determination
b. Slope of logarithmic calibration dependence (log I_p vs. log c)
c. L_D, limit of detection

3.3. Real sample

The practical applicability of the newly developed methods was tested on the sample of Evelor with declared *trans*-resveratrol content of 50 mg in each pastille (containing only analyte and electrochemically inactive compounds). The amount of *trans*-resveratrol in the original sample was determined by standard addition method with three additions. Determinations of *trans*-resveratrol were performed under the optimum conditions for developed methods (DPV, FIA at CFRE) and obtained results were compared with results using spectrophotometry, voltammetry at CPE, and FIA with

electrochemical detection at CPE, and spectrophotometric detection (306 nm). The resulting amperometric or spectrophotometric responses for the determination of *trans*-resveratrol in Evelor are depicted in Figure 4. Obtained average content of *trans*-resveratrol in three pastilles of Evelor is summarized in Table 3. Results from all methods are not statistically significantly different from each other and they also correspond to the declared 50 mg content of *trans*-resveratrol per one pastille. Obtained recovery from standard addition method with three additions were between 95.0 to 105.1 %.

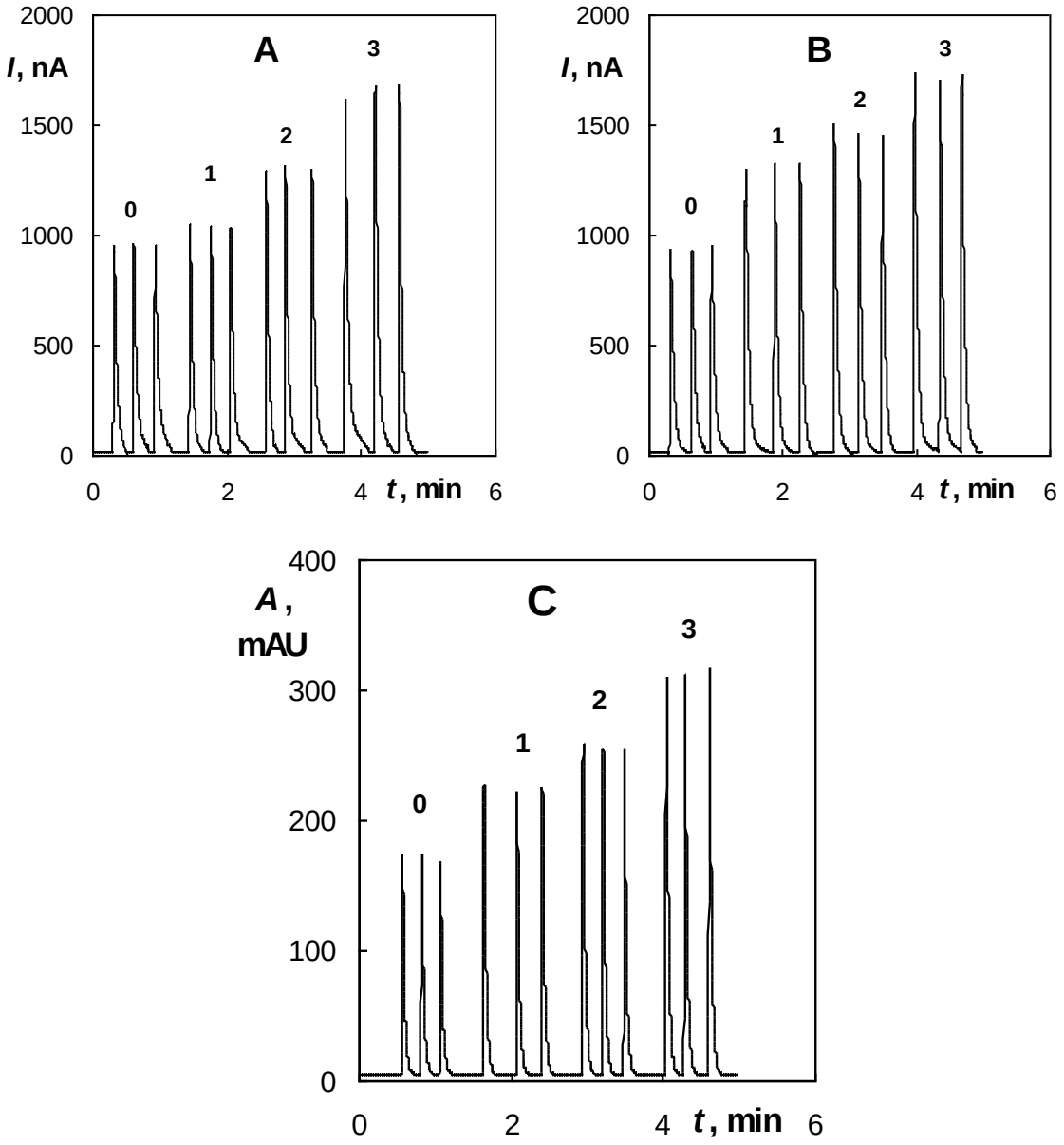

Figure 4. The response of *trans*-resveratrol at amperometric CFRE detector (A) or CPE detector (B), and spectrophotometric detector at 306 nm (C) in real sample of Evelor with addition 0 (0); 0.2 (1); 0.3 (2) and 0.5 (3) ml of standard solution of *trans*-resveratrol (c = 1× 10^{-3} mol L^{-1}) to final volume 10 ml. Carrier solution BR buffer pH 10 with methanol (1:1, v/v), flow rate 3 mL min^{-1}, E_{DET}= +1.0 V, injected 20 μL.

Table 3. Content of *trans*-resveratrol in Evelor pastille (declared content 50 mg of *trans*-resveratrol in each pastille) found using batch method (DPV, spectrophotometry) or FIA with spectrophotometric (SPD, λ = 306 nm) and amperometric (E = +1.0 V) detection at CPE and CFRE, supporting electrolyte BR buffer pH 2 (pH 10 for FIA) – methanol (1:1, v/v) medium.

