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Facile electrochemical determination and characterization of bioflavonoid hesperidin using disposable pencil graphite electrode – Application in pharmaceutical analysis

S. Šafranko[1], M. Banožić[1], A. Stanković[2], A. Asserghine[3], K. Aladić[1], M. Medvidović-Kosanović[2], S. Jokić[1]

[1] Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek, Croatia; **e-mail: silvija.safranko@ptfos.hr**

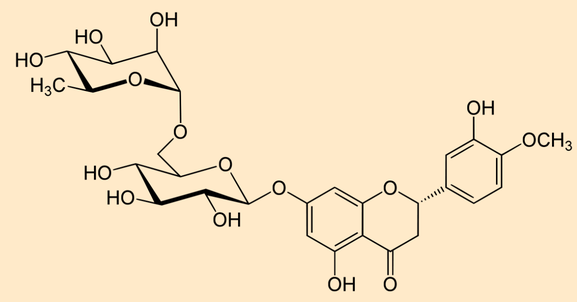
[2] Department of Chemistry, Josip Juraj Strossmayer University of Osijek, Ulica cara Hadrijana 8/A, 31000 Osijek, Croatia

[3] Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, 7624, Ifjúság u. 6. Pécs, Hungary

INTRODUCTION

This study is focused on simple voltammetric determination of hesperidin by using electroactivated PGE (disposable pencil graphite electrode; ePGE) in model systems and in real samples in order to evaluate the adequacy of the developed method in pharmaceutical analysis. Electroactivation of the electrode was performed in various potentials ranging from -1.4 V to 2.0 V in the phosphate buffer pH = 7.0. The electrochemical properties of hesperidin in model systems were studied with cyclic (CV) and differential pulse voltammetry (DPV). Identification and quantification of hesperidin in pharmaceutical formulation was obtained with DPV. The results have indicated that hesperidin is irreversibly oxidized, with the most pronounced oxidation at pH = 5.0. Oxidation process was under mixed diffusion and adsorption control and included the equal number of protons and electrons. The peak current and the hesperidin concentration showed linear dependence in the concentration range from $5x10^{-7}$ mol dm^{-3} to $1x10^{-5}$ mol dm^{-3}. Estimated limit of detection (LOD) was $5x10^{-7}$ mol dm^{-3}. The method was applied for determination of hesperidin in pharmaceutical formulation (containing active substance, hesperidin and excipients) and good correlation was obtained between experimentally obtained and expected hesperidin concentration (R^2 = 0.9462), which showed potential application of developed method for hesperidin detection in pharmaceuticals.

HESPERIDIN



Bioflavonoid

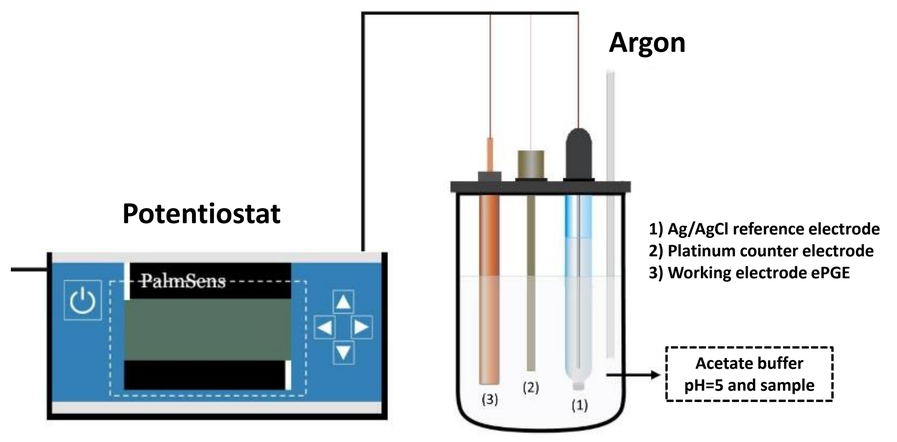
MATERIALS AND METHODS

All chemicals used in this study were of reagent grade and used as purchased from commercial sources: NaH_2PO_4, Na_2HPO_4, KCl, $[K_3[Fe(CN)_6]]$, $K_4[Fe(CN)_6]x3H_2O$, $C_{11}H_{12}FeO$ (FcMeOH).

