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Coupling of capillary electrophoresis with reaction detection for on-line determination of radical scavenging activity

Mantas Stankevičius,

Audrius Maruška*

Department of Biology,
Faculty of Natural Sciences,
Vytautas Magnus University,
Vileikos St. 8,
LT-44404 Kaunas, Lithuania

Chemical antioxidant activity assays can be classified into batch and separation-based antioxidant activity assays. Batch antioxidant activity assays measure the antioxidant compound ability to quench free radicals by a mixture of antioxidants. Batch methods are quantitative and do not provide information about individual compounds in a sample. Separation-based methods coupled with chemical antioxidant activity assays provide a possibility of the high resolution screening of individual antioxidants in the complex matrices. Capillary electrophoresis is a micro-method based on analyte separation according to the mass-to-charge ratio in a short time with high separation efficiency. Capillary electrophoresis coupled with the post-column radical scavenging reaction detection method can be applied for analysis of antioxidants and evaluation of their radical scavenging activity. In this study an interface for capillary electrophoresis coupling with radical scavenging detection was created using chromatographic components which are readily available in the laboratory. Two set-ups of the interface were tested. For the first set-up a ground electrode was inserted into the reagent vessel to close the electrical circuit before the reaction detector. In this case exclusively hydrostatic pressure was used for reaction mixture delivery through the reaction coil to the reaction detector. The second set-up was created grounding the outlet vial after the reaction detector. In this case high voltage and internal pressure was applied to the separation capillary and reaction coil for the separation of analytes and delivery of the reaction mixture to the reaction detector. For optimization of the reactants mixing ratio different values of hydrostatic pressure and high voltage were tested for both set-ups. The most effective mixing of the reactants is when the flow rate in the CE separation capillary is equal to the flow rate of the reagent solution into the reaction coil. Capillary electrophoresis coupled with radical scavenging reaction detection showed good linearity (R^2) (0.996 and 0.993) and repeatability (RSD 3.21 and 5.87%) for the CE detector and the reaction detector, respectively.

Keywords: capillary electrophoresis, reaction detection, 2,2-diphenyl-1-picrylhydrazyl, radical scavenging activity, polyphenolic compounds

INTRODUCTION

Changes in human dietary habits require new analytical methods and tools for prevention of health problems, which are related to oxidative stress. New requirements for higher nutritional value food products, food supplements and efficient pharmaceuticals increased the interest in natural antioxidants and their evaluation methods.

* Corresponding author. E-mail: a.maruska@gmf.vdu.lt

Antioxidant activity assays can be classified into biological and chemical assays [1]. Biological antioxidant activity assays involve biological systems of living cells and their constituents which naturally generate free radicals such as superoxide and hydrogen peroxide (O^{2-} or H_2O_2). Superoxide and hydrogen peroxide are naturally occurring inorganic products of normal aerobic metabolism in cells. The participation of transition metal ions, such as iron, converts poorly reactive oxygen molecules to highly reactive oxygen forms, which damage the cells. Most of cells have a natural antioxidant protection mechanism

against excessive production of these compounds [2]. Mitochondria are potent producers of cellular superoxide and mitochondrial superoxide production is a major cause of the cellular oxidative damage which may cause degenerative diseases and aging [3]. In the presence of flavonoids the superoxide formation in mitochondria is reduced. The flavonoid structure, particularly the 4-oxo, 5 position –OH group in the C ring influences the interaction with the mitochondrial membrane, decreasing its fluidity either inhibiting the respiratory chain of mitochondria or causing uncoupling [4]. It was shown that flavonoids are able to inhibit lipid peroxidation on the mitochondrial membrane [5]. The human hepatoma cell line is another object for antioxidant activity studies. The influence on human hepatoma cell line growth, cell viability and redox status when exposing to flavonoids, such as rutin, quercetin and apigenin, was demonstrated [6]. Nevertheless, biological antioxidant activity assays have some disadvantages. A special laboratory infrastructure, specific knowledge and experience are required. The mitochondrial superoxide generation depends on the metabolic state and varies considerably [7]. This causes additional dispersion of the results. Often biological assay data variation is ca. by one order higher compared to the chemical test results.

Chemical antioxidant activity assays can be classified into two main groups, i. e. (i) batch (non-separation based) and (ii) separation-based antioxidant activity assays. Batch assays measure the antioxidant ability to quench free radicals by a mixture of antioxidants. Such antioxidant or radical scavenging activity assays are widely spread and performed routinely in analytical laboratories. Non-separation based (i. e. batch) methods such as Trolox equivalent antioxidant capacity, methods utilizing synthetic 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) or 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical to evaluate antioxidant activity of a mixture of antioxidants are based on spectrophotometry for calibration of the assay and determination of data [8, 9]. Oxygen reduction antioxidant capacity and total radical-trapping antioxidant power methods are used for measurement of the inhibition of peroxyl radical by chain-breaking antioxidants based on a fluorescent probe and fluorescent spectroscopy [10–12]. Ferric reducing antioxidant power and copper reducing antioxidant capacity methods are similar to other antioxidant activity methods based on colorimetric measurements [13–15]. The latter methods are used to measure the ability of polyphenolic compounds to reduce an iron or copper complex into a colourful product. All the batch methods are quantitative and do not provide information about individual compounds in a sample. Noncomplex preparation of a synthetic free radical (or oxidant) solution, relative stability of the radical makes these methods attractive for coupling with separation methods and automated flow injection analysis methods. Separation based methods are highly selective and relatively robust. They provide information upon sample quality and quantity. Such techniques coupled with chemical antioxidant activity assays provide a possibility of the high resolution screening of individual antioxidants in the complex matrices. Terris van Beek and his research group (Koleva et al.; Dapkevicius et al.) introduced the coupled method employing high performance liquid chromatography (HPLC) with on-line post-column DPPH reaction detection for analysis of phenolic compounds and evaluation of their radical scavenging activity [16–17]. In order to carry out this analysis the unique chromatographic set-up with an additional reagent pump, a reaction detector and a reaction coil were employed.

