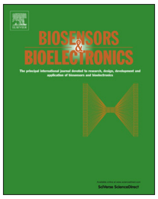
Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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



Highly selective colorimetric and electrochemical sensing of iron (III) using Nile red functionalized graphene film

Omer Sadak [a,1], Ashok K. Sundramoorthy [b,1], Sundaram Gunasekaran [a,b,*]

[a] Department of Materials Science and Engineering, University of Wisconsin-Madison, 1509 University Avenue, Madison, WI 53706, United States
[b] Department of Biological Systems Engineering, University of Wisconsin-Madison, 460 Henry Mall, Madison, WI 53706, Unites States

ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form
19 April 2016
Accepted 21 April 2016

Keywords:
Electrochemical sensor
Ferric iron
Nile red
Oxidized graphene
Visual detection
Wine

ABSTRACT

We report a highly selective method for identification and detection of iron (III) (ferric iron, Fe^{3+}) using Nile red (NR) as a complexing agent. Fe^{3+} preferentially binds with NR in dimethylformamide (DMF)/water (1:1) solution over other cations such as Fe^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} at room temperature. In the presence of Fe^{3+}, the color of NR solution changes from purple to dark brown, which is detectable with bare eyes. Using UV–vis spectroscopy, we could measure the amount of Fe^{3+} in the sample solution by monitoring changes in absorption from 540 to 580 nm; the linear range and the limit of detection are 30–1000 μM and 24.9 μM, respectively. Taking advantage of the NR selectivity, we treated partially oxidized graphene sheets (po-Gr) with NR to obtain po-Gr-NR dispersion by ultrasonication. The NR-treated po-Gr flakes (po-Gr-NR) were characterized by UV–vis, FT-IR, and Raman spectroscopies and FE-SEM, which indicated attachment of NR on po-Gr sheets. The po-Gr-NR hybrid film deposited glassy carbon electrode (po-Gr-NR/GCE) served as the Fe^{3+} sensor. Differential pulse voltammetry was used to investigate the detection of Fe^{3+} in 0.05 M HCl + 0.05 M KCl solution. The linear range and the limit of detection of Fe^{3+} were from 37.5 nM to 21.53 μM and 18.7 nM, respectively. Furthermore, this sensor was successfully used to measure Fe^{3+} content in red wine samples.

1. Introduction

Wine quality varies depending on its chemical content and composition, particularly the concentration of inorganic ions in wine is of great interest (Hernandez et al., 1996; Lopez-Lopez et al., 2015). In some cases, the presence of trace elements in wine, which is related to aesthetics of the final product, can be toxic at high concentrations. Iron is one of the most abundant metals in wine because it originates from various sources and processes such as soil, bloom covering grapes, grape harvesting, winery materials, transportation, fermenting and storing in tanks. As a result, iron concentration in commercial wines range from 0.5 to 5 mg L^{-1} (Esparza et al., 2006; Pyrzynska, 2004; Waterhouse and Laurie, 2006).

The determination of iron in wine products is critically important because if present above 10 mg L^{-1}, it may result in cloudiness or color change, which leads to haze formation commonly known as iron casse (Clark et al., 2011; Riganakos and Veltsistas, 2003). When protected from air, iron exists exclusively an as ferrous ion (Fe^{2+}), which is highly water soluble. However, when wine is aerated, the dissolved oxygen could oxidize Fe^{2+} to Fe^{3+}, which is responsible for the precipitation of coloring matter (blue casse) and for the cloudiness in white wine (white casse) (Lopez-Lopez et al., 2015; Wang and Mannino, 1989).

