

## ELECTROCHEMICAL DETECTION OF VITAMIN C IN REAL SAMPLES

Jelena Blažević<sup>1</sup>, Anamarija Stanković<sup>1</sup>, Silvija Šafranko<sup>2\*</sup>, Stela Jokić<sup>2</sup>,  
Darko Velić<sup>2</sup>, Martina Medvidović-Kosanović<sup>1</sup>

<sup>1</sup>Department of Chemistry, Josip Juraj Strossmayer University of Osijek, Ulica cara Hadrijana 8/A, 31000 Osijek, Croatia  
<sup>2</sup>Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 20, 31000 Osijek, Croatia

*original scientific paper*

### Summary

Electrochemical properties of vitamin C (also known as L-ascorbic acid) have been studied by cyclic and differential pulse voltammetry in the model systems in order to develop simple and suitable method for vitamin C detection in real samples. The results indicated that vitamin C oxidation is a quasi-reversible and diffusion-controlled process, as well that the oxidation product of ascorbic acid, dehydroascorbic acid is adsorbed on the glassy carbon electrode surface. Calibration curve was constructed and the linear response was obtained in a concentration range from 0.0025 mol dm<sup>-3</sup> to 1.0 mol dm<sup>-3</sup>. Vitamin C was successfully determined in real samples (fruit juices and food supplement) with cyclic voltammetry within concentration range from 0.034 mol dm<sup>-3</sup> to 0.340 mol dm<sup>-3</sup>. In addition, antioxidant activity of vitamin C in real samples was determined using DPPH assay with a good linear correlation obtained between the cyclic voltammetry results and the results evaluated by DPPH assay of the samples.

**Keywords:** vitamin C, detection, cyclic voltammetry, DPPH assay, fruit juices

### Introduction

Vitamin C (L-ascorbic acid) is water-soluble vitamin exhibiting many beneficial nutritional and medical properties. The biological activity of vitamin C is manifested through antioxidative capacity and ability to inhibit or quench free radical reactions, at the same time preventing cellular damage. Moreover, vitamin C is considered as biologically active compound and essential micronutrient widely present in fruits and vegetables, playing an important role in antioxidative capacity, especially in citrus fruits (Igual et al., 2011; Sdiri et al., 2019). Beyond the many beneficial effects, vitamin C is also participating in biological functions and metabolic pathways, such as a cofactor for enzymes involved in collagen synthesis and norepinephrine and adrenal hormones balancing, or as a reducing agent in the cellular metabolism (Skrovankova et al., 2015). According to the Recommended Dietary Allowances (RDA), the average and recommended daily intake of vitamin C is estimated to be 75 mg/day for adult women and 90 mg/day for men in order to maintain optimal health conditions (Chalmers et al., 1986). Therefore, deficiency or imbalance in vitamin C could cause severe health issues such as scurvy (lat. *scorbutus*), however the disease is not so widespread and rarely appears due to development of modern scientific medicine (Banan et al., 2013; Ly et al., 2004). The consequences of the scurvy disease include changes in the skin, mucous membranes, connective tissue and joints (Padayatty et al., 2003; Ravindran et al. 2018; Varvara et al., 2016).

Vitamin C is one of the most studied compounds among the all vitamins. However, due to its low stability vitamin C undergoes rapid oxidation process, forming one (ascorbyl radical) or two-electron oxidized product, known as dehydroascorbic acid (DHA). The oxidation processes of vitamin C can be enhanced by increasing temperature or pH value, while exposure to air, overcooking and storage temperature conditions showed significant effects on the compound stability (Oyetade et al., 2012). Ascorbic acid consists of highly reactive hydroxyl groups which are very sensitive to light, heating and the presence of oxidizing agents (Tadesse and Sirgawie, 2017). Above the pH 5.0, vitamin C exists predominantly in the form of ascorbate monoanion (AscH<sup>-</sup>), and in the alkaline environment above pH 12.0, fully dissociated form ascorbate dianion (AscH<sup>2-</sup>) is present. Therefore, the predominant product of vitamin C at physiological pH is undoubtedly in the form of monoanion AscH<sup>-</sup>, conferring the main antioxidative activity of the molecule in living organisms (García-Rodríguez et al., 2017). Due to its donating-electron or reducing ability (Ngai et al., 2013), antioxidant vitamin C is capable of chelating metal ions or acting directly by scavenging reactive oxygen species (ROS) and preventing oxidative damage electrons (Yuswan et al., 2015).