Miniaturized separation techniques, such as capillary electrophoresis (CE), have an advantage over conventional chromatographic techniques for being more rapid and using minute amounts of solvents, materials and samples. Additionally, the sample preparation for the capillary electrophoresis is less complex compared to HPLC, since there is no stationary phase and no risk of contamination of chromatographic columns with the complex natural matrices. Capillary electrophoresis is a micro-method based on analyte separation according to the mass-to-charge ratio in a short time with high separation efficiency. Kaljurand et al. used capillary electrophoresis for the determination of DPPH free radical scavenging activity of phenolic compounds in a model mixture and plant extracts [18]. Analytes were separated prior and after the reaction with DPPH free radicals. The electropherograms were compared and the peaks of the analytes which disappeared after reaction with free radicals were identified as potential scavengers of DPPH radicals. This miniaturized method requires repeating the analysis of the phenolic compounds with free radicals before and after the "pre-column" reaction, doubling the number of analyses needed to evaluate the sample. In our previous study we proposed on-line radical scavenging activity determination coupling CE with reaction detection via the reagent addition interface utilizing HPLC cross connection or funnel type connection [19]. The capillaries of separation and a reaction coil were fixed coaxially in the cross or funnel forming a gap between them for the supply of a reagent solution. The major problem of those set-ups is a difficulty to form the same gap between the capillaries when the interface is mounted/demounted and obtain the reproducible reagent flow after that. To overcome this problem another more robust setup of the radical scavenging interfacing is needed. Therefore, the task of the present study was to build up and test a robust gap type interface for coupling CE with reaction detection utilizing material readily available in the analytical lab.

MATERIALS, METHODS AND APPARATUS

All the experiments were performed using a model CE 3D automated capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) with an on-capillary diode array detector (DAD) and an on-line coupled model Linear 200 variable UV/Vis wavelength reaction detector (RD) (Linear Instruments, USA). The apparatus automatically performed all steps of the analysis protocol including capillary conditioning, sample introduction, voltage and pressure applications and

detection. The analysis was performed using 50 μg/ml DPPH as a synthetic radical reagent dissolved in 7:3 (v/v) methanol: sodium acetate buffer (100 mM, pH 5.5). The separation was performed in a fused silica capillary (50 μm i. d., Polymicro Technologies, Phoenix, USA) with an effective length of 22.5 cm and a total length of 67.5 cm. Prior to use, the capillary was rinsed with a 0.1 M NaOH solution, water and separation buffer for 5 min every. As a background electrolyte 40 mM disodium tetraborate (pH 8.2) was used. High voltage of 25 kV was used for CE separation. Reagent was added through a perforated sleeve, which was connecting the separation capillary with the reaction coil capillary and was immersed into a reagent solution container. The reagent solution from the reagent container was driven into the reaction coil by setting 50 mbar internal pressure, generated by the CE apparatus. To ensure a gap between the capillaries, the end of separation capillary was polished on a rotating fine sand paper disk to form a 30–35° edge (Fig. 1). The open area of the split between capillaries let the solution to be transported into the reaction coil when both capillaries were fixed coaxially close to each other.

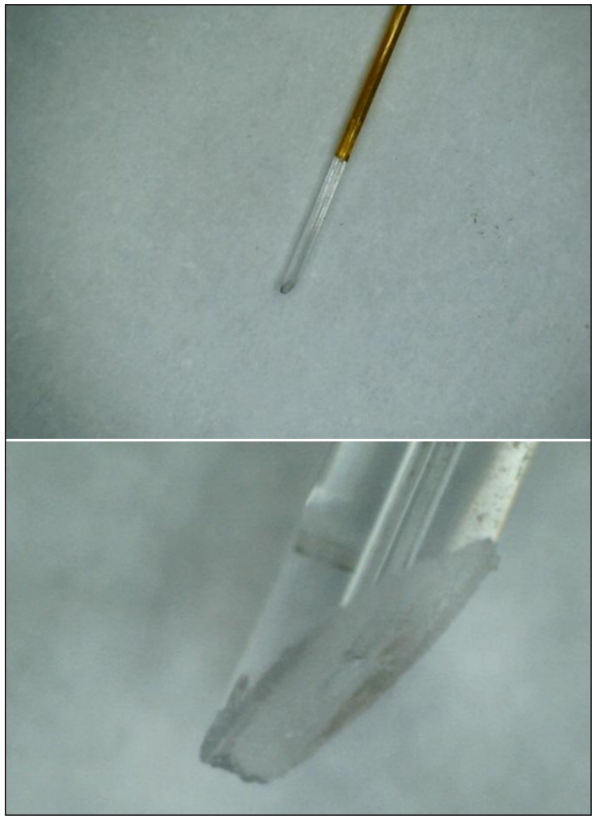

Fig. 1. Polished outlet of the separation capillary

EVALUATION OF SEPARATION PARAMETERS AND STATISTICAL EVALUATION OF THE DATA

Data calculation was made using MS Excel 2010 and OriginLab® v7.0. Each analysis was repeated three times and the relative standard deviation (%) for the rutin peak area was calculated. Limits of detection and limits of determination were calculated using the rutin standard when the signal to noise ratio was 2 and 4, respectively. A mixture of standard phenolic compounds was used, namely rutin, chlorogenic acid, trans-p-coumaric acid, ferulic acid and caffeic acid. Separation efficiency (N) and resolution (R_s) of the analyzed compounds was calculated according to standard equations. Linearity of the measurements was calculated using a calibration graph of rutin solution from 30 to 1000 μg/ml.

