Sensors and Actuators B 247 (2017) 155–162

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Ferric-*o*-phenanthroline adsorbed on a Nafion membrane: A novel optical sensor for antioxidant capacity measurement of food extracts

Mustafa Bener[a], Reşat Apak[a,b,*]

[a] Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, 34320 Istanbul, Turkey
[b] Turkish Academy of Sciences (TUBA),Piyade St. No: 27, Çankaya, Ankara, Turkey

ARTICLE INFO

Article history:
Received 3 December 2016
Received in revised form 11 February 2017
Accepted 2 March 2017
Available online 6 March 2017

Keywords:
Ferric-phenantroline
Optical sensor
Antioxidant capacity
Colorimetry
Fruit juices

ABSTRACT

With increasing interest in consumption of antioxidant-rich food for fighting oxidative stress-related diseases, practical and low-cost tests for total antioxidant capacity (TAC) assessment are on the rise. We developed a sensitive solid membrane optical sensor for screening the TAC of food extracts and plant materials. The sensor was based on immobilizing a chromogenic oxidant, Fe(III)-*o*-phenanthroline (Fe(III)-phen), onto a Nafion cation-exchange membrane, and colorimetric measurement of the change in 510 nm-absorbance associated with highly-colored Fe(II)–phen formation upon reaction with antioxidants. The calibration curves with respect to the Fe(III)-phen sensing method of individual antioxidants comprising vitamins C and E, polyphenols and flavonoids were constructed, and their apparent molar absorptivities and linear concentration ranges determined. The limit of detection (LOD) and quantification (LOQ) for Trolox using the sensor were 0.26 and 0.87 μM, respectively. The trolox equivalent antioxidant capacities (TEAC) of antioxidants found with the sensor were close to those of solution-phase Fe(III)-phen method, indicating that the immobilized reagent retained its reactivity toward antioxidants. This colorimetric sensor was validated through linearity, additivity, precision and recovery, showing its reliability and robustness. The sensor was tolerant to pH variations and turbidity, and used for screening the TAC values of some commercial fruit juices without pre-treatment. The sensor was more sensitive than the solution-phase method because the membrane concentrated the color from a larger volume solution. The sensor may be a good choice of field analytical chemists for rapid, simple and versatile determination of TAC of complex samples on-site (like a pH indicator-strip measuring H^+ activity).

1. Introduction

Antioxidant compounds are natural combat agents against oxidative stress–originated diseases such as heart disease, cancer, and diabetes [1,2]. Because of known health beneficial effects of food antioxidants, selective and sensitive assays for rapid sensing of antioxidants have gained importance. Antioxidant activity assays may be basically divided into two main groups as hydrogen atom transfer (HAT)– and electron transfer (ET)–based assays, without distinct boundaries. HAT–based assays generally measure the capability of an antioxidant to quench free radicals by H-atom donation; these assays include inhibition of induced low-density lipoprotein autoxidation, total oxyradical scavenging capacity (TOSC) [3,4], crocin bleaching [5,6], total radical trapping antioxidant parameter (TRAP) [7,8], and oxygen radical absorbance capacity (ORAC) [9,10] measurement. Spectrophotometric ET–based assays for total antioxidant capacity (TAC) measurement determine the capacity of an antioxidant in the reduction of a chromogenic oxidant that changes color when reduced [2,11]. The extent of color change within a fixed time indicates the level of antioxidants in the sample matrix. The most widely used ET-based assays in literature are ABTS/TEAC [12], Folin-Ciocalteu [13,14], DPPH [15,16], FRAP [17] and CUPRAC [18], each having different types of chromogenic oxidizing reagents with varying formal redox potentials.