Hence, simple and rapid methods for vitamin C determination could be study of interest. Nowadays, several analytical approaches have been established for vitamin C investigation in different samples: titrimetric, spectrophotometric, fluorimetric,

\*Corresponding author: silvija.safranko@ptfos.hr

chromatographic and electrochemical. Chromatographic and electrochemical techniques are considered as more sophisticated methods for chemical analysis providing selective and sensitive detection (Tadesse and Sirgawie, 2017). Chromatographic separation techniques most commonly include liquid chromatography coupled to mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC). However, these methods are more sensitive but quite expensive, therefore there is a constant search for cost-effective and reliable techniques for detection of certain analytes.

Electrochemical techniques can be used as an efficient alternative, providing an affordable and accurate approach for detection of compounds. Usually, electrochemical properties of electroactive molecules could be investigated by voltammetric techniques (cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, linear sweep voltammetry, chronocoulometry, chronoamperometry), UV-spectroelectrochemistry and by biosensors. Antioxidative capacity can be also evaluated by electrochemical techniques, as well by employing UV-Vis spectrophotometric methods using ABTS, FRAP, and DPPH (Medvidović-Kosanović et al., 2010).

In this study, cyclic and differential pulse voltammetry have been employed in order to study electrochemical properties of vitamin C in model systems. Developed method was applied for qualitative and quantitative investigation of vitamin C in real samples including commercial juices and food supplements, while antioxidative activity was assessed using DPPH radical scavenging assay.

## Materials and methods

### Materials

All chemicals were of reagent grade and were used without further purification. Standard solutions of L-ascorbic acid were prepared by diluting stock solution in water from MiliQ Millipore system (conductivity  $\leq 0.055 \mu\text{S/cm}$ ) and are further used for electrochemical measurements. Commercial fruit juices were analyzed as purchased, while instant orange powder drink was dissolved in pure water ( $\gamma = 5 \text{ g of powder/100 mL of water}$ ) and vitamin C supplement was also dissolved in pure water (1 tablet/100 mL of water). Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ) was purchased from Gram-mol (Zagreb, Croatia) and disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) was obtained from BDH Prolabo (Leuven, Belgium). Five samples were analyzed, sample 1 (home-made elderberry juice), sample 2 (instant orange powder

drink), sample 3 (commercial orange juice), sample 4 (vitamin C supplement) and sample 5 (commercial plum juice).

### Cyclic and Differential Pulse Voltammetry Measurements

Electrochemical experiments were performed in a three electrode cell on a PalmSens potentiostat/galvanostat (PalmSens BV, Utrecht, The Netherlands). Glassy carbon (geometrical area  $0.018 \text{ cm}^2$ ) was used as a working electrode, a platinum wire as a counter electrode and Ag/AgCl as a reference electrode. Before each measurement, the glassy carbon electrode was polished with  $\alpha\text{-Al}_2\text{O}_3$  ( $0.05 \mu\text{m}$ , ALS, Japan) and the system was purged with high purity argon, Ar5 ( $\phi_{\text{Ar}} = 99.999\%$ ). Sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ),  $c = 0.1 \text{ mol dm}^{-3}$  was used as electrolyte. Stock solution of vitamin C ( $c = 0.01 \text{ mol dm}^{-3}$ ) was prepared daily and diluted to specific concentrations for measurement purposes. Cyclic voltammetry scan rate varied from  $25 \text{ mV/s}$  to  $300 \text{ mV/s}$ . Conditions used for differential pulse voltammetry were: pulse amplitude  $25 \text{ mV}$ , scan increment  $5 \text{ mV}$ , scan rate  $5 \text{ mV/s}$  and pulse width  $70 \text{ ms}$ .

