SCREEN-PRINTED ELECTRODES AS "LAB-ON-A-CHIP" MONITORING TOOLS FOR ANTIOXIDANT CAPACITY OF FOODS

ELECTROZI SERIGRAFIAŢI CA INSTRUMENTE DE MONITORIZARE "LABORATOR-INTR-UN-CIP" PENTRU CAPACITATEA ANTIOXIDANTĂ A ALIMENTELOR

TRINCĂ Lucia Carmen[1*], TROFIN Alina[1], COZMA Andreea Paula[1], UNGUREANU Elena[1], ARITON Mirela[2]

[*]Corresponding author, e-mail: lctrinca@uaiasi.ro

Abstract. *The necessity for fast, accessible and reliable information in a complex-connected world generated in the last 40 years an impressive development of the "lab-on-a-chip" type technology based on miniaturized devices able to integrate in a single chip, one or several analyses. The areas of application for such type of devices are various, ranging from clinical diagnostic to evaluations of food substrates or environmental monitoring. The capabilities of "lab-on-a-chip" can be extended beyond the quasi-limited classical monitoring, thus providing valuable chemical and biological information that can be digitized by using centralized/decentralized facilities for data storage, remotely, thus being more easily available to users. The "lab-on-a-chip" materials and manufacturing technologies present net advantages (low cost, high parallelization, ease of use and compactness, reduction of human error, faster response time and diagnosis, low volume samples) but also and some limitations (miniaturization increases the signal-to-noise ratio so that most "lab-on-a-chip" technologies are not yet ready for industrialization, the mandatory external control system expands the final size and costs, the widespread accessibility can generate erroneous fears for an untrained public).*
Key words: lab-on-a-chip, screen printed electrodes, antioxidant capacity, foods

Rezumat. *Necesitatea obţinerii de informaţii rapide, accesibile şi fiabile într-o lume complex-conectată a generat în ultimii 40 de ani dezvoltarea impresionantă a tehnologiei de tip „laborator-intr-un-cip" bazată pe dispozitive miniaturizate care integrează într-un singur cip, una sau mai multe analize. Domeniile de aplicare pentru astfel de dispozitive sunt diverse, de la diagnostic clinic la evaluări ale substraturilor alimentare sau monitorizarea mediului. Capacităţilede tip "laborator-intr-un-cip" pot fi extinse dincolo de monitorizarea cvasi-limitată clasică, oferind astfel informaţii chimice şi biologice valoroase care pot fi digitalizate şi puse la dispoziţia utilizatorilor prin facilităţi centralizate/descentralizate pentru stocarea datelor, de la distanţă. Materialele şi tehnologiile de fabricaţie pentru sistemul de tip "laborator-într-un-cip" prezintă avantaje nete (cost redus, uşurinţă în utilizare*

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
[2] Research and Development Center for Cattle Breeding Dancu, Iasi, Romania

şi compactitate, reducerea erorilor umane, timp de răspuns şi diagnostic rapid, volum redus de probă) dar şi unele dezavantaje (miniaturizarea creşte raportul semnal-zgomot, astfel încât majoritatea tehnologiilor „laborator-într-un-cip" nu sunt pregătite pentru industrializare, sistemul de control extern obligatoriu măreşte dimensiunea şi costurile finale, accesibilitatea pe scară largă poate genera temeri eronate unui public neantrenat). Lucrarea prezintă rezultatele cercetării laboratorului nostru prin utilizarea de electrozi serigrafiți ca instrumente eficiente de tip „laborator-într-un-cip" de monitorizare a capacității antioxidante. Cuantificarea capacității antioxidante cu electrozi serigrafiați prin voltametria diferențială pulsatilă comparativ cu evaluarea spectrofotometrică a reacției CUPRAC cu acidul 6-hidroxi-2,5,7,8-tetrametilchroman-2-carboxilic s-a dovedit a fi o abordare promițătoare pentru caracterizarea capacității antioxidante a substraturilor alimentare datorită răspunsului rapid, rentabilității şi simplității de operare.

Cuvinte cheie: laborator-într-un-cip, electrozi serigrafiați, capacitate antioxidantă, alimente

INTRODUCTION

The necessity for fast, accessible and reliable information in a complex-connected world generated in the last 40 years an impressive development of the "lab-on-a-chip" type technology based on miniaturized devices that integrates in a single chip, one or several analyses (Dincer *et al.,* 2019).

