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Flow injection potentiometric determination of total antioxidant activity of plant extracts

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Abstract

A new flow injection potentiometric (FIP) method, rapid, reproducible and simple to apply, has been developed for the in vitro evaluation of antioxidative capacity of aqueous plant extracts. This method is based on the transient negative signal measurements with a flow-type platinum electrode detector due to the composition change of a $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox-reagent solution. The variables affecting the signal height such as composition and concentration of redox-reagent, injected sample volume, flow rates of carrier and redox-reagent solution streams were studied in details and the conditions were optimized. For the compounds under study, a linear relationship was stated between the potentiometric signal height and the logarithm of antioxidant concentration. It was stated that a wide antioxidant activity range from 1 μM to 10 mM could be determined by the changing concentrations of the hexacyanoferrate(III) from 5 to 0.01×10^{-4}. The present FIP method was applied to quantify relative antioxidant activity (RAA index) of the representative water-soluble antioxidants (ascorbic acid, pyrocatechol, pyrogallol, caffeic acid, chlorogenic acid, gallic acid, tannic acid, uric acid, L-cysteine, trolox). The high sampling rate ($100\,h^{-1}$) and a satisfactory reproducibility (R.S.D. = 0.7–1.8%, $n = 5$, 0.1 mM each compound) were obtained.

The method was also applied to estimate total antioxidant activity (TAA) of real samples (green and black tea infusions, herbal infusions and fresh fruit extracts) and the results were compared with those achieved using well-known in vitro testing methods.

Keywords: Flow injection; Potentiometry; Antioxidant; Total antioxidant activity; Plant extract

1. Introduction

There is considerable evidence that the antioxidants contained in plant food play a very important role in the maintenance of health [1]. In recent years, many published studies have stated that a diet rich in fruits, vegetables, herbs and cereals has a preventive effect against cardiovascular diseases, cancers, and other diseases related to ageing. This beneficial action in respect to health has been attributed to the phenolic compounds (phenolic acids, flavonoils and tocopherols) present in many plant materials, and largely to the antioxidant capability possessed by these compounds [2–5]. Tealeaves are considered important sources of such antioxidants as biophenolic compounds [6–8]. Non-fermented green tea contains more than 30% of their dry weight in polyphenols [9]. The main difference in composition of green and black tea is the relation of epicatechins to their oxidized condensation products [10]. Condensation products in black tea are responsible for the reddish color and the astringent effect, but they also possess strong antioxidant activity (AA) [11]. As far as antioxidant properties of dietary agents are concerned, there are situations in which knowledge of the total antioxidant activity (TAA) might be more useful than the individual antioxidant contents [12–14]. At present, TAA of mane natural products is considered as a significant parameter determining their dietary value.

There are few experimental approaches to evaluate or predict the antioxidative potency of individual biocompounds or complex matrices like fruits, vegetables, beverages, etc.: reducing power, free radical scavenging and metal chelating activities. In particular, a voltammetric sensor based on the process of electroreduction of oxygen at the mercury film electrode was proposed for evaluation of TAA in plant extracts [15]. The FRAP (ferric antioxidant power) assay was proposed by using ability of phenols to reduce Fe^{3+}-ions followed by detection of a colored

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complexes of Fe^{2+}-ions with 2,4,6-trypyridyl-s-triazine [16]. The FRAP values (in ascorbic acid equivalents) were applied to determine TAA in red wines [17]. However, the most frequently used methods for evaluation of this parameter are those in which free radicals are generated. In the presence of antioxidants, the amount of the free radicals detected is much less in comparison of a reference mixture. The main differences are in the nature of radical and the method of detection. The oxygen radical absorbance capacity (ORAC) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay and the trolox equivalent antioxidant activity (TEAC) assay are became to be most popular [18–21]. The TEAC assay was reported first by Miller et al. [22] and then modified by Re et al. [23]. This test is based on the oxidation of ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)] to the stable radical cation $ABTS^+$, measuring its absorbance at 734 nm. The oxidation is delayed, dependent on the concentration of the radical trapping substances, and adjusted to trolox, a water-soluble analogue of a-tocopherol, as standard. Improved ABTS assay was published for measuring TAA of wines, olive oils or fruits [24]. Guo et al. [25] have shown for several aqueous extracts of fruits and vegetables, a positive linear correlation between their oxygen radical absorbance capacity and their electrochemical behavior. In [26], a new version of the ABTS test was suggested, where the radicals were generated on-line by electrochemical oxidation of ABTS in the flow-through cell forming a part of the flow injection (FI) system. DPPH radical scavenger was used in FI procedure based on using an electron spin resonance spectrometry for analysis of beverages [27]. Lower detection of ascorbic acid was 10 μM (S/N = 4), sampling frequency was about 13 h^{-1}. Recently, determination of the antioxidant capacity of different food products with a new developed FI spectrofluometric detecting hydroxyl radicals was described [28].