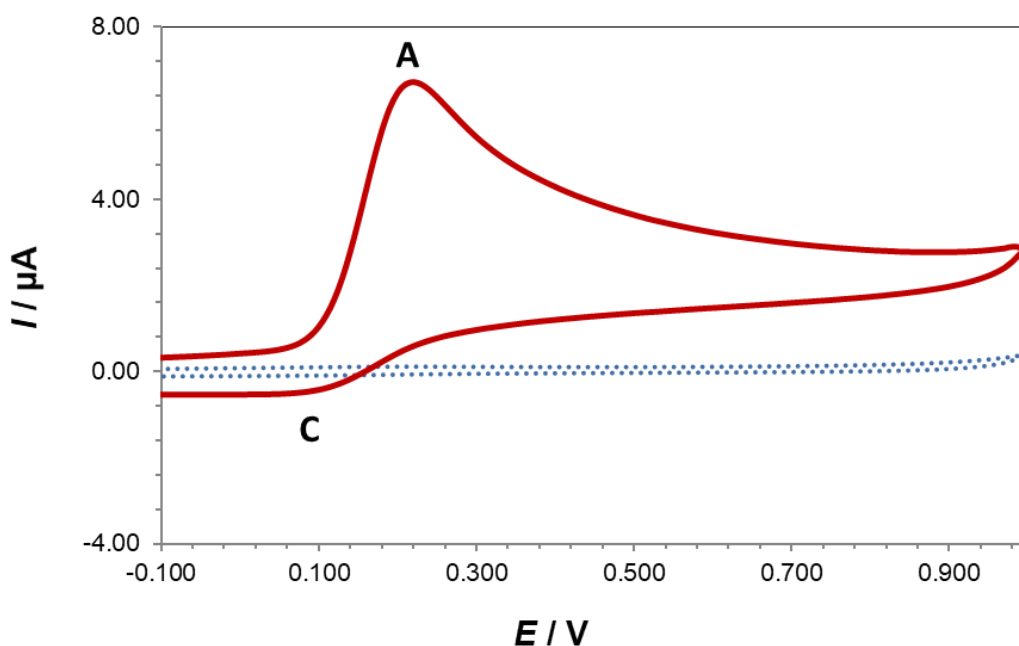
### DPPH Method (Brand-Williams Method)

Fresh DPPH ( $c(2,2\text{-diphenyl-1-picrylhydrazyl}) = 9.4 \times 10^{-5} \text{ mol dm}^{-3}$ ) solution was prepared in ethanol according to the Brand-Williams method (Brand-Williams et al., 1995). The reaction of radical scavenging is carried out by mixing the  $20 \mu\text{L}$  of vitamin C and  $980 \mu\text{L}$  of DPPH solution and samples were kept in dark covered with foil for 15 minutes. The UV-Vis measurements were performed using Shimadzu UV-2600 Spectrophotometer at  $\lambda_{\text{max}} = 515 \text{ nm}$ . The antioxidant activity of samples is evaluated by changes in colors from dark purple to pale yellow (Molyneux, 2004; Sharma and Bhat, 2009; Brand-Williams et al., 1995). The measurements were performed in triplicate and expressed as % scavenging activity (% DPPH).

## Results and discussion

### Cyclic voltammetry

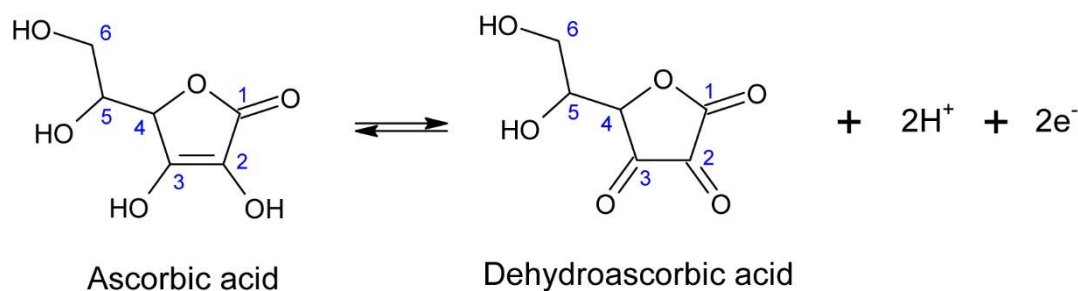
Electrochemical properties of L-ascorbic acid were first studied with cyclic voltammetry in a potential range from  $-0.1 \text{ V}$  to  $1.0 \text{ V}$  vs. Ag/AgCl reference electrode. In Fig. 1 cyclic voltammogram of L-ascorbic acid is shown.