RESULTS AND DISCUSSION

A gap reaction interface was built for coupling the CE apparatus and the reaction detector. It was designed as a non-complex and economical interface. It was constructed using labware and chromatographic parts readily available in the lab (Fig. 2). Two capillaries, namely the separation capillary and the reaction coil capillary, were positioned coaxially in front of each other and a certain gap was formed. Positioning of two perpendicularly cut capillaries coaxially creates a gap of ca. 10 μm, since the cut is never ideal. This gap provides an unsealed area of ca. 1.57×10^{-9} m^2 if the i. d. of the capillaries is 50 μm. Sharpening of the end of one of the capillaries (ca. 30°) increases the area of the gap to 6.8×10^{-9} m^2 which is ca. 4.5 times bigger compared to the area of perpendicularly cut capillaries connected coaxially. The separation capillary was of 50 μm i. d. and the reaction coil was 75 μm i. d. A bigger diameter reaction coil compared to the separation capillary was selected in order to ensure a sufficient background electrolyte and reagent solution flow into the reaction coil. The cross section areas of the channel of 50 and 75 μm i. d. capillaries differ 2 times. The reservoir of the free radical reagent was a polypropylene 20 ml reservoir, pierced close to the bottom with a 7 cm long 0.5 mm i. d. polyetheretherketone (PEEK) tubing sleeve. The sleeve was

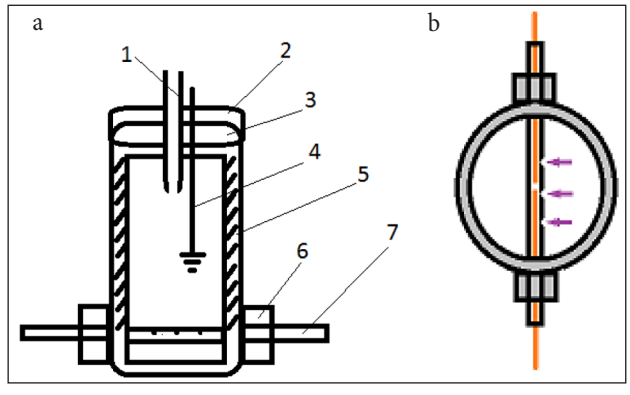

Fig. 2. A scheme of the gap interface reagent container used for coupling of capillary electrophoresis with reaction detection: (a) side view, (b) top view; (1) outlet of a Teflon tubing pressure line from the CE apparatus to the reagent container headspace, (2) screw cap, (3) Teflon coated silicon septum, (4) ground electrode, (5) reagent container (volume 15 ml), (6) fingertight nut screw, (7) polyetheretherketone capillary sleeve

fixed to the polypropylene vial walls at both sides with hot glue. The sleeve was perforated with three holes, so that reagent solution from the plastic container can penetrate it and reach the inserted fused silica capillaries. The inserted capillaries were fixed from outside of the plastic container using stainless steel nuts and finger tights at both ends of the sleeve. The internal pressure in the CE apparatus was set and supplied to the headspace of the reservoir of the reagent using an additional Teflon tubing line. This pressure was simultaneously applied to the inlet vial of the separation capillary and it forced the reagent solution to flow through PEEK sleeve holes together with effluent from the separation capillary into the reaction coil.

The system delivering reagent solution into the reaction coil plays an important role in capillary electrophoresis-reaction detection (CE-RD) analysis. Two solvent delivery modifications were applied to propel the reagent solution into the reaction interface in this study. The first interface set-up (I) was built with a ground electrode inserted into a reagent vessel to close the electrical circuit before the reaction detector. Another set-up (II) of the reaction interface was built closing the electric circuit after the reaction detector. In the first interface set-up (I) the reagent is delivered exclusively by hydrostatic pressure due to the height difference between the reagent vial and the outlet vial or by internal pressure generated by the CE apparatus. Using the second set-up (II) the reagent is delivered by pressure and additionally by the electroosmotic flow. The first designed CE-RD interface set-up (I) is presented in Fig. 3.

The siphoning effect based reagent transport was reached increasing the liquid level difference (Δh) at the reaction coil outlet reservoir (waste) and the reagent solution level in the regent container. To efficiently propel the reagent solution into the capillary the conditions were optimized. Increasing the height difference (Δh) the linear increase ($R^2 = 1$) of the flow rate was observed (Fig. 4). The maximum height of 39 cm possible for this particular set-up (I) resulted in a flow rate of 260 nl/min. At the height difference 12 cm, the flow rate of the reagent solution was 149.4 nl/min. At this flow rate the supply of the reagent into the reaction coil can be insufficient, since reacting antioxidants may deplete the radical leading to misinterpretation of the radical scavenging activity of the analyzed compounds. To avoid reagent depletion the sample can be diluted or the concentration of the DPPH solution can be increased prior to the separation.

