

## Supporting Information

# Sensitive and selective detection of iron-catalysed hydroxyl radical formation in water through an electrochemical method without chemical probes

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## 1. Experimental Section

### 1.1. Chemicals and Reagents

Nafion<sup>®</sup> (5 wt% in mixture of lower aliphatic alcohols and water, Aldrich), H<sub>2</sub>PtCl<sub>6</sub> (Sigma), H<sub>2</sub>O<sub>2</sub> 35% (Showa), FeSO<sub>4</sub> (Showa), ascorbic acid (AA), catechol (CA), pyrogallol, 4-HBA, GA (GA), (-)-epigallocatechin gallate (ECGC), (-)-epigallocatechin (EGC), and sulfuric acid were of ACS certified reagent grade. Nitrogen was obtained from Air Products and Chemicals (Taipei, Taiwan). Aqueous solutions were prepared with deionized water purified by a Millipore-Q purification system. Electrochemical experiments were performed on a CHI824b electrochemical workstation (Austin, TX, USA).

### 1.2. Preparation of the NPt-SPUME

The SPUME with 20 μm in width and 0.18 mm in length of working electrode was prepared as reported earlier by a built-in three-electrode pattern of alternating printed-layer of carbon, silver, and insulator on a non-conducting polypropylene substrate with the edge of the carbon and/or metal-sandwiched films between the insulator layers to serve as a band type ultramicroelectrode. The Pt nanoparticles were deposited on the SPUME at -0.6 V (vs. Ag/AgCl) for 190 s in 50 ppm H<sub>2</sub>PtCl<sub>6</sub>. The increase in mass transfer rate from the edge diffusion effect at ultramicroelectrode helps to result in homogeneous size and distribution of Pt nanoparticles without either protective or capped agents. After the electrodeposition of Pt nanoparticles, 3 μL

Nafion solution was drop-coated on the SPUME and dried in air to finish the preparation of the proposed gas sensor (i.e., NPt-SPUME). The thickness of Nafion layer and the average size of Pt nanoparticles were measured as  $4.5 \pm 0.5 \mu\text{m}$  and  $103 \pm 14 \text{ nm}$ , respectively. Note that, by measuring the reduction potential of platinum oxide in the presence of  $\text{H}_2\text{O}_2$ , we obtain a R.S.D. value of 2.79% ( $n = 40$ ) indicating the  $\text{Ag}_x\text{O}/\text{Ag}$  reference electrode is stable while detecting  $\text{H}_2\text{O}_2$  in  $\text{N}_2$  stream.

### 1.3. Measurement of $\text{HO}\cdot$ by Amperometric Method

Determination of  $\text{HO}\cdot$  was carried out by in situ monitoring of the oxidation peak current of  $\text{H}_2\text{O}_2$  using the gas phase NPt-SPUME system. All experiments were conducted at  $30^\circ\text{C}$  and 97% relative humidity by purging with  $\text{N}_2$ . As  $\text{H}_2\text{O}_2$  is highly soluble in water and hence can be easily carried out in  $\text{N}_2$  stream to reach the NPt-SPUME. Fenton reaction, i.e.,  $\text{H}_2\text{O}_2$  reacts with  $\text{Fe}^{2+}$ , was used to generate  $\text{HO}\cdot$  in  $\text{H}_2\text{SO}_4$  solution ( $\text{pH} = 3$ ). The decrease of  $\text{H}_2\text{O}_2$  concentration under Fenton reaction can consequently be monitored by the NPt-SPUME system and used as a probe for  $\text{HO}\cdot$  concentration.

