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Visual colorimetric 'turn-off' biosensor for ascorbic acid detection based on hypochlorite–3,3′,5,5′,-Tetramethylbenzidine system

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ABSTRACT

An 'turn–off' approach for the detection of ascorbic acid (AA) using 3,3′,5,5′,-tetramethylbenzidine (TMB) as a colorimetric probe is developed. The proposed method is based on the fact that hypochlorite (ClO^-) could oxidize TMB to oxidized TMB (oxTMB) along with a blue color and an absorption peak centered at 652 nm. However, the introduction of AA could cause the reduction of oxTMB, which results in a blue color fading and a decrease of the absorbance at 652 nm. Based on this finding, we proposed a sensitive colorimetric assay for quantitative determination of AA by UV–vis spectroscopy. Under the optimal conditions, a good linear response between the absorbance and AA concentration was achieved within the dynamic working range from 1 to 70 μM with a detection limit of 0.58 μM. More importantly, the proposed method offered advantages of simple, low-cost instruments and rapid assay without the utilization of nanomaterials and has also been successfully applied for the determination of AA in orange juice with satisfying recoveries over 97.0%. Furthermore, the obtained results were consistent with those measured by high performance liquid chromatography with the average relative error of 3.3%.

1. Introduction

Ascorbic acid (AA), an effective antioxidant, exists widely in human physiological environment [1]. It plays a key role in many biological processes in the human body such as a cofactor in enzymatic reactions, collagen synthesis, scurvy prevention, and immune system enhancement [2,3]. It is well known that amount of AA in physiological inner is associated with numerous clinical diseases such as scurvy, Parkinson's disease, urinary stone, diarrhea, stomach, as well as numerous types of cancers [4,5]. Additionally, because primates as human cannot obtain this species through their own biosynthesis, AA should be taken from external sources as a necessary nutrient [6]. This species exists in a variety of foods and drinks, and it is frequently detected for the evaluation of these sources. Hence, the sensitive determination of AA in foodstuffs and food preservatives is essential for the human health, food assurance, and quality control.

Up to date, numerous AA sensors have been developed and mainly rely on the following two strategies. One was electrochemical strategy, in which the presence of AA could generate a significant oxidation signal at electrode and thus lead to a typical amperometric behavior [7–10]. Unfortunately, electrodes used in electrochemical sensor inevitably suffered from a pronounced fouling affect due to the accumulation of oxidized products on electrode surface. Moreover, electrochemical oxidation peak potentials for dopamine and uric acid are similar to that for AA, which will seriously interfere in the determination of AA. The other was optical strategy, in which fluorescent sensors [11–15] and colorimetric sensors [16–20] were designed with specific and high response toward AA. Optical sensing using fluorescence is becoming increasingly important for quantitative analytical techniques because of its high sensitivity and low limit of detection. Various fluorescent probes including carbon dots (CDs) [11], gold nanoclusters (Au NCs) [12], copper nanoclusters [21], graphene quantum dots (GQDs) [13,22], graphitic carbon nitride (g-C_3N_4) nanosheets [23], MoS_2 quantum dots (MoS_2 QDs) [24] and CdTe quantum dots [25] have been developed, which provide an effective fluorescent sensing platform for the detection of AA. Meanwhile, colorimetric sensors have also aroused great interest due to the advantages of low background signal, direct measurement and no need of any advanced instrumentation, facilitating the detection of analytes by naked eyes and UV–vis spectrometer. For instance, Zhang et al. reported a label-free strategy for ascorbic acid (AA) sensing based on Fenton reaction with unmodified gold nanoparticles (AuNPs) as probe [16]. Wang et al. utilized Gold/Silver Core/Shell nanorod as a highly efficient signal amplification method for the detection of AA [18]. Peng et al. established a

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colorimetric method for determination of AA based on growth of silver nanoparticles by a simple and green photo-catalytic route [20]. Despite the importance of above optical sensors, their use still shows drawbacks. General speaking, these sensors suffered from the disadvantages of high cost, time-consuming and complex preparation or modification of nanomaterials. Furthermore, poor biocompatibility and instability of the probes also limited their practical applications. Consequently, developing a new colorimetric sensing platform with simplicity, sensitivity and efficiency for the detection of AA was still highly desired.

TMB (3,3,5,5-tetramethylbenzidine), a widely used chromogenic substrate, is utilized as a visualizing reagent in biosensing assays because it yields reaction products with high absorption coefficients and lacks carcinogenicity [26]. And the reaction of the oxidation of TMB by hydrogen peroxide (H_2O_2), as well as the catalyst of the reaction has been drawn a lot of attention. More recently, it has found that various nanomaterials (e.g., CuO/Pt nanocomposites [27], metal-organic frameworks material MIL-88 [28], Co_3O_4/CGM nanohybrid [29], Cu-Ag/rGO nanocomposite [30], PSS-rGO [31] and CuNPs@C nanocomposite [32]) exhibited a highly efficient peroxidase-like activity and has been successfully applied to the detection of AA. Compared to the above catalyst, the hypochlorite (ClO^-)-based colorimetric determination not only holds properties of fast response and high sensitivity, but also shows advantages over the peroxidase activity-mediated assay, such as convenience, low cost, and direct oxidation without the use of H_2O_2. But unfortunately, at present, its application field is still narrow, which only is confined to detect the concentration of ClO^- [33].