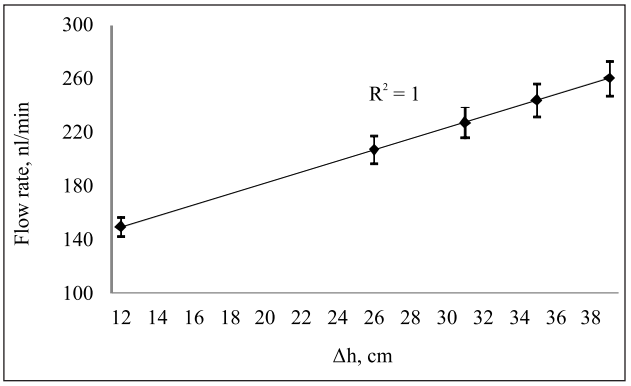

Fig. 4. The dependence of the flow rate in the reaction coil on the height difference (Δh) between the reaction interface reservoir and the outlet reservoir level using the first CE-RD interface set-up (I)

In the present interface set-up (I) the separation buffer is driven by several means, i. e. the internal pressure generated by the apparatus, which was set at 5kPa, and the electroosmotic flow generated by the voltage applied (15–30 kV). Such electrokinetic separation is commonly called pressure assisted separation. In order to obtain the best mixing quality of the effluent and the reagent solution, it was presumed that both should be supplied at the same flow rate. Different voltage values were tested. The voltage driven 40 mM working borate buffer (pH 8.2) flow rate was calculated by means of an electroosmosis marker, i. e. a neutral compound with UV absorbance (1% vol. acetone) (Fig. 5).

An increase of voltage increased the mobility of analytes and reduced the analysis time. In order to reach an equal flow rate for both the working buffer and the reagent solution, 20 kV voltage has been set. The measured flow rate was 266 nl/min and the reagent flow rate into the reaction coil generated by hydrostatic pressure was 260 nl/min, at the maximum height difference of the waste reservoir and the reagent reservoir set to 39 cm.

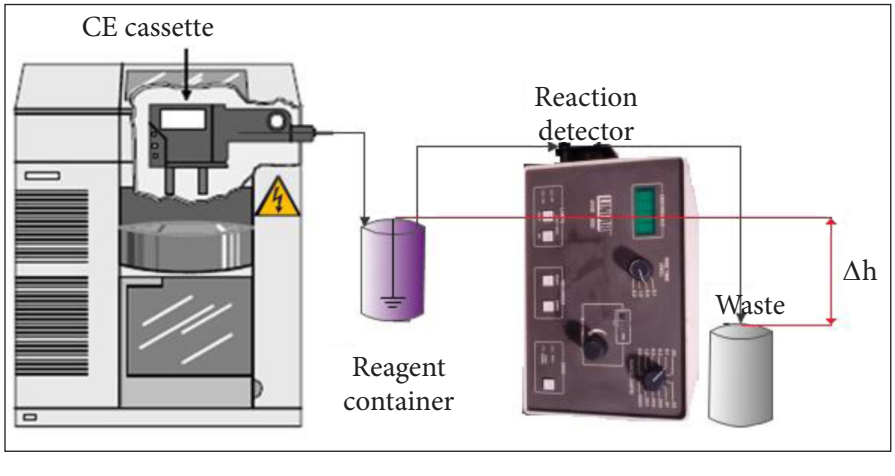

Fig. 3. Capillary electrophoresis with the reaction detection interface set-up (I). Reagent solution is driven by hydrostatic pressure

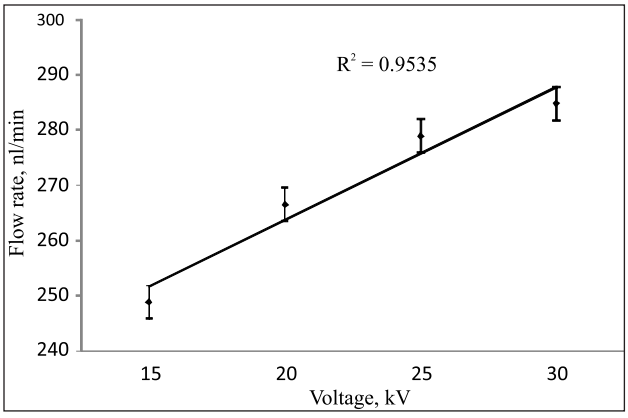

Fig. 5. The flow rate in the CE separation capillary dependence on the applied voltage. Interface set-up (I), inlet vial pressure 5 kPa, working buffer 40 mM borate, pH 8.2

The use of a funnel type reaction coil inlet (coaxial interface) was described in our previous work [19]. This set-up is not convenient in terms of mounting and demounting of capillaries and replenishment of the liquids. In this set-up the curved capillary additionally deforms a flow front and broadening of the peaks occurs.

The second modification of the CE-RD interface was set (II) in the present study, where the ground electrode was inserted into the outlet container closing the electric circuit after the reaction detector (Fig. 6). The gap interface was equipped with a manually operated pressure valve to allow switching pressure only on the inlet of the separation capillary or on both ends of the separation capillary. The injection was performed hydrodynamically, when the valve was switched to apply pressure only on the sample (inlet) vial.

