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Fabrication of paper based cerium oxide (CeO_2) square disc sensor for detection of standard antioxidants

Yugal Kishor Naik and Vivek Sharma

Abstract
The synthetic antioxidants like Butylated Hydroxyanisole, Tertiary Butyl hydroquinone are permissible to the tune of maximum 200 ppm in fats and oils. In the present study the possibility of using Cerium (IV) oxide nanoparticles (CeO_2) to detect adulteration of these antioxidants in milk fats. 20% dispersion of CeO_2 20 µL of CeO_2 as well as CeO_2 impregnated dried paper discs did not show a change in color after the addition of antioxidant BHA and TBHQ (0.02, 0.01, 0.005 and 0.0025%) solutions in acetonitrile. Disc sensor was fabricated by using whatman filter paper 4 and cut into small squares of approximately (0.5x0.5 cm) size by using a scissor. The CeO_2 20% dispersion was directly absorbed into square disc and evaporated at 85 °C for 5 min. The standard antioxidants were applied 20 µL in different concentration for BHA and TBHQ (0.0025, 0.005, 0.01and 0.02%) and for other antioxidants (0, 1, 2, 3, 4, 5 and 10 mM) into disc. The color change was observed in Gallic Acid, Ascorbic Acid and Ascorbyl Palmitate by using the same concentration of CeO_2 dispersion and dried paper discs.

Keywords: Fabrication, paper based CeO_2 disc, standard antioxidants

1. Introduction
In recent years, much attention has been drawn to antioxidants because of their supposed ability to fight cancer, promote health and prevent a wide variety of diseases including heart disease, aging, and neurodegenerative diseases such as Parkinson's and Alzheimer's. This has led FSSAI to standardize and make regulations on adding specific antioxidants to certain dairy and food products.

Several recent works have reported development of nanoparticle (NPs) based antioxidant assays, that monitor changes in the physicochemical properties of nanoparticles as they interact with antioxidants (Vasilescu, 2012). The most commonly used strategies are based on gold NPs (Scampicchio, 2006) [9, 12] in which detection of antioxidants is achieved indirectly by monitoring NP aggregation (Pal *et al*., 2007) [3], NP enlargement in the presence of $AuCl_4^-$ and the antioxidant compound (Qian and Ma, 2010) [4], formation of NPs by reduction of gold salts facilitated by antioxidants (Rios *et al*., 2011) [8], or by inhibition of H_2O_2 - mediated growth of gold nanostructures by antioxidants (Qian 2011) [5, 6, 7]. Changes in the physicochemical properties of the NPs in contact with antioxidants indicate antioxidant activity in the form of reducing power, which correlates well with the oxidation potential (Rios *et al*., 2011; Wang *et al*., 2006) [8, 12]. All colorimetric NP-based assays reported to date for the detection of antioxidants are carried out in colloidal dispersions. We report herein development of a fully-integrated colorimetric assay in which immobilized NPs of cerium oxide (CeO_2 or nanoceria) are used as color indicators. Due to the dual reversible oxidation state of cerium Ce (III)/Ce (IV) on the NP surface, nanoceria has the ability to change redox states and surface properties when in contact with antioxidants. These changes are accompanied by a color change that is used in this work to assess the total antioxidant capacity. To fabricate the sensor, ceria NPs are attached onto filter paper to create an active ceria-based sensing platform that provides a colorimetric readout which is inexpensive and easy-to-use. Introduction of antioxidant samples to the ceria sensor induces a color change that is proportional to the antioxidant concentration of the sample. The assay does not require reagents (except for the sample), specialized equipment or the use of an external power supply. Since the assay is easy-to-use and portable, it can be particularly appealing for remote sensing applications, where specialized equipment is not available, and for high throughput analysis of a large number of samples. Potential applications for antioxidant detection in remote locations and developing countries are envisioned.