**Fig. 1.** Cyclic voltammogram of blank solution (...) and vitamin C (—) ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{NaH}_2\text{PO}_4$  ( $I_c = 0.1 \text{ mol dm}^{-3}$ ) at scan rate 150 mV/s

One anodic peak (A) at the potential,  $E_{p,a} = 0.22 \text{ V}$ , which corresponds to the oxidation of ascorbic acid to dehydroascorbic acid and one cathodic peak (C) at the potential,  $E_{p,c} = 0.10 \text{ V}$  which correspond to reduction of dehydroascorbic acid to ascorbic acid can be

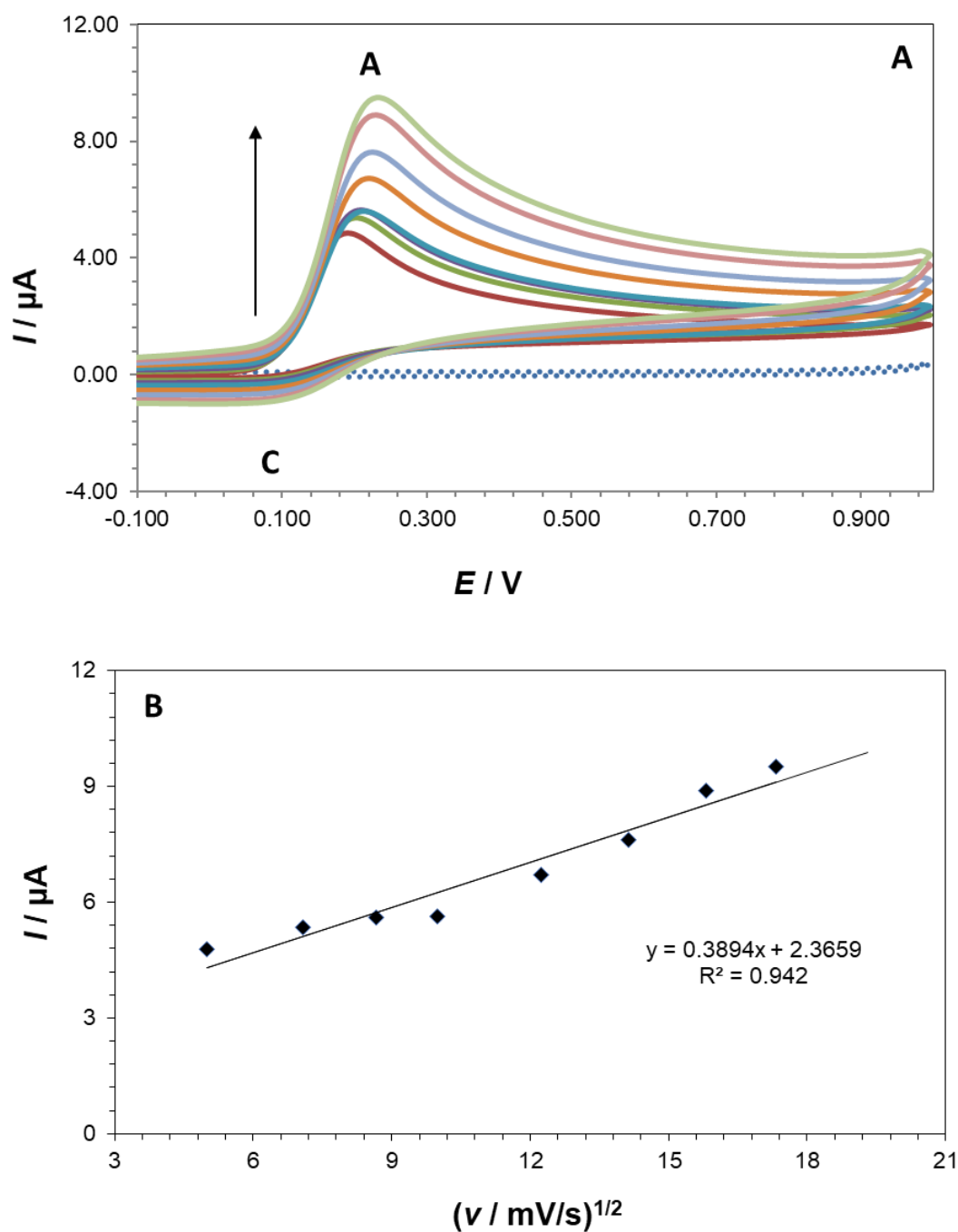
observed. Obtained  $\Delta E_p$  value was 120 mV which indicates quasi-reversible oxidation reaction. Oxidation mechanism of ascorbic acid, which includes transfer of 2 electrons and 2  $\text{H}^+$  ions, is shown in Fig. 2 (Ruiz et al., 1977; Phong et al., 2018).