To reduce the influence of different electrokinetic mobility of reaction products of separated compounds the length of the reaction coil was minimized. Additionally, an increased residence time of the analyte or the reaction product in the reaction coil causes a longitudinal diffusion of the concentration band, which is reflected in the decreased efficiency. On the other hand, it should be ensured that the reaction coil is long enough, so that radical scavenging reaction is completed before the reaction detection window. The cathode was installed in the outlet vial. Voltage in the reaction coil as an additional driving force of the reagent and the effluent helps to transport the reaction product to the reaction detector electrokinetically. It was determined that the length of the reaction coil decreases the efficiency of radical scavenging analysis and this influence can be minimized using a shorter piece of the reaction coil.

Analysis of a model mixture of five phenolic compounds was carried out using a 40 mM sodium tetraborate pH 8.2 (Fig. 7a) and 20 mM sodium dihydrogen phosphate buffer titrated with boric acid to pH 6.6 (Fig. 7b). Phenolic compounds are commonly analyzed in capillary electrophoresis using pH from 8 to 9 in order to ionize phenolic compounds and obtain higher resolution. However, in extremely acidic or extremely basic solutions the DPPH radical is highly reactive and unstable. Reproducibility of the results is higher at neutral or slightly acidic pH, therefore the phosphate buffer was selected for further experiments. In order to increase stability of the DPPH reagent solution pH 5.5 was selected. The close pH value of the separation buffer and the reagent solution increased robustness of the on-line radical scavenging measurement. As a result, the efficiency of separation in the CE capillary decreased from 77000 to 41000 m^{-1} for chlorogenic acid. The capacity of the reagent buffer was 3.5 times higher, so that mixing with the effluent would not change the reaction pH.

The flow rate of the working buffer and the reagent solution was measured at different voltage and CE apparatus internal pressure applied. The voltage was varied between 15 and 25 kV, while pressure was varied between 1 and 5 kPa. The results obtained for the separation capillary and the reaction coil are presented in Figs. 8 and 9, respectively.

As shown in Fig. 8, increasing the voltage, the flow rate of the working buffer (phosphate buffer 20 mM, pH 6.6) is increased. Based on the results obtained, the optimum conditions providing the maximum flow rate were selected for

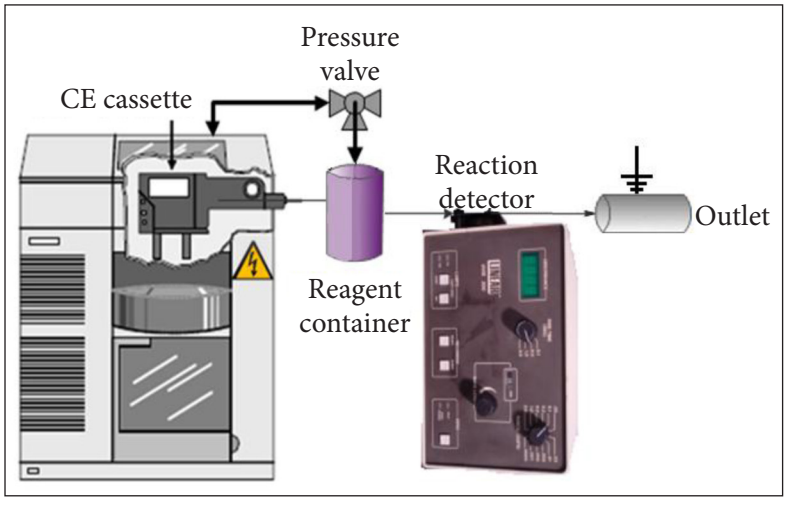

Fig. 6. CE-RD interface set-up (II) with pressure applied on the reagent solution container and the ground electrode inserted in the outlet container

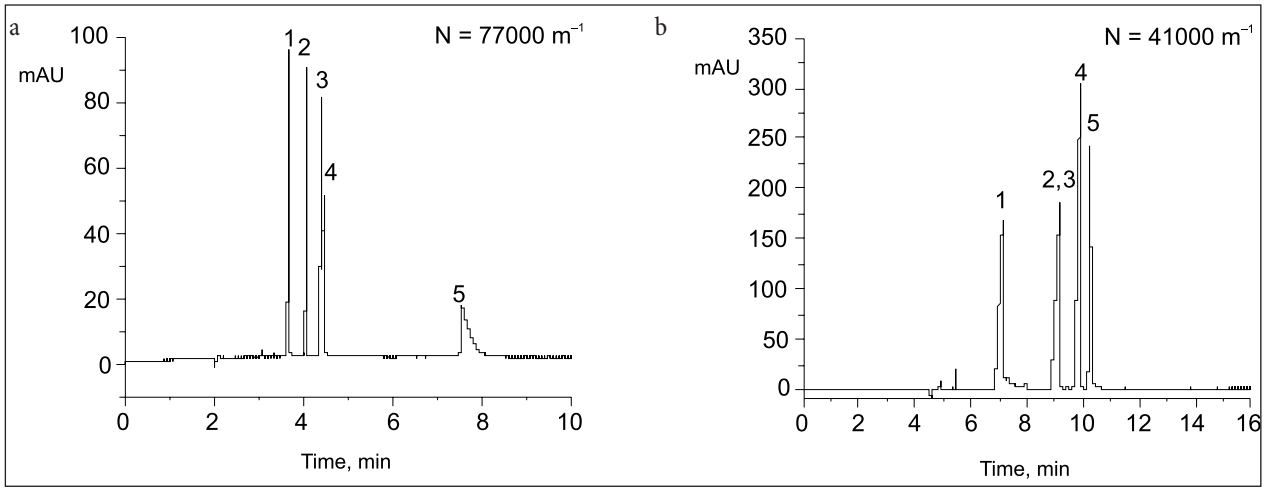

Fig. 7. Electropherogram registered with an on-capillary DAD detector (280 nm) using the CE-RD interface set-up (II) and different working buffers: (a) 40 mM sodium tetraborate, pH 8.2 and (b) 20 mM sodium dihydrogen phosphate buffer, pH 6.6. Voltage 20 kV. Sample mixture of five standard compounds: (1) rutin; (2) chlorogenic acid; (3) trans-p-coumaric acid; (4) ferulic acid; (5) caffeic acid, 1 mg/ml each, hydrodynamic injection 25 kPa * s

