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Paper-based assay of antioxidant activity using analyte-mediated on-paper nucleation of gold nanoparticles as colorimetric probes

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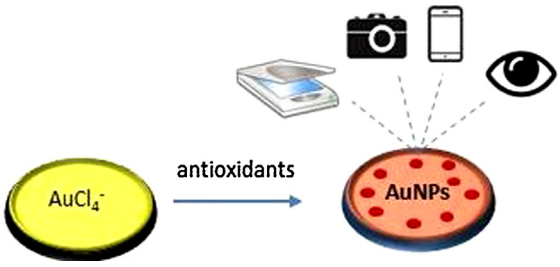
HIGHLIGHTS

- Paper-based sensor for the determination of antioxidant activity in real samples.
- On-paper formation of gold nanoparticles as colorimetric probes.
- Analyte-driven reduction of gold to gold nanoparticles on the paper surface.
- Determination of antioxidant activity in food samples without laboratory instrumentation.

GRAPHICAL ABSTRACT



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ABSTRACT

With the increasing interest in the health benefits arising from the consumption of dietary products rich in antioxidants, there exists a clear demand for easy-to-use and cost-effective tests that can be used for the identification of the antioxidant power of food products. Paper-based analytical devices constitute a remarkable platform for such expedient and low-cost assays with minimal external resources but efforts in this direction are still scarce. In this work we introduce a new paper-based device in the form of a sensor patch that enables the determination of antioxidant activity through analyte-driven on-paper formation of gold nanoparticles. The principle of detection capitalizes, for the first time, on the on-paper nucleation of gold ions to its respective nanoparticles, upon reduction by antioxidant compounds present in an aqueous sample. The ensuing chromatic transitions, induced on the paper surface, are used as an optical "signature" of the antioxidant strength of the solution. The response of the paper-based sensor was evaluated against a large variety of antioxidant species and the respective dose response curves were constructed. On the basis of these data, the contribution of each species according to its chemical structure was elucidated. For the analysis of real samples, a concentration-dependent colorimetric response was established against Gallic acid equivalents over a linear range of 10 μM–1.0 mM, with detection limits at the low and ultra-low μM levels (i.e. <1.0 μM) and satisfactory precision (RSD = 3.6–12.6%). The sensor has been tested for the assessment of antioxidant activity in real samples (teas and wines) and the results correlated well with commonly used antioxidant detection methods. Importantly, the sensor performed favorably for long periods of time when stored at moisture-free and low temperature conditions without losing its activity thus posing as an attractive alternative to the assessment of antioxidant activity without specialized equipment. The use of the sensor by non-experts for a rapid assessment of natural products in field testing is envisioned. Importantly, we demonstrate for the first time that analyte-mediated growth of nanomaterials directly on the paper surface could open new opportunities in paper-based analytical devices.

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1. Introduction

Many scientific reports have suggested that the intake of antioxidant compounds is an efficient way of combating undesired health risks associated with the presence of reactive oxygen species such as cardiovascular and neurodegenerative diseases, cancer, atherosclerosis, diabetes, etc [1–3]. For this reason, antioxidant-rich foods have gain considerable popularity and a variety of methods have been developed that can account for the antioxidant properties of natural products [4–8]. These methods can broadly be classified into those measuring the scavenging activity of samples against radical species through an hydrogen transfer mechanism (2,2-azino-di-(3-ethylbenzothia-lozine-sulphonic acid – ABTS, oxygen radical absorbance capacity – ORAC, chemiluminescent assays based on scavenging of H_2O_2 radical) and those that measure the reducing power of the sample based on electron atom transfer (cupric ion reducing antioxidant capacity – CUPRAC, Folin–Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl-DPPH, ferric reducing ability of plasma – FRAP). The first type assesses the ability of an antioxidant to scavenge specific free radicals technically created by synthetic chemicals not commonly found in natural systems, such as $ABTS^{\bullet+}$ or $DPPH^{\bullet-}$, while the other evaluate the reducing capacity of antioxidants on redox reactive metal ions such as Fe, Cu or Au [4–9]. In either case, signal transduction is provided by synthetic colorimetric dyes and fluorescence or chemiluminescence probes which provide spectroscopic evidence of the antioxidant capacity of the sample. However, the multiple reaction mechanisms and different principles of detection adopted in these methods, result in significant discrepancies in the hierarchy and ranking of antioxidant's power [4,10]. Therefore, multiple assays are needed to provide a complementary understanding of the antioxidant activity of a sample.