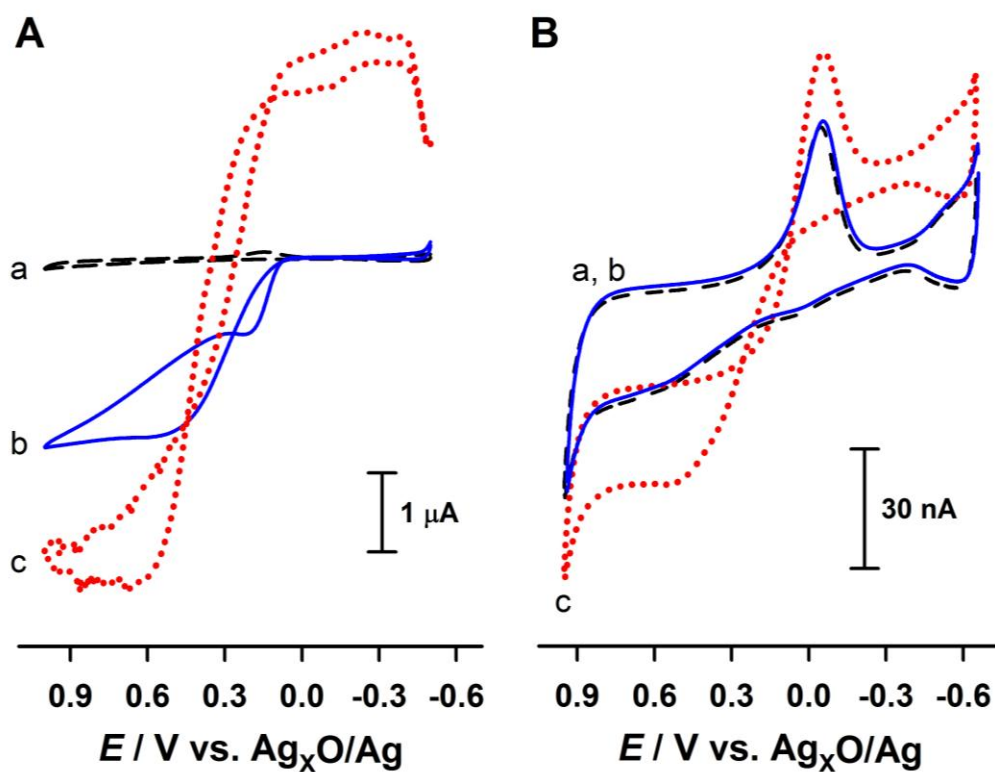
### 1.4. HPLC Analysis

The 4-HBA is commonly used as an assay for  $\text{HO}\cdot$  through the formation of 3,4-DHBA with a reaction rate constant of  $8.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  in aqueous media. The amount of 3,4-dHBA formed is measured by HPLC using a Hitachi L-2130 series with UV-visible detector at 295 nm. A Gemini-NX  $5\mu\text{C}18$  column (25 cm length  $\times$  4.6 mm id) was utilized for all separations with a mobile phase of  $\text{pH} = 3 \text{ H}_2\text{SO}_4$  solution. Elution was performed at a flow rate of 1 mL/min under a column temperature of  $25^\circ\text{C}$ . Various  $[\text{HO}\cdot]$  was produced via Fenton reaction under the conditions of  $25 \mu\text{M Fe}^{2+}$  and various concentration of  $\text{H}_2\text{O}_2$  ranging from  $25 \mu\text{M}$  to 1 mM with the addition of 50 mM 4-HBA in a final volume of 2 mL in 0.1 M  $\text{H}_2\text{SO}_4$

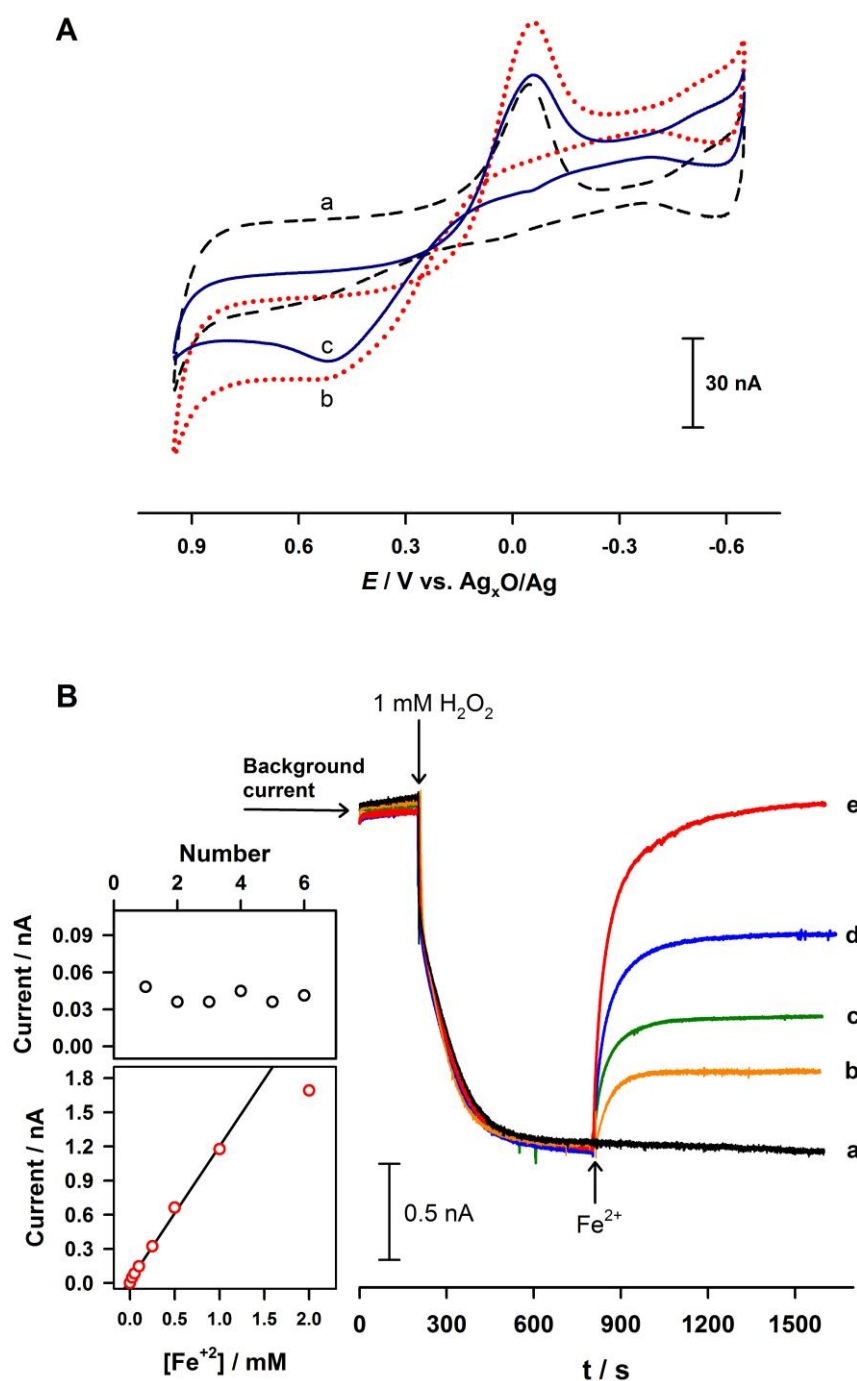
solution. After incubation time of 30 min, the analytes were injected into HPLC for 3,4-DHBA analysis.