Detection of Fe^{3+} via colorimetric methods such as visual color change or with a UV–vis spectrometer, have been widely studied for their advantages of easy and quick detection and low cost (Wei et al., 2011; Yang et al., 2015). However, the lack of photostability, biocompatibility, and sensitivity for Fe^{3+} have posed major challenges for colorimetric methods (Guo et al., 2015). Other spectrophotometric methods are also commonly used for the detection of total iron content in wine including flow injection analysis-chemiluminescence (FIA-CL) (Bowie et al., 1998; Elrod et al., 1991), inductively coupled plasma-mass spectroscopy (ICP-MS) (Grotti et al., 2009), chromatography (Dias et al., 2012), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Hirata et al., 1986), and X-ray fluorescence spectroscopy (X-ray-FS) (Norrish and Hutton, 1969). These methods are relatively expensive for use in ultra-trace determination of metal ions, time-consuming, difficult to adapt to automatic detection, and can suffer from interferences. Moreover, for measurement of iron content in wine via spectrophotometric methods, samples must be pretreated to avoid matrix effects (Boonchiangma et al., 2014). Alternatively,

---

[*] Corresponding author.
E-mail addresses: ashok.sundramoorthy@wisc.edu (A.K. Sundramoorthy), guna@wisc.edu (S. Gunasekaran).
[1] O.S. and A.K.S. have contributed equally.

http://dx.doi.org/10.1016/j.bios.2016.04.073
0956-5663/

electrochemical methods such as voltammetry and potentiometry offer significant advantages such as low limit of detection (LOD), high sensitivity and selectivity without any prior separation, rapid response time and inherent miniaturization and portability. Recently, the use of chemically modified electrodes coupled to electrochemical methods have shown a noteworthy potential for the detection of Fe^{3+} in aqueous media (Karimi and Hasheminasab, 2013; Lu and Compton, 2013; Pena et al., 2014; van Staden and Matoetoe, 1998). Therefore, Fe^{3+} can be detected in aqueous media using electrodes chemically modified with different organic and inorganic compounds (Compagnone et al., 1992; Gao et al., 1991; Motonaka et al., 1998; Oh et al., 2013; Pena et al., 2014; van Staden and Matoetoe, 1998; Wang and Mannino, 1989).

In recent years, graphene (Gr) has ignited increasing research interest, owing to its superior electrical conductivity, biocompatibility, and exceptional surface-to-volume ratio (Carbone et al., 2015; Favero et al., 2015; Gao and Duan, 2015; Hill et al., 2011; Lawal, 2015; Song et al., 2016; Sundramoorthy and Gunasekaran, 2014; Yasri et al., 2014). On the basis of these unique properties, various Gr-based probes have been explored (Bi et al., 2013; Seenivasan et al., 2015; Sundramoorthy and Gunasekaran, 2015; Wang et al., 2015; Yang et al., 2012; Yang et al., 2013). To enhance the electrochemical signal for analytical applications, Gr or Gr-functionalized redox mediators/nanomaterials can be incorporated on the working electrode surface (Mao et al., 2013; Seenivasan et al., 2015; Su et al., 2011; Wang et al., 2015; Yang et al., 2012, 2013; Zhou et al., 2013). Generally, Gr can be prepared from graphite by mechanical cleavage (Jayasena and Subbiah, 2011), chemical exfoliation (Zhang et al., 2010), thermal decomposition (Wang et al., 2012), or electrochemical exfoliation (Sundramoorthy et al., 2015). For electrochemical exfoliation, toxic, corrosive, oxidizing/reducing reagents or stabilizers are not required, thus electrochemical exfoliation of graphite is considered a simple, rapid, and green method.

We found Nile red (NR) dissolved in DMF/water (1:1 ratio) can bind selectively with Fe^{3+} which can be monitored by the purple to dark brown visual color change or via UV–vis absorption spectrophotometry. Other metal ions such as Fe^{2+}, copper (Cu^{2+}), lead (Pb^{2+}), mercury (Hg^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), zinc (Zn^{2+}), cobalt (Co^{2+}) and cadmium (Cd^{2+}) did not react with NR at the same condition. Taking advantage of the specificity of reaction of NR with Fe^{3+}, we treated electrochemically synthesized po-Gr with NR (po-Gr-NR) to selectively detect Fe^{3+} ions. Using differential pulse voltammetry (DPV), we demonstrated selective detection of Fe^{3+} in the concentration range of 37.5 nM to 21.53 μM with a limit of detection (LOD) of 18.7 nM at the po-Gr-NR modified glassy carbon electrode (po-Gr-NR/GCE). This po-Gr-NR based electrochemical sensor was applied to detect and measure Fe^{3+} content in red wine samples.