The areas of application for such type of devices are various ranging from clinical diagnostic to evaluations of food substrates or environmental monitoring (Tu *et al.,* 2019). The capabilities of "lab-on-a-chip" can be extended beyond the quasi-limited classical monitoring, thus providing valuable chemical and biological information that can be digitized and made available to users and centralized/decentralized facilities for data storage, remotely.

At the present moment "lab-on-a-chip" materials and manufacturing technologies presents net advantages (low cost, high parallelization, ease of use and compactness, reduction of human error, faster response time and diagnosis, low volume samples) but also some limitations (miniaturization increases the signal-to-noise ratio so that most "lab-on-a-chip" technologies are not yet ready for industrialization, the mandatory external control system increases the final size and costs, the widespread accessibility can generate erroneous fears for an untrained public).

Antioxidant capacity defines the body's ability to counteract the negative effects of oxidative processes through the own pool of antioxidant compounds able to neutralize various reactive oxygen or nitrogen species or other free radicals or unstable molecules. Antioxidant compounds include various enzymatic and non-enzymatic substrates, such as glutathione, coenzyme Q, vitamins C, A, and E, flavonoids, carotenoids, theine, usually found in fruits, vegetables, wine, meat, eggs, and other vegetal or animal products, or in food supplements. Standardization of an appropriate method to evaluate antioxidant capacity remains

a strong challenge both for fundamental and applicative research as well as for industry.

Actually, different colorimetric methods may be used to estimate antioxidant capacity based either on 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay, and ferric-reducing antioxidant power (FRAP) assay, as well as, Folin-Ciocalteu colorimetric method (measuring total phenol content), methods based on reactions with hydrogen transfer (measuring absorbance of the oxygen radical capacity, or methods based on reactions transferring electrons (measuring antioxidant capacity as Trolox equivalents -TEAC). These methods are rather complex and time-consuming and use high-price chemicals or test kits. Also, another serious limitation is the absorbing interference (of the main biochemical compound from the biological samples) at the same wavelength as the main reagent, generating many difficulties to precision and accuracy of the results (Apak *et al.*, 2016).

Complementary assays based on electrochemical methods were developed in the last decade allowing direct evaluations of the antioxidants based on their net electric charge measurement, by assuming the mandatary dilution of the real samples, thus minimizing the signal to-noise ratio due to unwanted contribution to the background current as the main impediment. Also, most biological compounds are readily oxidized at relatively low potential on platinum, whereas, the oxidation potential for the glassy carbon electrode is higher, thus enabling an undesirable co-oxidation. Moreover, precaution should be taken when selecting the dilution ratio to avoid the nonlinear response. Another impediment consists in the possible strong adsorption of the reduced / oxidized species on the surface of many classical electrode types, such as graphite or carbon paste electrodes.

Screen-printed electrodes (SPEs) are novel devices developed in the last five years, fact explaining why the electrochemical approaches using microsensors are less explored. Electroanalytical methods, using SPEs, present many advantages such as speed, low cost, simplicity, and low consumption of reagents when compared to other methods (Wang *et al.,* 2014).

This paper presents our laboratory initial research by using screen printed electrodes as lab-on-a-chip efficient monitoring tools for antioxidant capacity. Quantification of antioxidant capacity by using screen-printed electrodes was based on differential pulse voltammetry as compared with spectrophotometric measurement of the CUPRAC reaction with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid and was proven to be a promising approach (Ozyurek *et al.,* 2011, Cardenas *et al.,* 2014) for the characterization of antioxidant capacity of food samples due to the rapid response, cost-effectiveness and simplicity of operation.

MATERIALS AND METHOD

A buffer solution of ammonium acetate (1.2 mol/L), as well as an aqueous solution of copper chloride (0.012 mol/L) was prepared with deionized water. The solutions of neocuproine (0.001 mol/L) and Trolox (0.001 mol/L) were prepared as alcoholic solutions. The standards for the calibration curve were prepared with 0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively, of the 0.001 mol/L trolox solution, diluted with deionized water up to 4 mL, then completed to a total of 10 mL total mixture with 2 mL solution of ammonium acetate buffer (1.2 mol/L), 2 mL solution of copper chloride (0.012 mol/L), and 2 mL solution of neocuproine (0.001 mol/L). After 30 minutes, the absorbance of each Trolox standard versus blank was measured at 450 nm wavelength.

The absorbance measurements were recorded with a T90 + UV/VIS Spectrometer (PG Instruments Ltd.). The spectrophotometric measurements were recorded at room temperature (21 ± 1°C), by using quartz cells of a 1 cm optic path to measure the absorbance of the sample versus blank at 450 nm wavelength. For the spectrum recording, each Trolox standard and sample were tested separately using a one-cell measurement at the abovementioned same spectrophotometer.