Voltammetric measurements were performed in a three electrode voltammetric cell on PalmSens potentiostat/galvanostat. Working electrode: disposable pencil graphite electrode (ePGE) with a 0.05 cm diameter (Faber Castell 1HB). **Electroactivation: via cycling five times in phosphate buffer pH = 7.0 (I_c = 0.1 mol dm^{-3} KCl) in the potential range from -0.6 V to 2.0 V with a scan rate 50 mV/s.**

Detection of hesperidin in pharmaceutical product: a stock solution of pharmaceutical prepared in DMSO (c = 1.6×10^{-3} mol dm^{-3}); acetic buffer (pH = 5).

For validation of developed electrochemical method, the content of hesperidin in pharmaceutical formulation was examined using HPLC-DAD.



ELECTROCHEMICAL CHARACTERIZATION

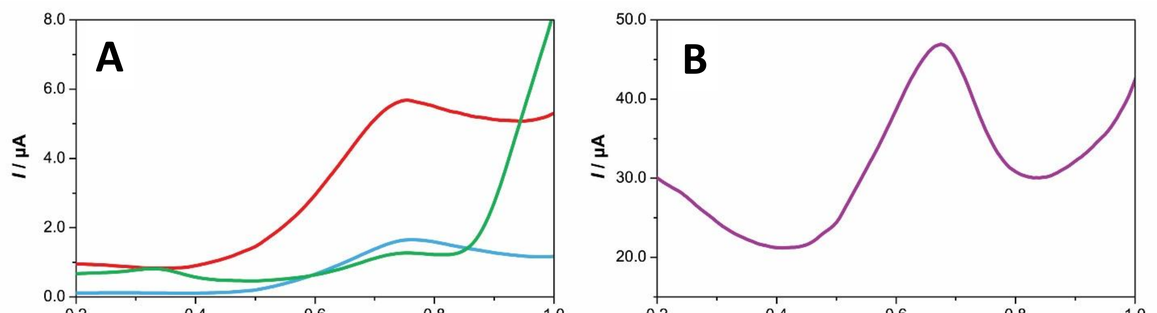


Fig. 1. Differential pulse voltammograms of hesperidin (c = 1×10^{-3} mol dm^{-3}) in phosphate buffer pH = 7.0 (I_c = 0.1 mol dm^{-3} KCl) recorded on A) platinum (–), GCE (–) and PGE (–) and B) ePGE (–).

