Potentiometric sensor system based on modified screen-printed electrodes for determining antioxidant activity

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Abstract. A potentiometric sensor system (PSS) was developed to assess antioxidant activity (AOA) using the potassium hexacyanoferrates ($[Fe(CN)_6]^{3-/4-}$) mediator system. The screen-printed indicator electrode was made of carbon ink modified with multi-walled carbon nanotubes (MWCNTs). The screen-printed silver electrode was potentiostatically modified with a silver chloride/ferricyanide precipitate and served as a reference electrode. The resulting PSS based on modified screen-printed electrodes was tested in the analysis of antioxidants and beverages. Beverage analysis results obtained using PSS and the Folin-Ciocalteu spectrophotometric method have a high positive correlation ($r = 0.97$, $p = 0.002$). The proposed PSS can be recommended for the analysis of pharmaceutical, cosmetic and clinical samples.

1 Introduction

Antioxidant activity/capacity (AOA/AOC) monitoring is widely used in healthcare, food, pharmaceutical and cosmetic technologies [1–4]. The need to standardize analytical methods for determining AOA/AOC was discussed in review [1]. To date, there is only one standard colorimetric method using the Folin-Ciocalteu reagent to determine the total polyphenol content in tea [5, 6]. However, the Folin-Ciocalteu reagent is capable of oxidizing compounds other than phenolic antioxidants [7, 8], so spectrophotometric analysis using this reagent can also be used to assess AOA/AOC [1, 2]. AOA and AOC were recommended to be used as kinetic and thermodynamic parameters, respectively [2]. In this work, we will use the term "antioxidant activity" (AOA) due to the following circumstances: 1) activity refers to the effective concentration of a substance; 2) activity is a parameter of the Nernst equation, which is used in potentiometry to calculate the redox potential.

Interest in electrochemical methods and sensors for assessing AOA is due to great achievements in miniaturization [4]. Compared to electroanalytical methods, electrochemical sensors demonstrate advantages in response time, detection limit, and analytical reproducibility. Electrochemical sensors for determining AOA are usually represented by modified carbon-containing electrodes. Souza et al. a modified carbon paste electrode made from graphite paste containing carbon nanotubes (CNTs) was used as a voltammetric sensor

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[9]. Nasri et al. developed a chronopotentiometric sensor based on a graphite rod electrode impregnated with paraffin, on the surface of which a redox component ($[Fe(CN)_6]^{4-}$) was immobilized in a matrix of a conducting polymer (poly-3,4-ethylenedioxythiophene) [10]. Ziyatdinova et al. carried out chronoamperometric determination of AOA using a glassy carbon electrode modified with multi-walled CNTs (MWCNTs) [11]. Raymundo-Pereira et al. developed a voltammetric sensor based on a glassy carbon electrode, which used a hybrid material consisting of Printex L6 nanocarbon and silver nanoparticles [12]. Tortolini et al. a screen-printed carbon electrode modified with a binary composite of MWCNTs and nanoceria particles was used as a voltammetric sensor [13]. David et al. used a screen-printed carbon electrode modified with gold nanoparticles for the voltammetric determination of AOA in the presence of hydrogen peroxide [14].

Brainina et al. proposed potentiometric sensor systems (PSSs) to evaluate AOA coupled with $[Fe(CN)_6]^{3-/4-}$, which used a new quasi-reference electrode with a stable potential in an environment with variable ferro/ferricyanide ion content [15–18]. The developed PSSs differed in the nature of the indicator electrode. Despite good analytical characteristics, the use of a screen-printed platinum electrode increased the cost of PSS and limited its one-time use [15, 16]. A screen-printed carbon electrode droplet-modified with gold nanoparticles was effective in assessing skin AOA using a microporous membrane [17], but its use in a stirred solution could serve to reduce the robustness of the analysis. A film electrode based on a carbon veil made it possible to obtain reliable results both in contact with a microporous membrane and in a stirred solution [18]. However, the manufacturing technology of this electrode (hot lamination) was different from the manufacturing technology of the reference electrode (screen printing), which made it difficult to integrate the two electrodes on a single substrate. In this work, we present an improved modification of PSS that uses a new indicator electrode, which is a screen-printed carbon electrode modified with MWCNTs. The effectiveness of the proposed PSS is demonstrated in the analysis of beverages, and the obtained results are compared with those of the standard Folin-Ciocalteu method.