Electrochemical experiments were performed with a potentiostat/galvanostat/EIS analyzer Palmsense 4® integrated with the PS Trace 5® software, Version 5.3.1127 Build 198586t. A Palm Spe-holder assured connection with the BVT-AC1.W4 R1 (BVT Technologies) screen-printed electrodes (of 7 by 25 mm dimensions and ceramic alumina as support material) with carbon as the working and counter electrode (Ø of the disc WE =1 mm, WE geometric area = 0.79 mm^2) and Ag/AgCl as the reference electrode.

The experimental conditions for cyclic voltammetry versus open-circuit potential (CV vs OCP) were as follows: t equilibration= 1 s, E begin = 0 V, Evertex1 = 0 V, Evertex2=1 V, Estep = 0.05 V, scan rate = 0.05 V/s, number of scans = 1, E begin vs OCP (t max. OCP =1 s, stability criterion = 1.0 m·Vs−1). For DPV, the pretreatment settings E condition = −0.6 V, t condition = 0 s, E deposition = −0.5 V, and t deposition = 0 s. DPV settings: t equilibration = 1 s, E begin = 0 V, Eend = 1 V, Estep = 0.005 V (Epulse = 0.0025 V, t pulse = 0.05 s), and scan rate = 0.025 Vs−1. For both CV vs OCP and DPV, the corresponding current of the oxidation peak was used to quantify the concentration of $[Cu(Nc)_2]^{1+}$. CV vs OCP curves have been modified by multiplying (30 times) and very high smoothing (25 times). DPV curves were processed through baseline subtraction, respectively, and linear baseline followed by moving average baseline (windows size =2 points; max. number of sweeps = 1001).

RESULTS AND DISCUSSIONS

The practical objective of the present work was focused in testing a screen-printed electrochemical sensor (with carbon as the working and counter electrode), as a lab-on-a-chip tool for the investigation of the antioxidant capacity. An important advantage of the SPEs with carbon as the working and counter electrode consists in the thin layers of carbon-dispersed particles acting as main components of working electrode (WE) and auxiliary electrode (AE) able to oxidize electroactive species at low potential thus minimizing the background current and favouring the signal-to-noise ratio. Also, the working technique based

on electrochemical CUPRAC method was adapted to minimize the adsorption process of reduced/oxidized species on the surface of electrodes.

The CUPRAC method involved the reduction of the copper-neocuproine complex $[Cu(Nc)_2]^{2+}$ by an antioxidant (AOX) in the presence of ammonium acetate to form a yellow compound, copper-neocuproine complex $[Cu(Nc)_2]^{1+}$, (with a maximum absorption at $\lambda = 450$ nm).

The recorded spectrum between the 400-500 nm wavelength for the Trolox standard series is showing the increase of the absorbance with the Trolox concentration (fig. 1a) thus allowing generation of the calibration curve (fig. 1b).

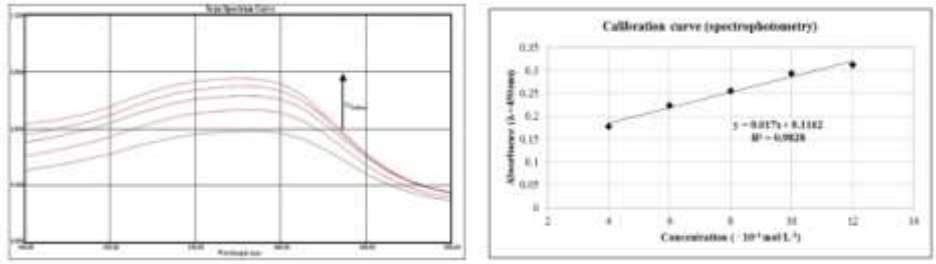

Fig. 1 a) Spectrum of the Trolox standards (4, 6, 8, 10, 12 x 10^{-4} mol·L^{-1}, b) Absorbance = f(concentration) calibration curve

The voltammograms obtained through cyclic voltammetry versus open circuit potential for the Trolox standards were processed by multiplying and smoothing with PSTrace software. CV vs OCP voltammograms of cyclic voltammetry versus open circuit potential tests (fig. 2a) showed that Trolox addition determined the shifting of the open circuit potential towards more negative values, results that may be explained using Nernst Equation as a consequence of the increasing ratio between main system's reducing $[Cu(Nc)_2]^{1+}$ and oxidant $[Cu(Nc)_2]^{2+}$ species. Trolox addition induced an oxidation peak (without a previous reduction process), as once the antioxidant is added, the generated $[Cu(Nc)_2]^{1+}$ species will be electrochemically oxidized and the oxidation current will be registered. Consequently, the oxidation peak current is expected to be proportional with the amount of added Trolox, as well as the measured current values corresponding to the oxidation peaks to generate CV vs OCP standard calibration curve.