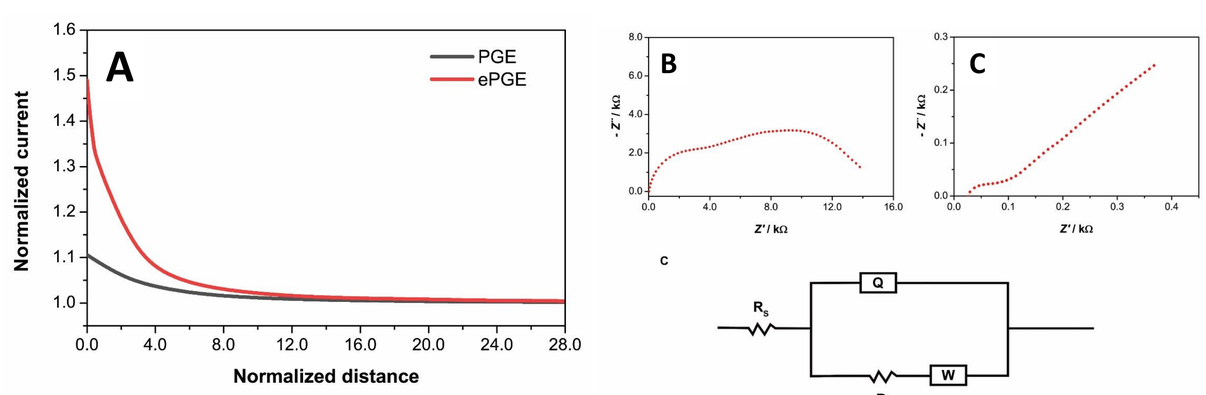
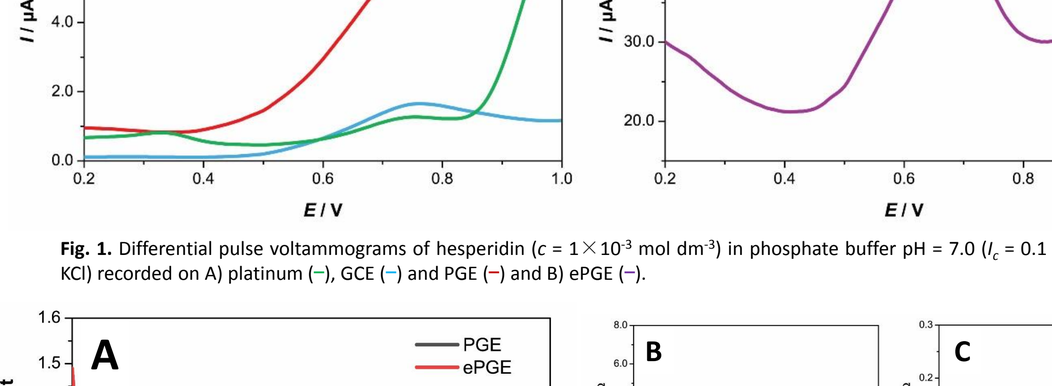


Fig. 2. D) SECM Z-approach curve recorded on the PGE (black line) and ePGE (red line) during immersion in 0.1 mol dm^{-3} KCl + 2 mmol dm^{-3} FcMeOH solution. Tip diameter: 15 µm; tip potential: +0.60 V Ag/AgCl/NaCl (c = 3 mol dm^{-3}); scan rate: 10 µm s^{-1}. Electrochemical impedance spectra of B) PGE, C) ePGE at the E_{oc} recorded with an excitation amplitude of 10 mV, in the frequency range from 50 kHz to 0.01 Hz, in 5 mmol dm^{-3} $[Fe(CN)_6]^{3-/4-}$ (I_c = 0.1 mol dm^{-3} KCl), C) equivalent electrical circuit used to fit EIS spectra for PGE and ePGE. R_s is the resistance of the solution, Q is double layer capacitance, R_1 is the electron transfer resistance and W is the Warburg element.



Two oxidation peaks were detected, the first one which corresponds to oxidation of phenolic hydroxyl group in ring B of hesperidin and includes the transfer of two protons and two electrons and the second oxidation peak which corresponds to irreversible oxidation of the hesperidin hydroxyl group in ring A and includes the transfer of one electron and one proton. Hesperidin is irreversibly oxidized on the disposable pencil graphite electrode, ePGE and its oxidation was the most pronounced at pH = 5 (data not shown). **The peak current and the hesperidin concentration showed linear dependence in the concentration range from 5×10^{-7} mol dm^{-3} to 1×10^{-5} mol dm^{-3}. Determined lower limit of detection (LOD) was 2×10^{-7} mol dm^{-3}, while limit of quantification (LOQ) was 8×10^{-7} mol dm^{-3} (data not shown).**