Detection method	Found *trans*-resveratrol[a] mg/pastille ± SD
Batch methods	
DPV-CPE	49.0 ± 1.2
DPV-CFRE	50.5 ± 1.1
SPD	49.6 ± 0.9
FIA methods	
ED-CPE	50.9 ± 1.2[b]
	49.1 ± 0.9[c]
ED-CFRE	50.2 ± 0.9[b]
	49.9 ± 0.8[c]
SPD	49.7 ± 1.1[b]
	49.4 ± 0.7[c]

a. Means (of three pastilles), calculated from three standard additions
b. calculated from peak height
c. calculated from peak area

If we compare our obtained results to previous works at carbon fiber epoxy composites, it is clear that CFRE is suitable electrode material probably more suitable than carbon fiber cloth [25]. Electrochemical behavior of various CFRE in supporting electrolytes (1M H_2SO_4 [24]; H_2SO_4 with 30% KOH (w/o) [23]; 0.1 M KCl [26]; BR buffers, 0.1 M NaOH, KCl, H_2SO_4 [27]) is comparable to glassy carbon electrode. The comparison of the obtained limits of detection and other results to the results published by other author is difficult. We can only compare results to our previous publication [27], where are the limits of detection for 5-amino-6-nitroquinoline CFRE (DPV: $L_D = 9.8 \times 10^{-7}$ mol L^{-1}) very similar to the limits of detection for *trans*-resveratrol (DPV: $L_D = 8.2 \times 10^{-7}$ mol L^{-1}). In flow systems the comparison of obtained results is also complicated, because in published work [26] dealing with capillary HPLC ring-disk CFRE and different flow cell design were used and applied to different analytes. Nevertheless, the obtained limits of detection are comparable confirming the general usefulness of this new electrode material.

4. CONCLUSIONS

A simple, sensitive and selective voltammetric and FIA methods for the determination of *trans*-resveratrol at CFRE have been developed. Optimum conditions were BR buffer pH 2 – methanol (1:1, v/v) for voltammetry (except for AdSDPV BR buffer pH 10 – methanol, 95:5, v/v) and BR buffer pH 10 – methanol (1:1, v/v) medium at the working potential +1.0 V or wavelength 306 nm for FIA. The

methods exhibit good sensitivity, appropriate selectivity and they are applicable for the determination of *trans*-resveratrol content in pharmaceuticals.

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