It might be mentioned, however, that various experimental methods show extreme diversity. Comparative studies of the methods of antioxidant activity have been published, and all conclude that each methodology gives different responses for the same compounds or samples [29,30]. Some assays are suitable only for hydrophilic antioxidants, other for hydrophilic and lipophilic substances. In addition, different solvents and pH values for the same assay led to different antioxidant activity in some cases. For this reason, there is a clear requirement for the development of accelerated methods for evaluation of the "antioxidant power" of food products.

It appears that FI electrochemical methods are particularly appealing for the fast screening of the antioxidant properties of plant foods, because they combine a relatively simple and low-cost instrumentation with high sensitivity. Even though the selectivity of electrochemical detectors are not adequate for any practical determination of TTA values or individual antioxidants in real samples, their applicability for the determination of total phenolics seems to be generally accepted. Recently, a FI procedure using amperometric detection of oxidizable substrate (e.g., α-tocopherol plus phenolics) has been developed [31]. Advantages claimed for the proposed procedure are that it does not involve accelerated test conditions. A screening protocol for the amperometric determination of total polyphenolics in food was also proposed by using the FI system with a glassy carbon electrode [32]. An "electrochemical index" was evaluated as a novel alternative to the widely used Folin–Ciocalteu (FC) spectrophotometric protocol [33]. It might be mentioned, however, that the FC test permits to determine the total content of phenolics (polyphenols and monophenolics), while the "electrochemical index" is considered to be more selective since less total phenolics have been obtained. At the same time, an advantage of the proposed electrochemical protocol is that it can provide quantitative information on different fractions of polyphenolics, by simply changing the applied potential of the working electrode. A novel approach for a fast screening of total flavonols (quercetin, kaempferol, myricetin) in wines, tea-infusions and tomato juice using adsorptive stripping voltammetry in a FI system was described [34]. It is based on the property of flavonols to be pre-concentrated on carbon paste electrodes where diphenylether is used as the pasting liquid. Mixtures of flavonols resulted into a single anodic peak, which was used for the calculation of the total flavonols concentration expressed in terms of "quercetin equivalent". The sample throughput was about 13 h^{-1} and the overall reproducibility of measurements was 3–6%. Of course, all these electrochemical protocols could be used as measure of antioxidant capacity when correlation between "total (poly)phenolics" and TAA is estimated.

In our previous investigations [35], it was demonstrated that the oxidative/antioxidative capacity of different compounds can be evaluated by using a $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox-reagent. The present paper describes a systematic study performed by FI potentiometry (FIP), utilizing the measurement of transient electrode response generated by the simple redox reaction between water-soluble antioxidants and $[Fe(CN)_6]^{3-}$-ions contained in a $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox-couple solution. Based on the findings, the novel FIP technique was developed for the rapid evaluation of the in vitro antioxidative capacity of several primary (electroactive) antioxidants and "antioxidant power" of aqueous extracts of tea leaves, herbal mixtures and fresh fruits, in term of L-ascorbic acid equivalent ($Asc._{eq}$).

2. Experimental

2.1. Reagents and solutions

All antioxidants (L-ascorbic acid, Asc.; pyrocatechol, Cat.; caffeic acid, Caf.; chlorogenic acid, Chl.; pyrogallol, Pyr.; gallic acid, Gal.; tannic acid, Tan.; uric acid, Ur.; L-cysteine, Cys.; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or trolox, Tr.) used in this study were the purest commercially available from various sources (Fluka, Sigma–Aldrich) and were used as received. Stock solutions of the antioxidants (0.010 M) were prepared by dissolving weighed amounts of the substances in appropriate volume of water and they were kept in dark vials in a refrigerator. More dilute solutions were freshly prepared when needed by dilution of the stock solutions with water.

Stock solutions (0.1 M) of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were prepared by dissolving weighed amounts of the substances in appropriate volume of 0.2 M phosphate buffer solution (PBS) with pH 2.5–10.5. The working $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$