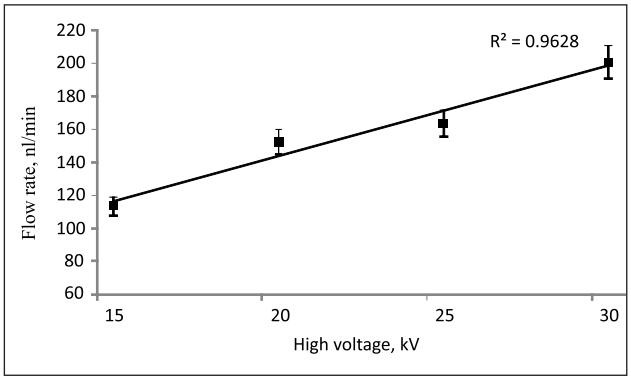

Fig. 8. The dependence of the flow rate in the CE separation capillary on the applied voltage using the CE-RD interface set-up (II). Conditions: 20 mM sodium dihydrogen phosphate working buffer, pH 6.6, CE-DAD detection at 280 nm

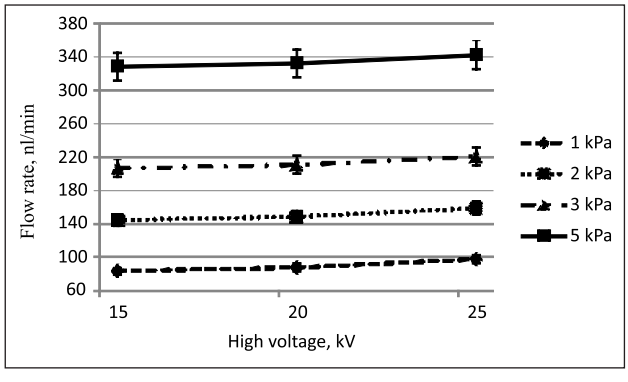

Fig. 9. Dependence of the flow rate in the reaction coil of the interface set-up (II) on the applied voltage and CE apparatus internal pressure. Working buffer, 20 mM sodium dihydrogen phosphate buffer, pH 6.6. UV detector 280 nm

further investigation: internal pressure of 5 kPa and 25 kV voltage. The maximum voltage generated by the CE apparatus (i. e. 30 kV) was not chosen for safety reasons, since the ground electrode is positioned after the reaction detector but the reagent reservoir was not ground (see Fig. 6). The electrophoretic separation process of the analytes and reaction products continues in the reaction coil. Somewhat different pH can change the selectivity. However, the reaction detector is selective only for the reagent bleaching (517 nm) occurring due to the radical reaction with radical scavengers. Therefore different mobility of the reaction products should not affect the final result. Reaction kinetics can somewhat reduce separation pattern efficiency for slow reacting compounds, since their zone is electrophoretically shifting relatively to a neutral DPPH reagent moving in the reaction coil [20]. In this particular interface set-up (II) the reaction detector monitors the signal of reagent absorbance, but not the signal of reaction products. Therefore, different mobility of analytes and reaction products in the reaction coil will not provide additional selectivity.

For optimization of the flow rate in the CE separation capillary and the reaction coil to obtain higher efficiency and resolution of phenolic compounds, instead of a free radical reagent solution the working buffer of 20 mM sodium dihydrogen phosphate (pH 6.6) was added to the reagent container. The CE-DAD detector and the reaction detector were set to 280 nm simultaneously. As shown in Fig. 9, the flow rate in the reaction coil is increasing when the internal pressure and voltage increase. For the best mixing of the effluent and reagent solution the flow rates from the separation capillary and the reagent solution container should be comparable. Capillary electrophoresis separation and reaction coil qualitative characteristics, such as resolution and efficiency, depend on hydrodynamic flow and electroosmotic flow generated by pressure and voltage.

In the reaction coil hydrodynamic pressure forms a parabolic laminar flow profile, therefore separation efficiency