Ferric ion–based TAC assays depending on Fe(III)-Fe(II) reduction (in the presence of ligands such as tripyridyltriazine (TPTZ), ferricyanide, 1,10-phenanthroline, and 4,7-diphenyl-1,10-phenanthroline, also known as *batho*-phenanthroline) are popular because they are easy, inexpensive, linearly responsive and do not need specialized equipment. However, due to the slow kinetics of high-spin Fe(III) having half-filled d-orbitals, Fe(III) oxidations of certain hydroxycinnamic acids and especially thiols may be

* Corresponding author at: Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, 34320 Istanbul, Turkey.
E-mail address: rapak@istanbul.edu.tr (R. Apak).

http://dx.doi.org/10.1016/j.snb.2017.03.017
0925-4005/

incomplete [19]. The most widely used ferric-based assay, FRAP [17], has been criticized for its unrealistically acidic medium (pH 3.6) because most phenols are not dissociated at this pH, which may underestimate their true TAC values. A simple and sensitive spectrophotometric Fe(III)-*o*-phenanthtroline method, originally developed for the determination of ascorbic acid by Besada [20], was later modified by Berker et al. [21] so as to include a greater variety of antioxidants and accelerate their oxidation reactions by elevated temperature incubation for a longer time.

Compared to classical instrumental methods, optical sensors offer a number of advantages in many applications, such as speed, affordability, field use, simplicity of measurement and handling of results [22]. Chemical sensors are responsive to changes in the qualitative and quantitative composition of the system [23]. Optical sensors can evaluate analytical information with the use of optical transduction techniques (absorbance, reflectance, *etc.*). Reagent–based sensors (optrodes) are suitable for simple, rapid and low-cost analysis with high sensitivity/selectivity, and additionally enable the evaluation of the response of the sensitive layer not only by instrumental methods but also by the naked eye so as to be used in the field in the absence of specialized equipment [24,25]. Colored complex formation on the surface of a solid sensor may enable more sensitive spectrophotometric determinations, especially in turbid solutions, than in aqueous solutions of much larger volume. Nafion, a perfluorosulfonate-type cation exchange membrane (having R-{-O-CF_2-$CF(CF_3)$-}$_x$-$OCF_2)_2SO_3H$ functional groups), has been demonstrated to be one of the useful polymeric support materials in different optical sensors, because it has great thermal stability, mechanical strength, insolubility in water/ethanol and high transparency in the UV-VIS-NIR region [26].

Examples of optrode sensing of the TAC of complex samples are relatively few in literature. Bener et al. developed [27] and patented [28] the first versatile colorimetric TAC sensor having a linear response over a wide range of antioxidant concentrations [26]. This sensor works on the principle that copper(II)-neocuproine (Cu(II)-Nc) reagent electrostatically held on a cation-exchanger Nafion membrane is reduced by antioxidants *in situ* to the highly colored Cu(I)-Nc chelate, and this color change can be followed colorimetrically at 450 nm [27] or reflectometrically at 530 nm [29] for quantitative evaluation of TAC values. Steinberg & Milardovic immobilized two chromogenic radicals, *i.e.* DPPH• (2,2-diphenyl-1-picrylhydrazyl) and galvinoxyl radical (GV•), on polymeric PVC (*poly*(vinyl chloride)) for the design of antioxidant-sensing films [30], but this sensor suffered from the problems associated with relative radical instability under ambient conditions and nonlinear responses with respect to Log(concn.) and time. The catechin-group antioxidants were selectively detected among other polyphenolics by Apak et al. using an indophenol dye sensor on nanometric-sized TiO_2 particles [31], however there were linearity and additivity problems in TAC determination. Portable nano-ceria sensor (NanoCerac) [32], fabricated of immobilized CeO_2 nanoparticles on filter paper [33], was developed by Sharpe et al. for rapid and sensitive detection of food antioxidants, however the sensitivity was relatively low. A gold nanoparticles on paper sensor for TAC determination, though with problems of reproducibility and sensitivity, was designed by making use of the reduction reaction ($AuCl_4^- \rightarrow Au^0$) by antioxidants [34].