**Fig. 2.** Oxidation mechanism of vitamin C (L-ascorbic acid)

The effect of scan rate on L-ascorbic acid oxidation was also studied. Cyclic voltammograms of L-ascorbic acid ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $I_c = 0.1 \text{ mol dm}^{-3} \text{ NaH}_2\text{PO}_4$ ) recorded at different scan rates ( $\nu = 25 - 300 \text{ mV/s}$ ) are shown (Fig. 3A). As shown, both anodic and cathodic peak currents increase with the increase of scan rate. Vitamin C

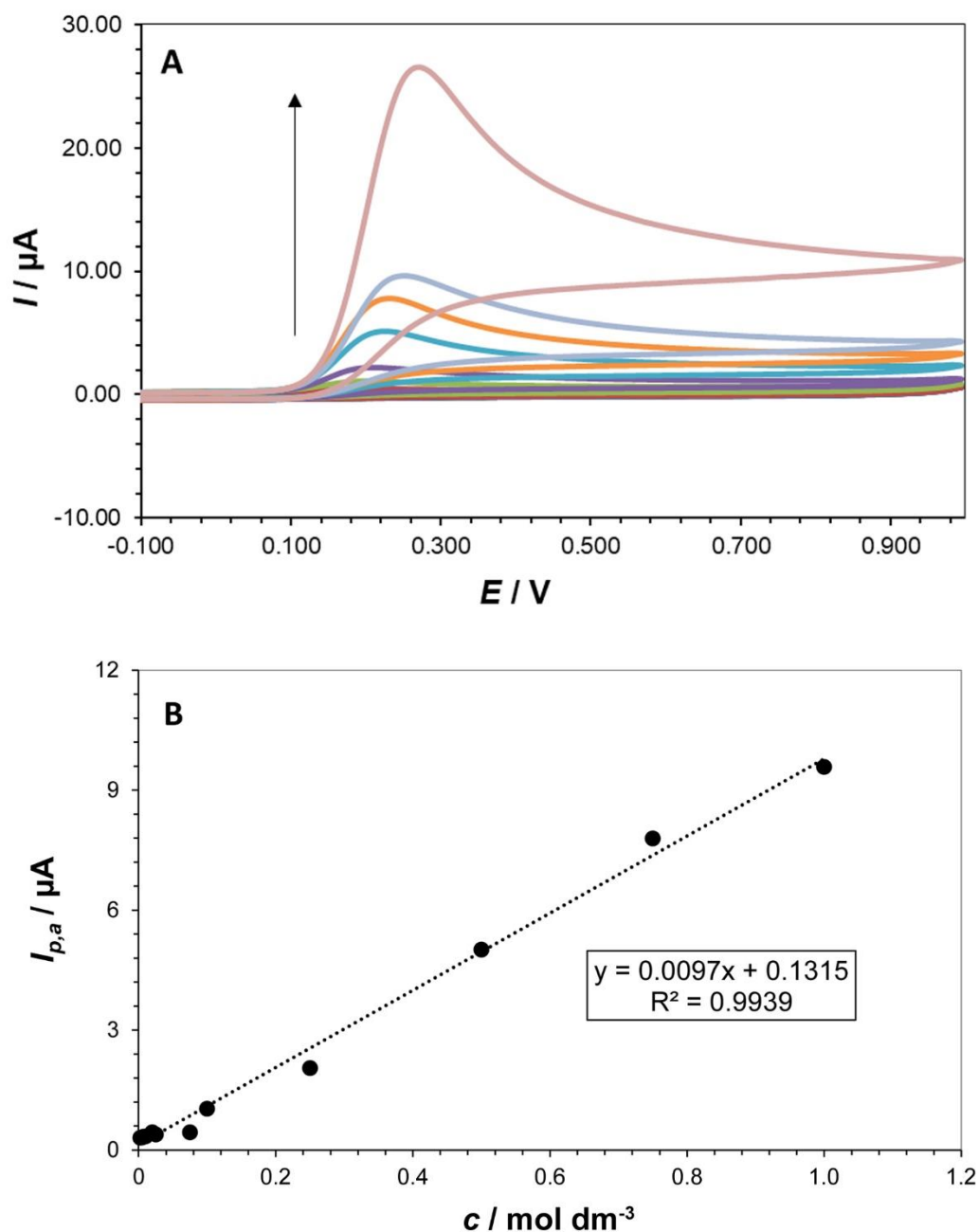
oxidation is diffusion-controlled process since linear correlation between anodic peak current and square root of scan rate was obtained (Fig. 3B). Linear correlation can be expressed with equation:  $I_{p,a} (\mu\text{A}) = 0.3894 \nu (\text{mV/s}) + 2.3659$  with  $R^2 = 0.942$ , where  $I_{p,a}$  is oxidation peak current and  $\nu$  scan rate.