Recently, there has been an increasing interest in nanoparticle (NP)-based antioxidant assays. These assays either exploit the ability of antioxidants to reduce noble metals towards the formation of the respective nanoparticles (e.g. $AuCl_4^-$ to AuNPs, Ag^+ to AgNPs) [11–14] or use antioxidants as reducing agents in the seed-mediated growth technique, where small NP seeds serve as nucleation centers to the growth and further enlargement of nanoparticles [15,16]. Since the absorbance of the colloidal NP suspensions depends on the properties of the surface resonance plasmon bands, intense colorimetric and chromatic transitions are observed which are used as a measure of antioxidant activity.

Despite their wide acceptance, both conventional and nanoparticle-based assays are not portable and they are performed under controlled laboratory conditions. The requirement for multiple experimental steps, sometimes involving a sequence of time-based reactions, further perplexes their routine use, often results in lengthy turnaround times, and increased costs. More serious still, the requirement for instrumental detectors and benchtop equipment (e.g. thermostated baths, well pates and microplate readers, etc), which are necessary to obtain quantitative evidence on the antioxidant properties of the sample, sometimes renders their use prohibitive in non-specialized laboratories and without trained personnel.

In recent years, there has been a strong interest in the development of portable sensing platforms that can function autonomously of external energy sources under non-laboratory conditions. Such stand-alone platforms hold great promise as an alternative to the mainstream laboratory-based analytical technologies in providing qualitative and quantitative information even to non-expert users in resource limited areas. Among various technologies, paper-based analytical devices is an attractive and challenging technology, where paper matrices are used as an inexpensive platform for performing analytical assays, in a two or three-dimensional configuration [17–19]. To date most research on PADs focuses on medical and environmental applications [20–25], while assays for food quality are still scant [26,27].

In this work we describe a nanoparticle-based assay of antioxidant activity integrated in a paper-based device in the form of a sensor patch. The assay relies on the ability of antioxidants to reduce gold ions to AuNP species giving rise to chromatic transitions in which intensity depends on the structure and concentration of antioxidants. In this manner, we show for the first time that the formation and growth of AuNPs can take place directly on the paper surface, needless of additional reagents or reaction steps other than sample introduction. This feature significantly simplifies the analysis, reduces reagents consumption and increases sample throughput. Importantly, the results of this method correlate well with common assays of antioxidant activity thus it can be used as an alternative to more cumbersome assays or in conjunction with other methods to assess more comprehensively the antioxidant properties of real samples. We demonstrate the usefulness of this method for the detection of antioxidants in teas and wines and further discuss its advantages as a tool for the detection of antioxidants in field testing.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade. Folin–Ciocalteu reagent, potassium dihydrogen phosphate and L-ascorbic acid were obtained by Merck. Hydrogen tetrachloroaurate trihydrate, cetyl trimethyl ammonium bromide (CTAB), *o*-coumaric acid, cinammic acid, ferulic acid, (+)-catechin, caffeic acid, vanillic acid, gallic acid monohydrate and (±)-α-tocopherol were from Sigma–Aldrich. Phosphate buffer (pH 8.00) was prepared by appropriate dissolution of potassium dihydrogen phosphate in distilled water and the pH was adjusted by the addition of 0.1 mol L^{-1} NaOH (Titrisol, Merck). Whatman No. 1 (0.18 mm, 87 g m^{-2}) and No. 3MM (0.34 mm, 189 g m^{-2}) chromatography papers were procured from Whatman (Maidstone, Kent, UK). Filter papers were obtained from Munktell-Ahlstrom (Bärenstein, Germany) (Grade 34N, 0.18 mm, 60 g m^{-2}) and VWR (Wien, Austria) (Grade 600, 0.15 mm, 64 g m^{-2}).

2.2. Equipment

For imprinting the devices we used a Xerox Phaser 8560N printer that dispenses ink (melted wax) in the form of liquid droplets of 50–60 μm in diameter. The wax material is composed of a mixture of hydrophobic carbamates, hydrocarbons, and dyes which cool and solidify instantaneously without further spreading. For capturing colored images of the devices (JPEG format, 300 dpi) we used a desktop scanner (Canon LIDE 20) because it generally provides more reproducible results than digital cameras due to the constant focus of the image and the lighting conditions [17].

2.3. Fabrication of the sensor

We designed the paper sensors on a white background [28] as circles of 0.6 cm-diameter enclosed within lines (barriers) of 3 pt. (0.1056 cm) thickness. After heating, the total diameter of the device was 0.8 cm, with internal diameter of 0.4 cm (hydrophilic-detection zone) and 0.2 cm line (wall) thickness (Fig. S1 – Supporting information). These dimensions were decided on the basis of preliminary trials in order to accomplish a compromise between easy handling of the devices and minimum consumption of reagents and sample volume requirements [29]. The hydrophobic barriers were printed with solid wax in high printing quality