ANALYSIS IN PHARMACEUTICALS

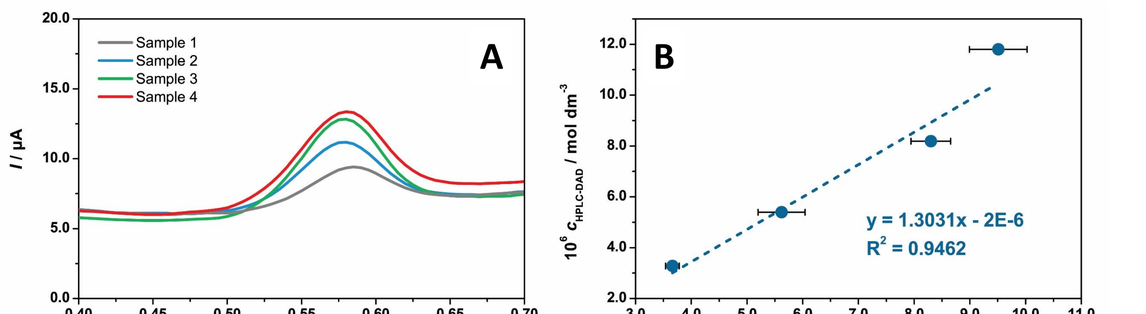


Fig. 3. A) Differential pulse voltamograms of hesperidin in pharmaceutical samples recorded on ePGE in acetate buffer pH = 5 (I_c = 0.1 mol dm^{-3} KCl), scan rate 5 mV/s. $c_{theoretical}$ (hesperidin) = 3.3 (sample 1), 5.5 (sample 2), 8.2 (sample 3) and 10.9 (sample 4) µmol dm^{-3} and B) Hesperidin concentration ($c_{HPLC-DAD}$) determined with HPLC technique as a function of experimentally determined hesperidin concentration with ePGE (c_{exp}).

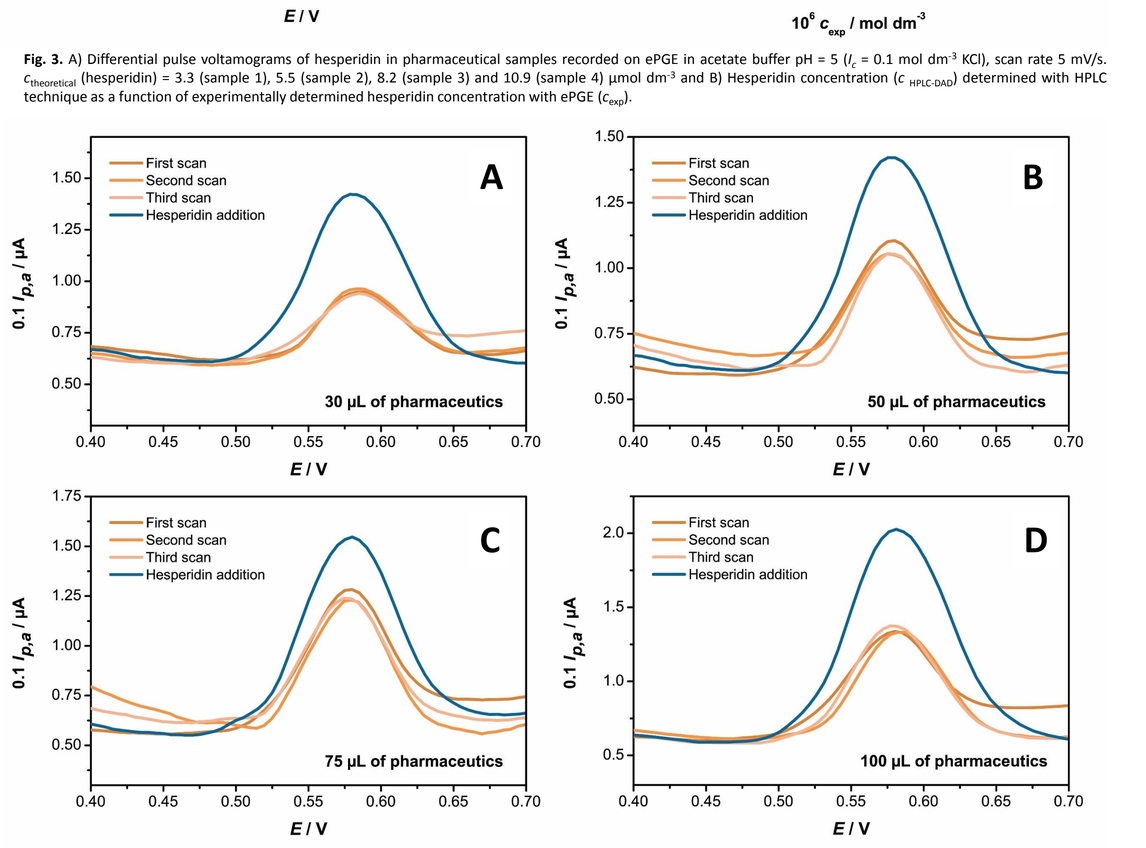
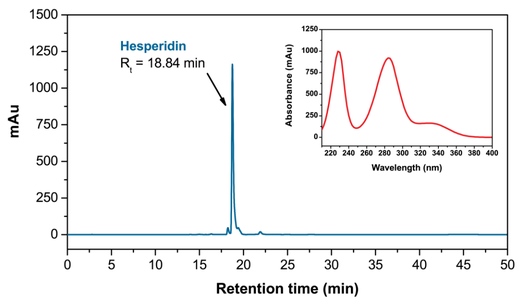