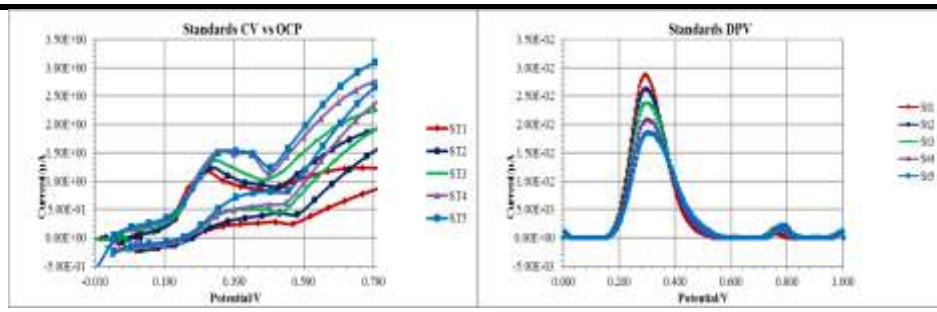

Fig. 2 Voltammograms of the Trolox standards (4, 6, 8, 10, 12 x 10^{-4} mol·L^{-1}) a) by cyclic voltammetry versus OCP b) by differential pulse voltammetry

The voltammograms of the Trolox standards obtained through differential pulse voltammetry (fig. 2b) were processed for the baseline adjustment. The maximum registered values for the current expressed in μA were considered to generate the standard calibration curve. Correlations of CV vs OCP and DPV results showed that once the antioxidant capacity determined from DPV increases, the experimental data obtained from CV vs OCP results tend to a plateau, highlighting that CV vs OCP does not discriminate against blocking of the electrode reaction by the adsorbed species. Thus, the DPV technique gaved a better response in solution since the detection of faradaic current was discriminated against the effects that are constant before and after pulse application (Rebelo *et al*., 2013). Also, a weaker correlation was registered between cyclic voltammetry vs. OCP and spectrophotometry (fig. 3a) when compared with the one between differential pulse voltammetry and spectrophotometry results (fig. 3b).

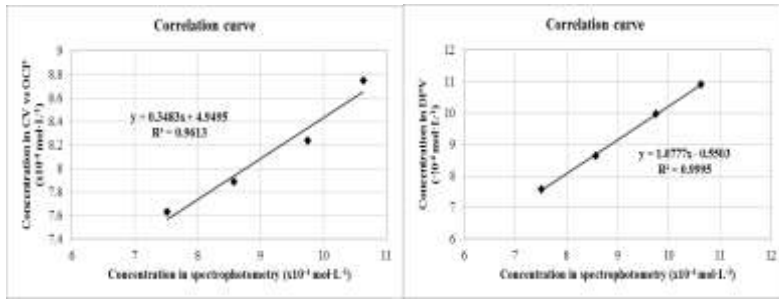

Fig. 3 Correlation curve between the results of a) cyclic voltammetry (vs. open circuit potential) and spectrophotometry b) differential pulse voltammetry and spectrophotometry

The practical application was focused on the analysis of the antioxidant capacity of various black and green tea infusion samples. Five of each black (B1–B5) and green (G1–G5) tea samples of different brands were purchased from the commercial network. The infusions were made by adding 0.5 g of packed tea into 10 ml of deionized water at 95°C and maintained there for 5 minutes. After removing the satchel, the resulting infusion was let to cool down at room temperature. The 10 ml final volume CUPRAC samples contained 4 ml infusion, 2 ml buffer, 2 ml $CuCl_2$ and 2 ml Neocuproine solutions.

The spectrophotometric and electrometric measurements of the tea samples were conducted exactly 30 minutes after preparation and the sample's concentrations were calculated by using calibration curve. The antioxidant capacity of the infused tea samples ranged from $7.5 \cdot 10^{-4}$ mol/l and $10.6 \cdot 10^{-4}$ mol/l Trolox (Fig. 4), thus fitting into the normal domain of the variability reported by similar researches (Kilmartin *et al.,* 2003; Roginsky *et al.,* 2003, Apak *et al.,* 2006; Korotkova *et al.,* 2002) that mentioned variations conditioned by the characteristics of the tea type as well as by the type of extraction method.