2 Materials and Methods

Chemical reagents $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]\cdot 3H_2O$, KCl, NaCl, $Na_2HPO_4\cdot 12H_2O$, KH_2PO_4, $Na_2CO_3\cdot 10H_2O$ were supplied by Chemreaktivsnab JSC (Russia). L-ascorbic acid, L-glutathione reduced, (+)-catechin and quercetin were purchased from Sigma-Aldrich Co. (USA). L-cysteine hydrochloride, gallic acid monohydrate, Folin-Ciocalteu reagent 2 N and MWCNTs were obtained from Panreac Quimica S.A.U. (Spain), MP Biomedicals LLC (USA), Firm Syntacon LLC (Russia) and MST-Nano LLC (Russia), respectively. The 0.5 mm thick polyethylene terephthalate substrate was purchased from Fellowes Inc. (USA). Guangzhou Print Area Co., Ltd. (China) and Shenzhen Welsolo Electronic Technology Co., Ltd. (China) supplied Ceres carbon ink and Mechanic DJ912 silver ink. Deionized water obtained using an Akvalab-UVOI-MF-1812 unit (RPC Mediana-Filter JSC, Russia) was used as a solvent.

The PSS electrodes were fabricated using scalable screen printing technology. The indicator electrode was made of carbon ink containing 1 or 2 wt. % MWCNTs. The resulting electrodes were dried in an oven at 110 °C for 30 minutes. Since the electrode made with 2 wt. % MWCNTs showed a decrease in ductility, then an electrode made with 1 wt. % was subsequently used. MWCNTs. The reference electrode was fabricated according to the procedure described previously [15]. Briefly, silver ink was applied to a substrate and dried in an oven at 110 ºC for 30 minutes. The resulting electrode was immersed in stirred phosphate buffered saline (PBS) pH 7.4, containing 10 mM $K_3[Fe(CN)_6]$ and 0.1 mM $K_4[Fe(CN)_6]$, and polarized at a constant potential of 0.325 V (vs. Ag/AgCl, 3.5 M KCl) for 120 seconds. Under these conditions, a deposit consisting of silver chloride and silver

ferricyanide was formed on the surface of the silver printed electrode. Electrodeposition of the mixed sediment was carried out in a three-electrode cell of a stripping voltammetric analyzer IVA-5 (RPIE Iva LLC, Russia) using a silver chloride reference electrode EVL-1M3.1 (Gomel Plant of Measuring Devices JSC, Belarus) and an auxiliary electrode GC-2000 (RI Grafit JSC, Russia).

Potentiometric measurement of AOA was performed using a developed PSS complete with a pH/ions meter TA-Ion (RPE Tomanalyt LLC, Russia). PBS pH 7.4 containing 10 mM $K_3[Fe(CN)_6]$ and 0.1 mM $K_4[Fe(CN)_6]$ was used in the measurements. The calculation of AOA has been described previously [15–19]. Experimental stoichiometric coefficients were calculated as the ratio of AOA to the concentration of the analyzed antioxidant. Spectrophotometric AOA measurement was performed using the standard Folin-Ciocalteu method [6] using gallic acid (GA) as a standard on an Ecoview UV-1200 spectrophotometer (Shanghai Mapada Instruments Co., Ltd., China).