2. Material and Methods

2.1 Chemicals

Cerium (IV) oxide (CeO_2) nanoparticles/ceria i.e., 20% colloidal dispersion in 2.5% acetic acid (CH_3COOH) were purchased from Merck Specialities Pvt. Ltd., Mumbai, India;

2.2 Solvents

Ethanol absolute and Acetonitrile (HPLC grade) were purchased from S.D Fine- chem. Ltd. Mumbai, India.

2.3 Synthetic antioxidants

Butylated Hydroxyanisol (BHA), Butylhydroquinone (TBHQ) and Ascorbic acid were purchased from Sigma Aldrich, St. Louis, USA; Butylated Hydroxytoluene (BHT) was procured from Thermo Fisher Scientific India Pvt Ltd; Gallic Acid was procured from central Drug House (P) Ltd, New Delhi, India: 6-O-Palmitoyl-L-ascorbic Acid was procured from Tokyo Industry CO., LTD 6-15-9 Toshima, Kita-Ku, Tokyo, Japan;

2.4 Filter papers

Whatman filter paper no-1 and 4 and thick Chromatographic filter paper (3 mm CHR) were purchased from Whatman International Ltd., Kent, England.

2.5 Reagents

a. **Working solution of BHA and TBHQ:** Dissolved 20 mg of synthetic antioxidant (BHA & TBHQ) separately in acetonitrile and volume was made to 100 ml in volumetric flask, as stock solutions. The above-mentioned stock solution was used to prepare the working solutions of lower concentrations (0.01, 0.005 and 0.0025%) in acetonitrile.

b. **Working solution of 6-O-Palmitoyl-L-ascorbic Acid (AsP):** Dissolved 50 mg of 6-O-Palmitoyl-L-ascorbic Acid in small quantity of ethanol and volume was made up to 100 ml in volumetric flask, as stock solution. The said stock solution was used to prepare the working solutions of lower concentrations (0.02, 0.01, 0.005 and 0.0025%) in ethanol.

c. **Working solution (10 mM) of 6-O-Palmitoyl-L-ascorbic Acid (AsP):** Dissolved 41.45 mg of 6-O-Palmitoyl-L-ascorbic Acid with ethanol and methanol, separately and made up the volume to 10 ml with respective solvents, as a stock solution. The above said stock solution was used to prepare the working solutions of lower concentrations (8, 6, 4, 2 and 1) mM in their respective solvents, separately.

d. **Working solution of Ascorbic Acid:** Dissolved 17.612 mg of Ascorbic Acid in a 10 ml volumetric flask with ethanol as a stock solution, as stock solution. The above said stock solution was used to prepare the working solutions of lower concentrations (8, 6, 4, 2 and 1) mM were prepared in ethanol.

e. **Working solution of Ascorbic Acid:** Dissolved 30 mg of ascorbic acid in ethanol and volume was made up to 100 ml in volumetric flask, as stock solution. The above said stock solution was used to prepare the working solutions of lower concentrations (0.02, 0.01, 0.005 and 0.0025) % were prepared in ethanol.

f. **Working solution of Gallic Acid:** Dissolve 3.4 mg of Gallic Acid in a 10 ml volumetric flask with acetonitrile, as a stock solution. The above said stock solution was used to prepare the working solutions of lower concentrations (1.5, 1 and 0.5) mM were prepared in acetonitrile

g. **Working solution Gallic Acid:** Dissolve 20 mg of Gallic Acid in a 100 ml volumetric flask with acetonitrile as a stock solution. Further, the working solutions of lower (0.01, 0.05 and 0.0025) % were prepared by diluting the stock solution with acetonitrile in each concentration, separately.

h. **Fabrication of square disc sensor:** Disc sensor was fabricated according to the method given by Sharpe et *al.* (2012) [11]. In this s experiment whatman filter paper 4 was used. The paper was cut into small squares of approximately (0.5x0.5 cm) size by using a regular pair of scissors. The CeO_2 20% dispersion was directly absorbed and evaporated at 85 °C for 5 min. (Fig 1.0).