In this study, we report a facile and effective colorimetric 'turn-off' sensing platform for the quantitative detection of AA based on ClO^-–TMB system. Previous reports have shown that ClO^- can oxidize TMB to induce a blue color and an absorption peak centered at 652 nm [33]. AA are well-known reducing agents due to the existence of phenol hydroxyl group which can induce the reduction of oxidized TMB (oxTMB) and result in the fading of the blue color and the decrease of the absorbance at 652 nm. Based on the above facts, a rapid and sensitive method for AA detection was established and successfully applied to detect AA in orange juice samples. To the best of our knowledge, this is the first method in which ClO^-–TMB system has been used for the detection of AA.

2. Experimental

2.1. Reagent and Chemicals

All chemicals used were at least of analytical reagent grade and used without further purification. TMB was purchased from Biosharp (Hefei, China). Ascorbic acid (AA), glucose (Glu), fructose (Fru), mannose (Man) and citric acid (CA) acquired from Sigma-Aldrich. Sodium hypochlorite (NaClO), sodium chloride (NaCl), potassium chloride (KCl), magnesium sulphate ($MgSO_4$), calcium chloride ($CaCl_2$), sodium acetate (NaAc) and acetic acid (HAc) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Orange juice came from Minute Maid (www.coca-cola.com.cn). Ultrapure water (18.2 M cm^{-1}) prepared by a Millipore system (Millipore, USA) was used throughout the whole experiments.

2.2. Instruments

UV–vis spectra were recorded using an Agilent 8453 UV–vis spectrometer (Agilent, USA). A characteristic absorption peak of oxidized TMB at 652 nm was used for quantitative analysis. The high performance liquid chromatography (HPLC) system was the LC-20A series (Shimadzu, Japan), including a quaternary pump, a vacuum degasser, a thermostated column compartment, a prominence diode array detector (DAD), a manual sample valve injector with a 20 μL loop, and an analytical column (Shim-pack ODS, 250 × 4.6 mm – i.d. 5 μm; Shimadzu, Japan). The pH value of buffer was adjusted by titrating with HAc and controlled by a PHS-3C pH meter (Leici, Shanghai).

2.3. Procedures for AA Sensing

To a series 5 mL calibrated test tubes, 50 μL of 4 mM NaClO solutions and 50 μL of 4 mM TMB dissolved in absolute alcohol were diluted to 900 μL with HAc/NaAc buffer (50 mM, pH 3.5). Then, 100 μL of AA standard solutions with different concentrations were added to each of the mixture solutions, which were shaken and equilibrated at room temperature for 30 s. Finally, the mixed solution was transferred separately into 1 cm quartz cuvette and directly monitored by UV–vis spectrometer.

2.4. Selective Detection of AA

Several potential interfering solutions coexisting in commercial orange juice, including glucose, fructose, mannose, citric acid and 4 kinds of cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) were prepared in a concentration of 2.5 mM, respectively. To evaluate the selectivity of the proposed method, two groups of experiments were performed. On the one hand, 50 μL of 4 mM TMB, 50 μL of 4 mM NaClO and each 100 μL of the above solutions were added sequentially in 800 μL of 50 mM HAc/NaAc buffer solutions. On the other hand, each 100 μL of the above solutions was pre-mixed with 100 μL of 0.5 mM of AA, which was then separately added to the ClO^-–TMB sensing system. Photographs and UV–vis spectroscopy were recorded after reaction for 30 s at room temperature.

2.5. Analysis of AA in Orange Juice

Commercial orange juices were bought from a local supermarket (Hefei, China). Firstly, orange juice samples were filtered with 0.22 μm Millipore filter to remove the insoluble components and then forty-fold diluted by ultrapure water. Following that, the diluted orange juice samples were added into the ClO^-–TMB sensing system as mentioned. Finally, content of AA in orange juice samples was calculated according to a linear equation obtained from a standard solution of AA.

3. Results and Discussion

3.1. Design and Principle of the Sensing System

The working principle of the designed method for sensing the AA is schematically shown in Scheme 1. Previous report have shown that TMB as a colorimetric probe could be oxidized by ClO^- to form an oxidizing product (oxTMB), thereby inducing a blue color and a strong characteristic absorption peak centered at 652 nm. Subsequently, addition of AA into ClO^-–TMB aqueous solution could cause the reduction of oxTMB, which results in a blue color fading and a decrease of the absorbance (A detailed reaction process was provided in Fig. S1). The sensing system could be visualized by naked eyes or measured with UV–vis spectrophotometer. Thus, a new colorimetric sensor for the detection of AA was established by coupling two reactions, which can be expressed by the following equations:

$$C1O^- + TMB \xrightarrow[pH3.5]{NaAc/HAc} C1^- + oxTMB \quad (1)$$

$$oxTMB + AA \rightarrow TMB + DHAA + H^+ \quad (2)$$

In order to verify sensing mechanism, several control tests under different conditions were implemented by using visual color readout and UV–vis absorption. As indicated from Fig. 1, no characteristic absorption peak from 300 to 800 nm was observed at pure TMB solution. In contrast, two strong absorption peaks were simultaneously achieved after