**Fig. 3.** A) Cyclic voltammograms of blank solution (...) and vitamin C ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{NaH}_2\text{PO}_4$  ( $I_c = 0.1 \text{ mol dm}^{-3}$ ) recorded at different scan rates ( $v = 25 - 300 \text{ mV/s}$ ). B) Oxidation peak current,  $I_{p,a}$  as a function of square root of scan rate,  $v^{1/2}$

The effect of L-ascorbic acid concentration was also studied and it was determined that anodic peak current increases linearly with the increase of ascorbic acid concentration (Fig. 4A). Based on data obtained from cyclic voltammograms, calibration curve was constructed and the linear equation:  $I_{p,a} (\mu\text{A}) = 0.0097 c (\text{mol dm}^{-3}) + 0.1315$

with  $R^2 = 0.9939$  was obtained, where  $I_{p,a}$  is oxidation peak current and  $c$  designates L-ascorbic acid concentration (Fig. 4B). Linear response was obtained in a concentration range from  $0.0025 \text{ mol dm}^{-3}$  to  $1.0 \text{ mol dm}^{-3}$ .

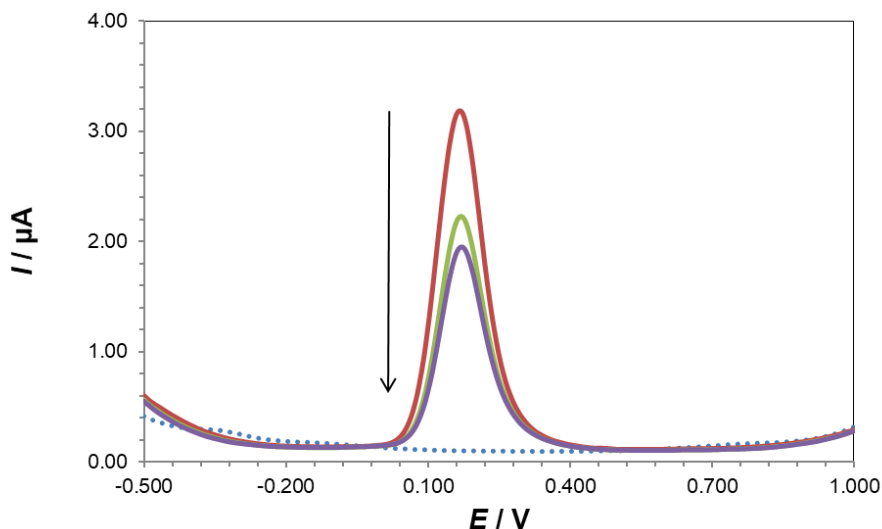


**Fig. 4.** A) Cyclic voltammograms of vitamin C ( $c = 0.0025 - 1.0 \text{ mol dm}^{-3}$ ) in  $\text{NaH}_2\text{PO}_4$  ( $I_c = 0.1 \text{ mol dm}^{-3}$ ) recorded at scan rate 150 mV/s. B) Calibration curve for determination of vitamin C

#### Differential Pulse Voltammetry

Differential pulse voltammograms of L-ascorbic acid ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ) recorded are shown in Fig. 5. One oxidation peak at the potential,  $E_{p,a} = 0.17 \text{ V}$ , corresponding to the oxidation of L-ascorbic acid can be observed. It is indicative that the oxidation peak current decreases with successive scans which shows that the oxidation product of L-ascorbic acid,

dehydroascorbic acid is adsorbed on the glassy carbon electrode surface.