### **1.5. Antioxidative Activity Analysis**

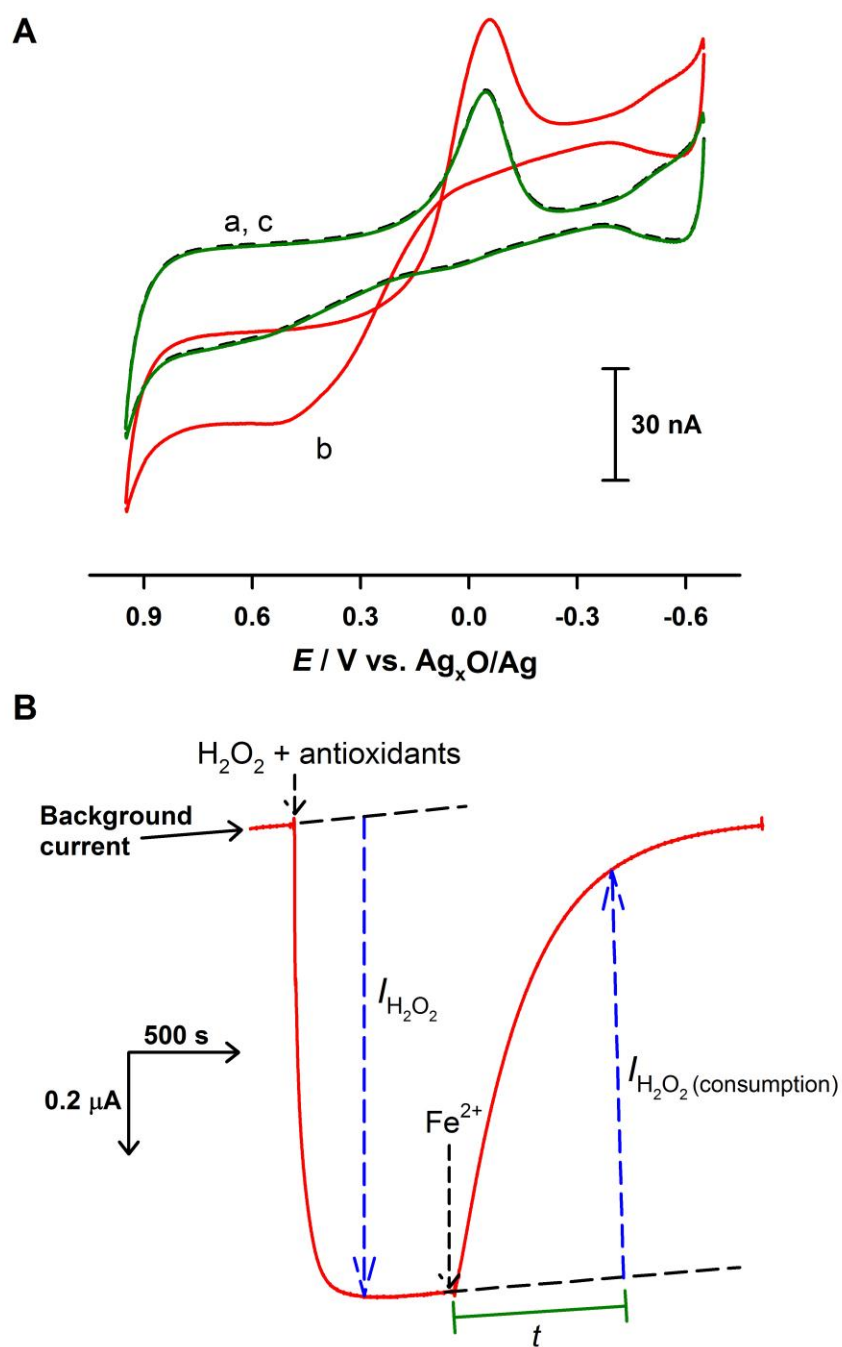
To validate the applicability of the proposed method, the antioxidative activity of several phenolic compounds and catechins were evaluated. Due to the much higher antioxidative activity of catechins, the studies were divided into two groups of: (1) AA, pyrogallol, CA, GA and (2) AA, EGC, EGCG, 4-HBA. For easy comparison, a solution of 1 mM H<sub>2</sub>O<sub>2</sub> (2 mL) containing 25 μM Fe<sup>2+</sup> was taken into the reaction for group (1); whereas, 50 μM Fe<sup>2+</sup> was used for group (2). The consumption rate of H<sub>2</sub>O<sub>2</sub> oxidation current at the NPt-SPUME was monitored after the addition of 10 mM antioxidants in 0.1 M H<sub>2</sub>SO<sub>4</sub> solution.