The single use of ferric-phenanthroline in the manufacture of a TAC sensor was made by Gavrilenko et al. [35] by immobilizing the complex into a *poly*-methacrylate matrix, and measuring the absorbance changes at 510 nm due to the formation of Fe(II)-phen with antioxidants. Although the analytical figures of merit were not reported, the sensor seemingly showed quite low sensitivity against standard antioxidants (*i.e.* the apparent molar absorptivity of ascorbic acid was ≈3450 M^{-1} cm^{-1}, as seen from the calibration graph). This low sensitivity probably stemmed from the embedding of the coordination complex in a polymer matrix by inactivating the redox-active centre: Fe(III). Thus, the aim of this work is to use the highly sensitive Fe(III)-phen reagent in a TAC sensor formulation so as not to block the coordination centre. The Fe(III)-phen cationic chelate can be easily immobilized electrostatically on a Nafion membrane without any preliminary modification process of the membrane, owing to the anionic sulfonate groups of Nafion. The proposed sensor was successfully applied to pure antioxidants, synthetic antioxidant mixtures and commercial fruit juices. The results obtained with the optical sensor assay were well correlated to those found by the pre-established solution-phase ferric-phen assay. The additional advantages of the membrane sensor assay over the solution-based one was that it worked in turbid solutions and did not require any preliminary heating for speeding up the redox reactions with antioxidants.

2. Experimental

2.1. Materials

Trolox (TR), caffeic acid (CFA), rosmarinic acid (RA), ferulic acid (FA), L-ascorbic acid (AA), α-tocopherol (TOC), and Nafion® 115 perfluorinated membrane (thickness 0.005 in): Aldrich (Steinheim, Germany); (+)catechin (CAT), DL-homocysteine (HCYS), L-cysteine (CYS), HCl, NH_4Cl, and: Fluka (Buchs, Switzerland); gallic acid (GA), rutin (RT), uric acid (UA), quercetin (QR), L-glutathione reduced (GSH), glacial acetic acid: Sigma (Steinheim, Germany); ethanol (EtOH), bilirubin (BIL), 1,10-phenanthrolinium chloride monohydrate, NaOH, NaH_2PO_4, Na_2HPO_4, CH_3COONa (NaAc), and ammonia solution (25%, by wt.): Merck (Darmstadt, Germany); Lipton black tea was purchased from CAYKUR-Cay Isletmeleri Genel Mudurlugu (Rize, Turkey), orange, cherry, peach, apple, tomatoes, and pomegranate juices from Meysu Gıda San. Tic. AS (Kayseri, Turkey).

2.2. Instrumentation

The visible spectra and absorption measurements were recorded using a Varian CARY Bio 100 UV–vis spectrophotometer (Mulgrave, Victoria, Australia). Additionally, absorbance measurements were made with the use of an Apple iPhone 6s Plus smartphone (Cupertino, California, USA) with colorimeter application, according to the procedure defined by Kuntzleman and Jacobson [36]. Quartz cuvettes with 1 cm path length for solution phase, and 2 mm path length for sensor measurements were used for the UV–vis measurements. Other related apparatus and accessories were BIOSAN Programmable rotator-mixer Bulti Bio RS-24 (Riga, Latvia) and Elektromag vortex stirrer (Istanbul, Turkey).

2.3. Preparation of solutions

Two milliliters of 1 M HCl were added to 0.160 g of $NH_4Fe(SO_4)_2{\cdot}12H_2O$; a suitable mass of *o*-phenanthroline was dissolved in distilled water so as to make its final concentration 1.0×10^{-2} M. These two solutions were mixed and diluted to 100 mL with distilled water to form the 'Fe(III)-phen' reagent solution.

The stock solutions of the tested phenolics were freshly prepared in EtOH, while CYS, HCYS, and AA in water, at 1 mM concentration. Uric acid and bilirubin were dissolved in NaOH, and the excess base was neutralized with a suitable volume of HCl solution. All stock solutions were stored at +4 °C in a refrigerator prior to analysis. The following buffer solutions were prepared: 0.1 M acetate buffer at pH 5, 0.1 M phosphate buffer at pH 7, and 0.1 M ammonia buffer at pH 9.