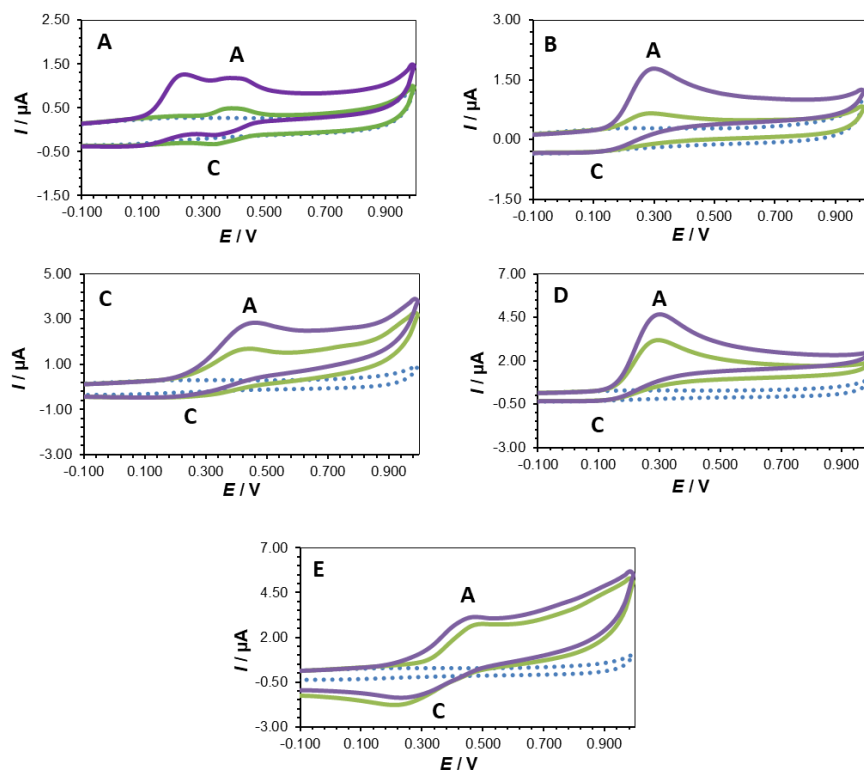


**Fig. 5.** Differential pulse voltammograms of blank solution (...) and vitamin C ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{NaH}_2\text{PO}_4$  ( $I_c = 0.1 \text{ mol dm}^{-3}$ ), scan rate 5 mV/s. 1<sup>st</sup> scan (—), 2<sup>nd</sup> scan (—) and 3<sup>rd</sup> scan (—)

*Analysis of real samples*  
*Cyclic voltammetry*

In Fig. 6 cyclic voltammograms of five investigated real samples are shown. In all investigated samples one oxidation peak (A) around the potential  $E_{pa} = 0.36 \text{ V}$  and one reduction peak (C) around the potential,  $E_{pc} = 0.22 \text{ V}$ , which corresponds to oxido-reduction processes of

vitamin C, were observed. Addition of  $0.15 \text{ μL}$  of  $0.01 \text{ mol dm}^{-3}$  L-ascorbic acid in each sample has confirmed that the vitamin C can be detected in each sample. It was determined that sample 4 (vitamin C supplement) has the highest ( $0.340 \pm 0.028 \text{ mol dm}^{-3}$ ) and sample 1 (home-made elderberry juice) the lowest ( $0.034 \pm 0.0020 \text{ mol dm}^{-3}$ ) vitamin C content among the investigated samples.



**Fig. 6.** Cyclic voltammograms of blank solution (...), vitamin C in real samples (—). Sample 1 (A), sample 2 (B), sample 3 (C), sample 4 (D), sample 5 (E) and with added  $0.15 \text{ μL}$  of vitamin C standard solution ( $c = 0.01 \text{ mol dm}^{-3}$ ) (—) in  $\text{NaH}_2\text{PO}_4$  ( $I_c = 0.1 \text{ mol dm}^{-3}$ ) recorded at scan rate 150 mV/s

In Fig. 7 concentrations of vitamin C in all investigated real samples detected with cyclic voltammetry are shown.