Chamomile tea (Tri Lista LLC, Russia) and rose hip tea (Kamelia-LT LLC, Russia) were purchased from a retail pharmacy and classified as dietary supplements. Aqueous infusions of herbal teas were prepared in the following way: 1 g of phytopreparation was poured into 100 ml of hot (95 ± 3 ºC) deionized water, infused for 15 minutes, cooled in a water bath and filtered through a paper filter. ArtshAni orange nectar (Arshani LLC, Russia), dry white wine "Tsinandali", dry red wine "Saperavi" (Badagoni JSC, Georgia) were purchased at a retail chain. Freshly squeezed orange juice was directly cold pressed and analyzed for comparative purposes.

3 Results and Discussion

The oxidation of reference antioxidant compounds by potassium ferricyanide under experimental conditions was studied using the developed PSS. The obtained experimental stoichiometric coefficients are listed in Table 1, and they are in good agreement with literature data [19–21].

Table 1. Stoichiometric coefficients of antioxidants in the reaction with $K_3[Fe(CN)_6]$.

Antioxidant	Stoichiometric coefficient	
	Theoretical [19–21]	Experimental (n = 3)
Cysteine	1	1.07 ± 0.03
Glutathione reduced	1	0.98 ± 0.03
Ascorbic acid	2	1.93 ± 0.04
Catechin	4	3.93 ± 0.07
Gallic acid	4	4.31 ± 0.11
Quercetin	5	4.86 ± 0.08

Beverage analysis results obtained using PSS and the Folin-Ciocalteu spectrophotometric method are presented in Table 2. Good reproducibility was observed for both methods. The relative standard deviation of the results (S_r) obtained using PSS and the Folin-Ciocalteu method does not exceed 8 and 7%, respectively.

Table 2. Results of determination of AOA of drinks (n = 3).

Beverage	PSS with $[Fe(CN)_6]^{3-/4-}$		Folin-Ciocalteu assay [6]	
	mM-eq L^{-1}	S_r	mg GAE L^{-1}	S_r
Chamomile tea	1.37 ± 0.09	0.07	124.06 ± 5.32	0.04
Rosehip fruits tea	4.95 ± 0.41	0.08	142.86 ± 10.63	0.07
Orange nectar	1.98 ± 0.15	0.08	244.36 ± 5.32	0.02
Orange juice freshly squeezed	6.41 ± 0.31	0.05	278.19 ± 10.63	0.04
White dry wine	3.85 ± 0.29	0.07	154.13 ± 8.54	0.05
Red dry wine	25.47 ± 1.21	0.05	729.32 ± 44.43	0.06

Figure 1 shows a correlation showing the relationship between the results of the analysis of drinks obtained using the PSS and the Folin-Ciocalteu method. Pearson's linear correlation coefficient is r = 0.97 (p = 0.002), indicating a very high positive relationship between the two methods.

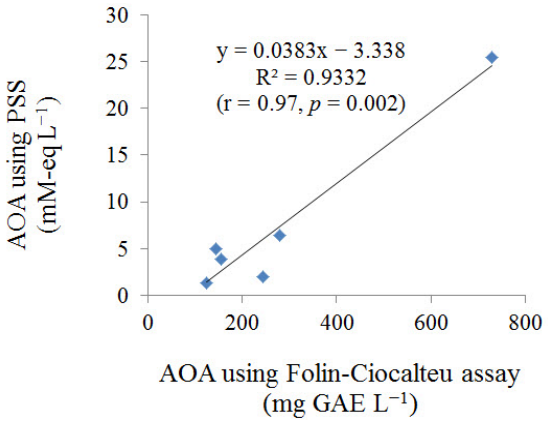

Fig. 1. Relationship between beverage analysis results obtained using PSS and the Folin-Ciocalteu method.

4 Conclusion

A new PSS based on modified screen-printed electrodes has been developed, which has been successfully used in the determination of antioxidants and assessment of AOA of drinks. Beverage analysis results obtained using PSS are highly reproducible and correlate with results from the Folin-Ciocalteu method. Based on the data obtained in this work, the proposed PSS is recommended for use in the analysis of pharmaceutical, cosmetic and clinical objects.

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