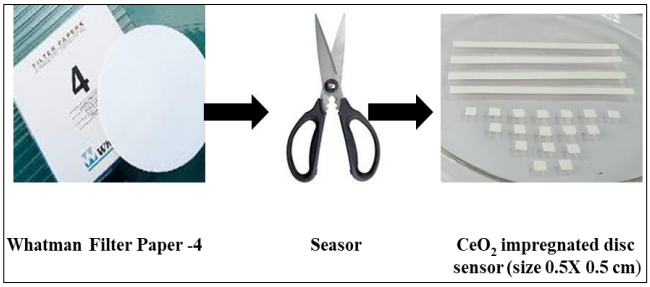
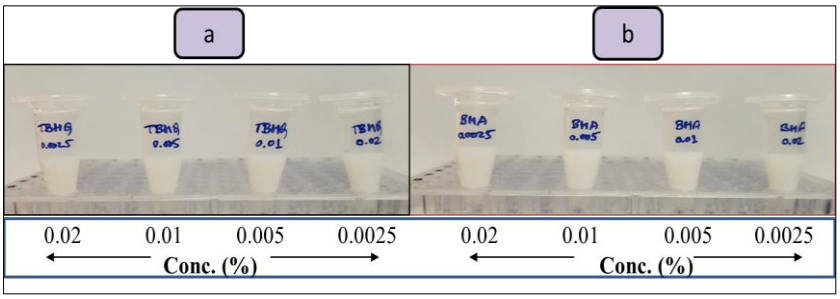


Fig 1: Flow chart for fabrication of square disc sensor

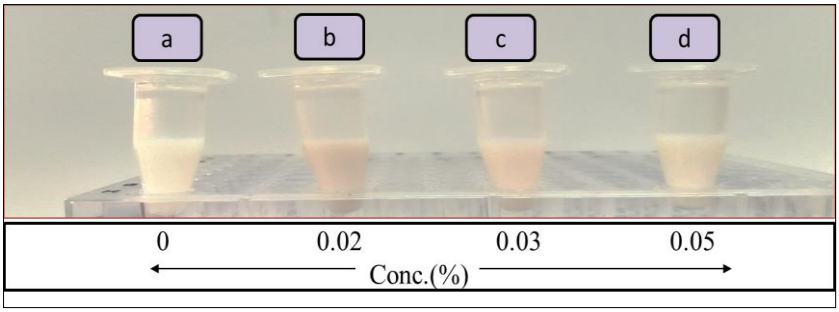
4. Result and Discussion

4.1 Optimization of cerium oxide (CeO_2) colloidal dispersion for efficient visual color change in the presence of antioxidants

In this experiment, 20 µL of CeO_2 colloidal dispersion in 20% acetic acid were added into 600 µL of standard antioxidants solutions in acetonitrile for BHA (0.02%), TBHQ (0.02%), and GA (0.02%); in ethanol for AA (0.03%) and Asp (0.05%). It is evident from the results (Fig 2.0) that in case of BHA and TBHQ, there were no color changes but for antioxidants like GA, AA and AsP there was a change in color from white to yellowish or brownish (Fig 2.0). This can be attributed to the fact that GA, AA and AsP have ability to chelate metal ions which was responsible for showing a color change (Sharp *et al.*, 2012 & 2014) [10, 11]. However, the other two antioxidants (BHA and TBHQ) lack the ability to chelate metal ions and therefore could not bring a change in color. The color concentration/intensity was varied for the types of antioxidants, reducing capacity of Ce^4 to Ce^3 and binding ability to the ceria surface. The response of the sensors fabricated using CeO_2 were directly proportional to the oxidation potential and the chemical structure of antioxidants like size, position (*-ortho*) and number of OH substituents (Sharp *et al.*, 2012 & 2014) [10, 11].