Fig. 4. Differential pulse voltammograms of hesperidin in pharmaceutical samples recorded on ePGE in acetate buffer pH = 5 (I_c = 0.1 mol dm^{-3} KCl), scan rate 5 mV/s. c_{th} (hesperidin) = 3.3 (A), 5.5 (B), 8.2 (C) and 10.9 (D) mmol dm^{-3} V(added hesperidin) = 150 ml.

Table 1. Determination of hesperidin in pharmaceutical formulations with HPLC-DAD and voltammetric method with ePGE

Sample	c_{th} / mmol dm^{-3}	$c_{HPLC-DAD}$ / mmol dm^{-3}	c_{exp} / mmol dm^{-3}	RSD / %
1	3.3	3.28 ± 0.05	3.83 ± 0.12	3.18
2	5.5	5.39 ± 0.09	5.94 ± 0.42	7.12
3	8.2	8.19 ± 0.03	8.40 ± 0.36	4.07
4	10.9	11.80 ± 0.01	9.95 ± 0.52	5.20



Our idea was to test the analytical performance of ePGE electrode in these real samples of diluted pharmaceutical and to compare the obtained results with HPLC method. Differential pulse voltammograms in prepared pharmaceutical solutions were recorded three times with ePGE.

Appearance of one oxidation peak confirmed that hesperidin can be detected in investigated pharmaceutical formulations. The experimental concentration (c_{exp}) obtained by voltammetric analysis were compared with total hesperidin concentration ($c_{HPLC-DAD}$) determined with standard HPLC method as shown in Fig. 3B. The obtained coefficient of determination, R^2 = 0.9462 while the overall results are presented in **Table 1**.

Fig.5. Chromatogram of High Performance Liquid Chromatogram with Diode-Array detection (HPLC-DAD) of hesperidin (R_t = 18.84 min). Inset: UV spectra of hesperidin standard.

CONCLUSIONS

1) Electrochemical properties of hesperidin were studied on the electroactivated disposable graphite electrode (ePGE).
2) Differential pulse voltammetry with ePGE was applied for the determination of hesperidin in pharmaceutical formulation (containing active substance, hesperidin, and excipients) - good correlation between experimentally obtained and hesperidin concentration determined by standard HPLC method (R^2 = 0.9462).
3) Developed electrochemical method using ePGE could be suitable method for hesperidin detection in pharmaceutical analysis.

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ByProExtract

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