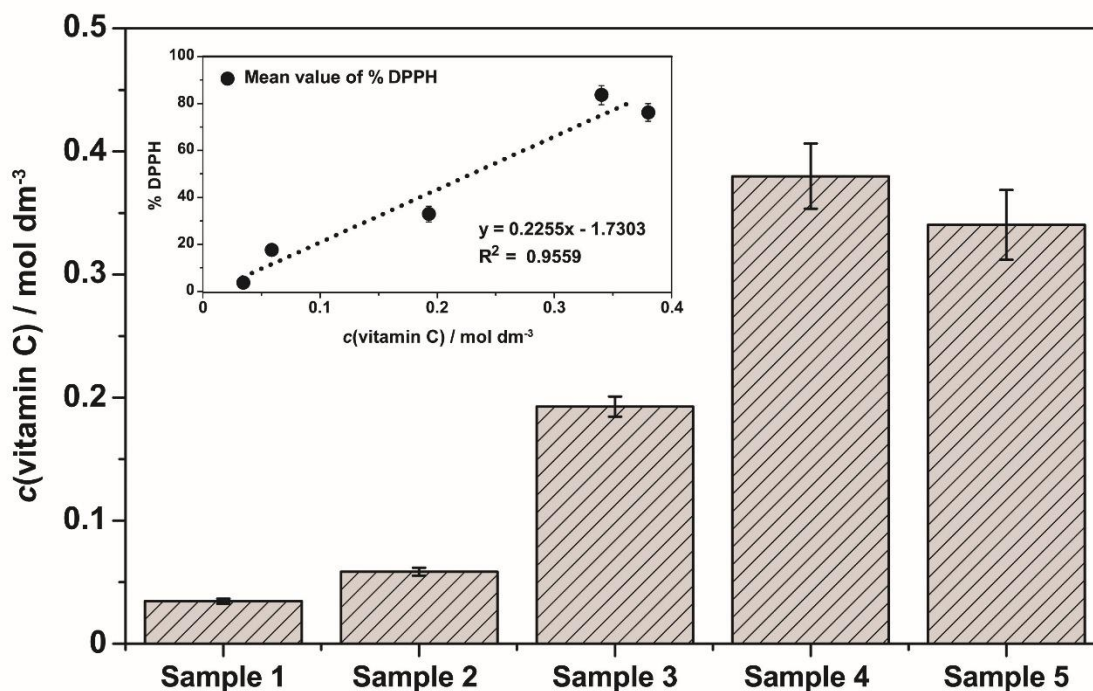


Fig. 7. Column graphs showing concentration of vitamin C in real samples determined with cyclic voltammetry (inset: correlation between % DPPH inhibition percentages and determined vitamin C concentrations)

#### Spectrophotometric Measurement – Radical Scavenging (DPPH<sup>•</sup>) Assay

Antioxidant activity of vitamin C in real samples was evaluated by DPPH assay. The results are shown in Fig. 7, and compared with the cyclic voltammetry results. At the lowest concentration of vitamin C determined by cyclic voltammetry ( $0.034 \pm 0.0020$  mol dm<sup>-3</sup>), the inhibition percentage of DPPH radical scavenging activity of vitamin C was the lowest as well as the standard deviation value ( $3.56 \pm 0.29$  %). The DPPH activity follows this linear trend, until reaches the highest concentration of vitamin C ( $0.340 \pm 0.028$  mol dm<sup>-3</sup>), where higher standard deviations were obtained. This variation in standard deviation values could be explained that due to high concentrations of vitamin C, the adsorption of vitamin C oxidation product to the electrode surface was more enhanced compared to samples with lower vitamin C concentration (Fig. 7). In addition, increased electrochemical signal in samples with higher vitamin C concentration, could be produced due to the presence of interfering substances from complex mixture consisting of multiple polyphenols, as it is the case in real samples. Moreover, the highest % DPPH activity obtained was at determined concentration of  $0.340 \pm 0.028$  mol dm<sup>-3</sup> and it was

estimated as  $83.54 \pm 4.03$  %. A good correlation with the results of two methods was observed with the obtained coefficient of correlation of  $R = 0.9770$  and corresponding coefficient of determination of  $R^2 = 0.9559$  and evident linear dependance of vitamin C content in samples with measured % DPPH activity.

#### Conclusions

The results of this study demonstrated that in all investigated systems reversible oxidation step of L-ascorbic acid to dehydroascorbic acid occurs, with high tendency of vitamin C adsorption to a glassy carbon electrode surface at higher concentrations. Oxidation was determined to be diffusion-controlled process as linear relation between anodic peak current and the square root of scan rate was observed. Adsorption of the ascorbic acid oxidation product (dehydroascorbic acid) on the glassy carbon electrode was confirmed with differential pulse voltammetry. Vitamin C was detected in model systems where linear response was obtained in a concentration range from  $0.0025$  mol dm<sup>-3</sup> to  $1.0$  mol dm<sup>-3</sup>. It was also successfully detected in real samples within concentration range from  $0.034 \pm 0.0020$  mol dm<sup>-3</sup> to  $0.340 \pm 0.028$  mol dm<sup>-3</sup>. DPPH assay results showed good correlation with the results obtained with cyclic

voltammetry, as % DPPH inhibition values were dependent on the detected concentrations of analyzed vitamin C.

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