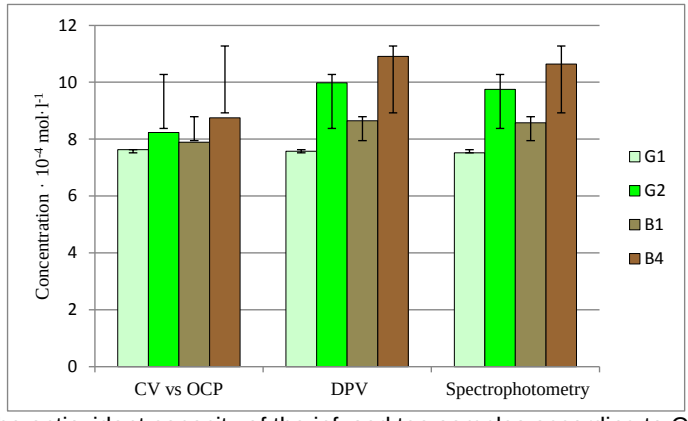

Fig. 4 The antioxidant capacity of the infused tea samples according to CUPRAC-Neocuproine spectrophotometric and electrometric methods

CONCLUSIONS

1. The analysis from the present study were employed to find an alternative to classical CUPRAC spectrophotometric method in order to quantify the antioxidant capacity by using voltametric technique with screen-printed electrodes.

2. The response of the screen-printed electrodes was linearly correlated with the sample's Trolox contents.

3. Analytical results of the antioxidant capacity (mol L^{-1} Trolox equivalents) showed a better agreement between the spectrophotometry and differential pulse voltammetry (R^2 >0.99) results as compared with cyclic voltammetry vs OCP (R^2 >0.96).

4. Work in progress is done in order to validate DPV with screen-printed electrodes based on CUPRAC-NEOCUPROINE reaction to evaluate tea's antioxidant capacity, according to ISO 17015:2017 requirements.

REFERENCES

1. **Apak R., Güclü K., Özyüreket M. Al., 2006** - *The Copper ion reducing antioxidant capacity and polyphenolic content of some herbal teas,* International Journal of Food Science and Nutrition,volume 57, pp. 292-304.
2. **Apak R., Özyürek M., Güçl K. ü, et. al., 2016** - *Antioxidant activity/capacity measurement. 1. classification, physicochemical principles, mechanisms, and electron transfer (et)-based assays,* Journal of Agricultural and Food Chemistry, volume 64, issue 19, pp. 997-1027
3. **Cárdenas A., Gómezand M., Frontana C., 2014 -** *Development of an electrochemical Copper reducing antioxidant capacity method (CUPRAC) for antioxidant analysis,* Electrochimica Acta, volume 128, 113-118, pp. 2014-2019.
5. **Dincer C., Bruch R., Costa-Rama E., Fernández-Abedul M.T., Merkoçi A., Manz A., Urban G.A., Güder F., 2019** - *Disposable sensors in diagnostics, food, and environmental monitoring*. Advanced Materials. 2019 Jul;31(30):1806739.
6. **Kilmartin P. A., Hsu C.F., 2003**- *Characterisation of polyphenols in green, oolong and black teas and in coffee, using cyclic voltammetry,* Food Chemistry, volume 82, issue 4, pp. 501-512.
7. **Korotkova E. I.,. Karbainov Y. A, Shevchuk A. V., 2002**- *Study of antioxidant properties by voltammetry,* Journal of Electroanalytical Chemistry, volume 518, pp. 56−60.
8. **Ozyurek M., Guclu K., Tutemet E. al., 2011** - *A comprehensive review of CUPRAC methodology,* Analytical Methods, volume 3, issue 11, pp. 2439–2453.
9. **Roginsky V., Barsukova T., Hsu C. F. et. al., 2003** - *Chain-breaking antioxidant activity and cyclic voltammetry characterization of polyphenols in a range of green, oolong, and black tea,* Journal of Agricultural and Food Chemistry, volume 51, issue 19, pp. 5798-5802.
10. **Tu J., Torrente-Rodríguez R.M., Wang M., Gao W., 2019** - *The Era of Digital Health: A Review of Portable and Wearable Affinity Biosensors*. Advanced Functional Materials. pp. 1906713.
11. **Wang Y., Wei X., Wang F. et. al., 2014 -** *Sensitive voltammetric detection of caffeine in tea and other beverages on a DNA-functionalized single-walled carbon nanotube modified glassy carbon electrode,* Analytical Methods, volume 6, issue 18, pp. 7525-7531