2. Materials and methods

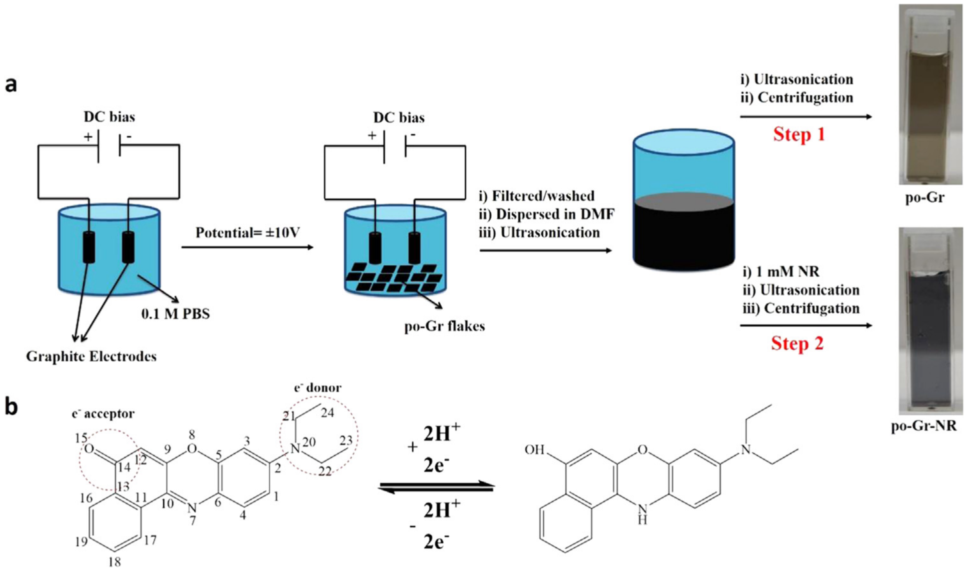
2.1. Electrochemical exfoliation and NR treatment

The po-Gr flakes were synthesized via electrochemical exfoliation as illustrated in Scheme **1a** (*see* Supporting Information *for additional experimental details*). Briefly, graphite sheets were used as anode and cathode electrodes in 0.1 M phosphate-buffered saline (PBS) solution (pH 7.0) as a supporting electrolyte. After applying ±10.0 V across the electrode for 30 min, the exfoliated po-Gr flakes were collected. Then PBS was removed from the resulting product by vacuum filtration (using Whatman filter paper with a pore size of 6 μm) and followed by washing with deionized (DI) water several times. The obtained po-Gr flakes were dried at 60 °C for one hour, and then dispersed in 10 mL of dimethylformamide (DMF) with a probe-sonicator for 30 min to obtain po-Gr/DMF dispersion.

5 mL of po-Gr dispersion was mixed with 5 mL of 1 mM NR/DMF, and was probe-sonicated for 15 min in an ice bath. The top fraction of the po-Gr-NR dispersion was collected after centrifugation at 3000 rpm for 30 min. For control studies, 5 mL of the po-Gr dispersion (without NR) was probe-sonicated again for 15 min. The po-Gr dispersion was centrifuged at 3000 rpm for 30 min and the supernatant was collected.

2.2. Preparation of Fe^{3+} sensor

Glassy carbon electrode (GCE) was polished with alumina powder (0.05 μm) on a micro-cloth polishing pad to obtain mirror-like surface. Then, 6 μL po-Gr-NR dispersion or po-Gr (control) was placed on the GCE and dried at 60 °C in an oven for 30 min to evaporate solvent (DMF). The modified electrodes (po-Gr/GCE and po-Gr-NR/GCE) were gently washed by immersing in DI water to remove unbounded materials from the electrode surface.



Scheme 1. (a) Electrochemical synthesis of po-Gr and treatment of NR with po-Gr. (b) Proposed redox mechanism of Nile Red.

Please cite this article as: Sadak, O., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.04.073