**Fig. S1** Typical cyclic voltammograms for (A) liquid phase and (B) gas phase in (a) 0.1 M PBS (pH = 7), (b) 10 mM AA, and (c) 10 mM  $H_2O_2$  + 10 mM AA at the NPt-SPUME under a scan rate of 20 mV/s.



**Fig. S2** (A) Typical cyclic voltammograms of the NPt-SPUME under 100  $\mu$ L/min  $N_2$  stream in the presence of (a) 0, (b) 10 mM  $H_2O_2$ , and (c) 10 mM  $H_2O_2$  + 5 mM  $Fe^{2+}$  under a scan rate of 20 mV/s. (B) FIA responses of 5 mM  $H_2O_2$  at the NPt-SPUME under  $N_2$  stream and with the spike of (a) 0.1, (b) 0.25, (c) 0.5, (d) 1, and (e) 2 mM  $Fe^{2+}$ , respectively. The insets show FIA responses for six injections of 25  $\mu$ M  $Fe^{2+}$  and the obtained calibration curve.



**Fig. S3** (A) Typical cyclic voltammograms of the NPt-SPUME under 100  $\mu L/min$   $N_2$  stream in the presence of (a) 0, (b) 10 mM  $H_2O_2$ , and (c) 10 mM  $H_2O_2$  + 1 mM  $Fe^{2+}$  under a scan rate of 20 mV/s. (B) FIA response for the measurement of  $I_{H_2O_2(consumption)\%}$ .

**Table S1. Antioxidative Activities of Phenolic Compounds and Catechins**

AA*		Pyrogallol*	CA*	Gallic acid*
5%	39.29	32.43	47.20	50.40
10%	64.71	57.60	84.64	97.28
30%	168.49	176.42	249.28	299.87
50%	304.69	333.78	460.96	566.53
70%	503.91	580.93	762.40	1021.13
90%	916.96	1141.76	1275.36	1806.12
EGC**		AA**	EGCG**	4-HBA**
5%	25.33	39.28	29.06	566.33
10%	43.76	59.65	53.02	869.46
30%	112.34	138.81	155.85	1483.78
50%	200.11	231.06	290.47	1878.00
70%	333.02	366.93	491.50	2254.58
90%	602.24	646.43	738.24	2816.67

The values were calculated by the time for 5–90% consumption of H<sub>2</sub>O<sub>2</sub> oxidation current at the Nf/Pt-SPUME. The solution was 0.1 M H<sub>2</sub>SO<sub>4</sub> (pH = 3) in the presence of 1 mM H<sub>2</sub>O<sub>2</sub> and 50 mM antioxidants and the addition of (\*) 25 μM and (\*\*) 50 μM FeSO<sub>4</sub>. Time unit is second.