(A)



(B)

Fig 2: Effect of antioxidants solution on the color development in 20 µL of Cerium Oxide 20% dispersion solution: (A) a. TBHQ in acetonitrile (600 µL) & b. BHA in acetonitrile (600 µL) and (B) a: Control, b:GA in acetonitrile (600 µL), c: AA in ethanol (600 µL) & d: Asp in ethanol (600 µL)

In another experiment CeO_2 was impregnated on filter paper to see the effect of antioxidants on dried cerium oxide. On adding 20 µL of the above said antioxidants (0.02, 0.01, 0.005 and 0.0025% solutions were added), the color change on the CeO_2 impregnated paper was observed. It can be observed from the results (Fig 3.0) that BHA and TBHQ could not bring any change in color whereas AA (1, 2, 3, 4, 5, 10) mM, GA (0.5, 0.1, 1.5 and 2.0) mM and AsP (1, 2, 4, 6, 8, 10) mM, were able to bring a change in color and intensity of color change increased as the concentrations of AA, GA and AsP was increased. The above standard antoxidants given the same colour change result in contact with ceria sensor or dispersion. The sensor was applied and observed the same colour change in variety of tea, mushroom, acai berry juice and Merlot wine samples reported by Sharp *et al*. (2012) [11].

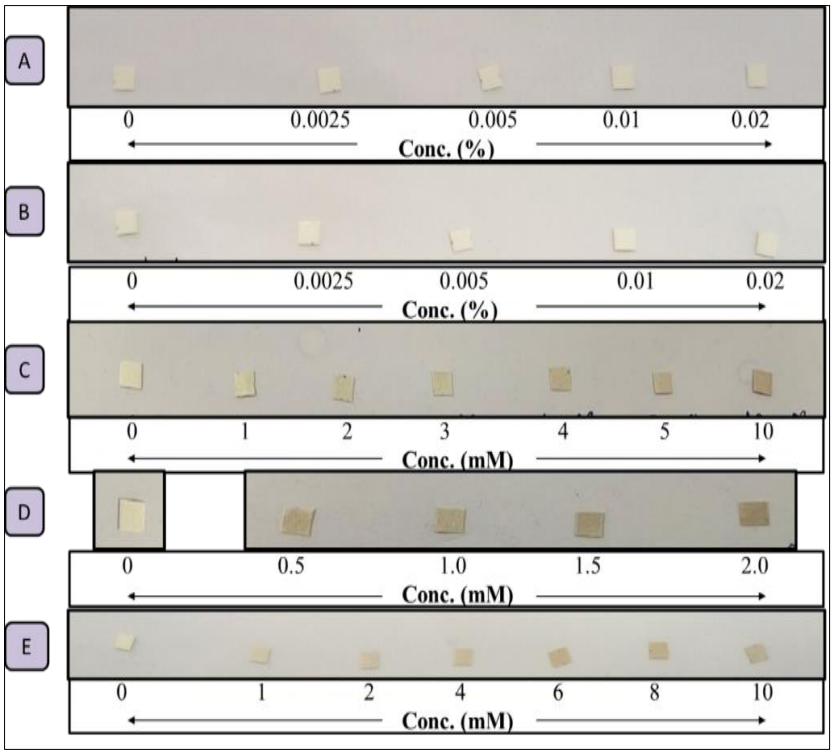


Fig 3: Color development in Cerium Oxide impregnated dried paper disc in the presence of (A) BHA, (B) TBHQ, (C) Ascorbic Acid (D) Gallic Acid and (E) Ascorbyl Palmitate

5. Conclusion

The present study has been summarized as following points:

1. 20 µL of 20% nanoceria ($CeO_{2)}$ dispersion, as well as impregnated dried paper-based disc did not show color change in the presence of BHA and TBHQ concentrations from (0.02, 0.01, 0.005 and 0.0025) %.
2. 20 µL of 20% CeO2 dispersion and CeO_2 impregnated dried paper disc showed color change in GA, AA & AsP.

Therefore, it can be concluded that above mentioned CeO_2 (dispersion and impregnated dried disc) can be effectively used for detection of some of antioxidants like Gallic Acid, Ascorbic Acid, and Ascorbyl Palmitate in ghee, vegetable fats, and other fats. Further research is required for detection of antioxidants like BHA and TBHQ in fats and oils.

6. References

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