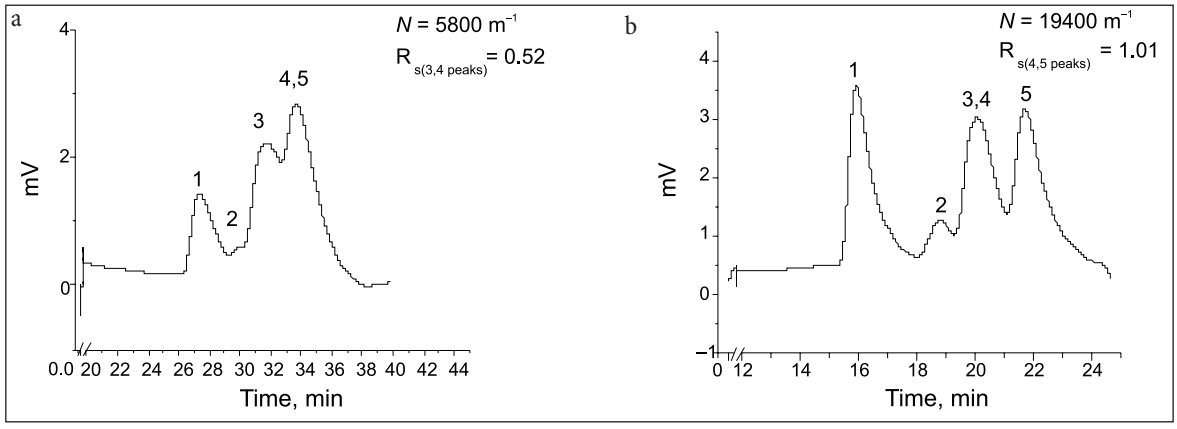

Fig. 10. The CE-RD interface set-up (II) reaction detector signal (280 nm) profile of polyphenols mixture. Internal pressure: (a) 3 kPa, and (b) 5 kPa. Mixture of standards: (1) rutin; (2) chlorogenic acid; (3) trans-p-coumaric acid; (4) ferulic acid; (5) caffeic acid, 1 mg/ml each, working buffer 20 mM sodium dihydrogen phosphate, pH 6.6, high voltage 5 kV

and resolution are decreased (Fig. 10a). An increase of pressure (from 3 to 5 kPa) decreases longitudinal diffusion in the capillary, therefore the peak width is decreased and the efficiency of separation is increased from 5800 to 19400 m^{-1} for ferulic acid (Fig. 10b).

The mixture of five standard polyphenolic compounds was analyzed with capillary electrophoresis – the DPPH free radical scavenging reaction detection interface set-up (II) (Fig. 11). Transportation of the analytes in the separation capillary was exclusively due to electroosmosis. Internal pressure was applied to the inlet and outlet of the CE separation capillary simultaneously. The reagent was delivered to the reaction coil by internal pressure applied to the headspace of the reagent container and additionally by the high voltage generated electroosmosis.

The biggest contribution to radical scavenging activity was from chlorogenic acid and caffeic acids; the smallest was from ferulic acid and trans-p-coumaric acids. This is related to the molecular mass and structure of antioxidant compounds and steric availability to a DPPH molecule. Ferulic acid is a small molecule (Mr = 194), in its structure it has one hydroxyl group and one methoxy group. Bigger molecules, such as rutin (Mr = 610) and chlorogenic acid (Mr = 354) with five hydroxyl groups in the molecule, are able to scavenge the DPPH radical more actively. Caffeic acid of a relatively low molecular mass (Mr = 180) contains two hydroxyl groups in a molecule, therefore can give a big contribution to DPPH radical scavenging activity.

Method validation was carried out for the CE-RD gap interface using an external standard method. The regression coefficient (R^2) for the CE diod array detector (DAD) and the reaction detector were 0.996 and 0.993, respectively. The relative standard deviation for the peak area in the separation capillary was 3.21% and in the reaction coil it was

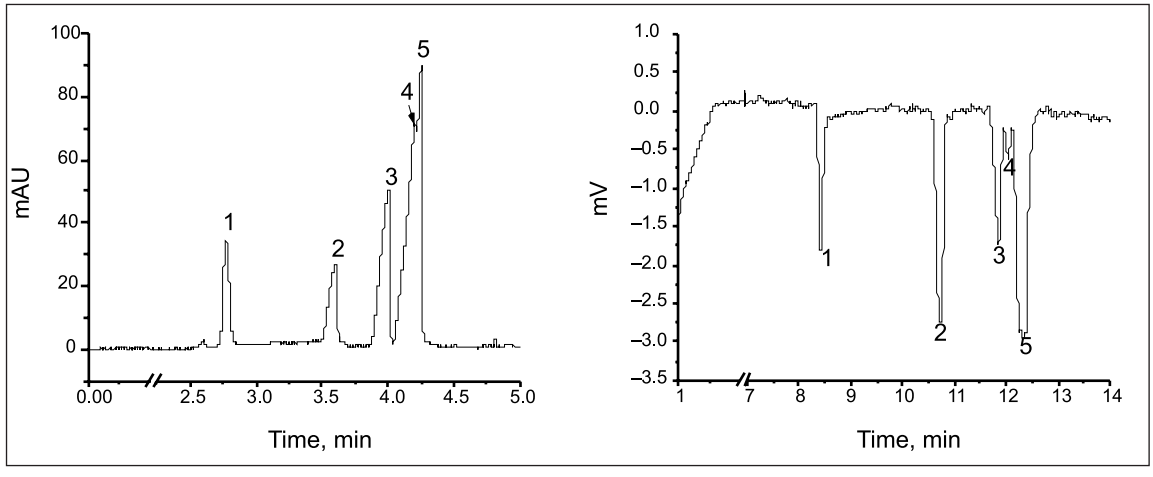

Fig. 11. CE-RD analysis using the interface set-up (II). Signals from both detectors: CE-DAD detector (a) 280 nm and reaction detector (b) 517 nm. Mixture of standards injected: (1) rutin; (2) chlorogenic acid; (3) trans-p-coumaric acid; (4) ferulic acid; (5) caffeic acid, 1 mg/ml each. Working buffer, 20 mM sodium hydrogen phosphate pH 6.6, reagent 0.1 mM DPPH dissolved in 100 mM sodium acetate pH 5.5 and methanol (7:3 v/v), voltage 25 kV, internal pressure 5 kPa, hydrodynamic injection 25 kPa * s

5.87%. Efficiency calculated from an electropherogram registered with the CE detector was from 14500 m^{-1} for chlorogenic acid to 61000 m^{-1} for ferulic acid and with the reaction detector from 13500 m^{-1} for ferulic acid to 48500 m^{-1} for rutin. Resolution (R_s) was calculated using a mixture of standard polyphenolic compounds. Resolution in the separation capillary between the 3rd and the 4th peak was 1.48 and for the reaction detector it was 1.22. Resolution between the 4th and the 5th peak for CE was 0.54 and for RD it was 0.93. The limits of detection were 7.3 and 26.3 μg/ml for CE-DAD and RD detectors, respectively. The limits of determination were 14.6 and 52.6 μg/ml.

CONCLUSIONS

1. A non-complex, robust and cost-effective interface for capillary electrophoresis coupling with radical scavenging detection was created using parts readily available in the laboratory. To ensure sufficient background electrolyte access, flow rate into the reaction coil and efficient mixing with the reagent solution smaller i. d. separation capillary compared to the reaction coil was used (50 and 75 μm i. d., respectively) and its outlet was sharpened.

2. Two set-ups of the interface were constructed and tested. In the first set-up (I) the ground electrode was inserted into the reagent vessel close to the electrical circuit before the reaction detector. In this case exclusively hydrostatic pressure was used for reaction mixture delivery through the reaction coil to the reaction detector. The second set-up (II) was created grounding the outlet vial after the reaction detector. In the set-up (II) high voltage applied to the reaction coil enchances the flow rate of the effluent and increases the efficiency of the reaction coil by forming a perpendicular flow profile instead of an elongated parabolic purely laminar flow profile.

3. For optimization of the reaction mixing ratio different values of hydrostatic pressure and high voltage were tested for set-ups (I) and (II). Set-up (II) showed to be more convenient for reagent delivery to the reaction coil. This set-up is less limited to the instrumental set-up.

4. Capillary electrophoresis coupled with radical scavenging reaction detection showed good linearity (R^2) (0.996 and 0.993) and repeatability (RSD 3.21 and 5.87%) for the CE detector and the reaction detector, respectively. Efficiency for the separation capillary was from 14500 m^{-1} for chlorogenic acid to 61000 m^{-1} for ferulic acid and for the reaction detector it was from 13500 m^{-1} for ferulic acid to 48500 m^{-1} for rutin. The limits of detection were 7.3 and 26.3 μg/ml for DAD and for RD detectors, respectively, using the rutin standard.

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Mantas Stankevičius, Audrius Maruška

KAPILIARINĖS ELEKTROFOREZĖS IR REAKCIJOS DETEKCIJOS SUJUNGIMAS NUSTATANT TIESIOGINĮ RADIKALŲ SURIŠIMO AKTYVUMĄ

Santrauka

Cheminiai antioksidacinio aktyvumo tyrimai gali būti grįsti skirstymo ir neskirstymo metodais. Neskirstymo metodais paremtais antioksidacinio aktyvumo tyrimais galima įvertinti antioksidantų gebėjimą mišinyje surišti laisvuosius radikalus. Šie metodai yra kiekybiniai ir nesuteikia informacijos apie atskirų komponentų antioksidacines savybes. Skirstymo metodai, sujungti su cheminiais antioksidacinio aktyvumo metodais, suteikia galimybę ištirti sudėtingose gamtinėse matricose esančių atskirų antioksidantų gebėjimą surišti laisvuosius radikalus ir pasižymi dideliu atrankumu. Kapiliarinė elektroforezė yra efektyvus mikrometodas, pagrįstas analičių atskyrimu pagal jų masės ir krūvio santykį per trumpą laiką. Kapiliarinės elektroforezės, sujungtos su laisvojo radikalo surišimo reakcijos detekcija, metodas gali būti panaudotas antioksidantų analizei ir radikalų surišimo aktyvumui įvertinti. Šio darbo metu sukurta kapiliarinės elektroforezės ir reakcijos detektoriaus sąsaja, panaudojus cheminės analizės laboratorijoje esančius įprastus chromatografinius komponentus. Sukurtos dvi sąsajos modifikacijos. Pirmojoje modifikacijoje (I) įžemintas elektrodas patalpintas reagento inde, uždarant elektros grandinę prieš reakcijos detektorių. Šiuo atveju tėkmė reakcijos kilpoje buvo dėl hidrostatinio slėgio, susidariusio dėl aukščio skirtumo reakcijos kilpos galuose. Antroji sąsajos modifikacija (II) sukurta įžeminus buferio indą, esantį už reakcijos detektoriaus. Tokiu atveju tėkmę generavo iš kapiliarinės elektroforezės aparato tiekiamas slėgis ir aukštos įtampos sukeltas elektroosmozinis srautas. Metodo sąlygoms optimizuoti siekiant efektyvaus darbinio elektrolito ir reagento sumaišymo išbandytos skirtingos slėgio ir įtampos reikšmės. Kapiliarinės elektroforezės su reakcijos detekcija metodas pademonstravo gerą tiesiškumą: kapiliarinės elektroforezės UV detekcijos ir reakcijos detekcijos regresijos koeficientas (R^2) buvo atitinkamai 0,996 ir 0,993. Metodo pakartojamumas išreikštas santykiniu standartiniu nuokrypiu kapiliarinei elektroforezei ir reakcijos detekcijai – atitinkamai 3,21 ir 5,87 %.