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ORIGINAL PAPER

Ultrasensitive non-enzymatic glucose sensing at near-neutral pH values via anodic stripping voltammetry using a glassy carbon electrode modified with Pt_3Pd nanoparticles and reduced graphene oxide

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Abstract We describe an anodic stripping voltammetric (ASV) method for glucose sensing that widely expands the typical amperometric i-t response of glucose sensors. The electrode is based on a working electrode consisting of a glassy carbon electrode modified with Pt-Pd nanoparticles (NPs; in an atomic ratio of 3:1) on a reduced graphene oxide (rGO) support. The material was prepared via the spontaneous redox reaction between rGO, $PdCl_4^{2-}$ and $PtCl_4^{2-}$ without any additional reductant or surfactant. Unlike known Pt-based sensors, the use of Pt_3Pd NPs results in an ultrasensitive ASV approach for sensing glucose even at near-neutral pH values. If operated at a working voltage as low as 0.06 V (vs. SCE), the modified electrode can detect glucose in the 2 nM to 300 μM concentration range. The lowest detectable concentration is 2 nM which is much lower than the LODs obtained with other amperometric i-t type sensing approaches, most of which have LODs at a μM level. The sensor is not interfered by the presence of 0.1 M of NaCl.

Introduction

Stripping methods, particularly anodic stripping voltammetry (ASV) methods, are widely applied as alternatives to spectroscopic techniques in the determination of trace metal ions, because of their accuracy and precision, as well as the low cost of instrumentation for these methods [1, 2]. Their remarkable sensitivity is attributed to the prior electrolytic accumulation of the compounds to be determined on the working electrode (WE) [3, 4]. In most cases, Hg based electrodes are used as the WE, when the analytes are metal ions because most metal ions are easily deposited on and stripped from the Hg film. Because of the high toxicity of Hg, since 2000 bismuth film electrodes (on glassy carbon and carbon fiber substrates) have been introduced for ASV [5]. The mechanism and application ranges are almost the same as those using Hg-based electrodes. In the early stages of the 21st century, new materials for ASV and extended the range of application are challenging and meaningful [6].

Diabetes mellitusis a world-wide public health problem and it was estimated that in 2014 9 % of adults 18 years and older had diabetes. In 2012 diabetes was the direct cause of 1.5 million deaths (data from the WHO) http://www.who.int/mediacentre/factsheets/fs312/en/index.html. The diagnosis and control of diabetes mellitus requires close monitoring of blood glucose levels. As a result, the increasing demand for glucose sensors with high sensitivity and selectivity, good stability, fast response, and low cost has driven tremendous research efforts for several decades [7]. Depending on the usage of glucose

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oxidase, electrochemical glucose sensors can be divided into enzymatic or non-enzymatic types. Among them, the amperometric method (i-t) has commonly been used for glucose sensing. Generally, the lowest detectable concentration of glucose reaches μM level [8, 9] and a more sensitive approachs rarely reported for an electrochemical glucose sensor.

In non-enzymatic glucose sensors [10, 11], nano-sized noble metals such as Pt [12, 13], Pd [14, 15] and Au [16, 17] have been used because of their high catalytic activity. The mechanism of electrocatalytic glucose oxidation using Pt group metals has been well studied. At a potential lower than −0.3 V (vs a saturated calomel electrode), dehydrogenation of the glucose molecule at the hemiacetalic carbon 1 atom (C1) occurs, and adsorption of the glucose molecule onto the Pt surface follows. When the potential is positively moved forward, accompanied by the generation of catalytically active OH_{ads} and subsequently PtO, catalytic oxidation of the absorbed glucose and the bulk glucose solution occur, and the two anodic oxidation current peaks are used to quantify the glucose concentration [18–20].

Alloys usually exhibit better catalytic activity than monometal catalyst. Pt and Pd share the same face-centered cubic (fcc) structure, together with good miscibility and a minor lattice mismatch of 0.77 %, it is feasible to optimize the performance of Pt-Pd nanostructures [21, 22]. In previous studies, we report the spontaneous redox activity between $PdCl_4^{2-}$ and graphene oxide (GO) [23], $PtCl_4^{2-}$ and reduced GO (rGO) [24]. In this study, using rGO as a reductant, clean Pt/Pd nanoparticles (NPs) supported on rGO (Pt₃PdNPs/rGO, the atomic ratio of Pt:Pd is 75.3 : 25.7, close to 3:1, from the following characterization) were synthesized without any additional surfactant or reductant. Taking into account the electrocatalytic procedures in the non-enzymatic glucose sensing, including the adsorption of the glucose molecule onto the Pt surface at lower applied potential and the catalytic oxidation of absorbed glucose at a higher potential, we found that the procedure was much similar to that of ASV. In this paper, we reported an ultrasensitive non-enzymatic glucose sensor using ASV combined with a Pt₃PdNP/rGO modified electrode for the first time. Unlike known Pt-based sensors, the use of Pt₃Pd NPs results in an ultrasensitive ASV approach for sensing glucose even at near-neutral pH values.

Experimental

Materials and reagents

Graphite powder was purchased from Lvyin Co. (Xiamen, China); $KMnO_4$, H_2SO_4 and HNO_3 were obtained from the Chemical Reagent Company of Shanghai (China); K_2PtCl_4 and K_2PdCl_4 were purchased from Wake Pure Chemicals,

Co. Ltd. (Osaka, Japan); and glucose was from the Chemical Reagent Company of Guangzhou (China). 0.05 M phosphate buffer (pH 7.4) was employed as a supporting electrolyte. The rod glassy carbon electrode (GCE, diameter 3.0 mm) was from BAS Co. Ltd. (Japan). All other reagents were of analytical grade and used without further purification. The pure water for solution preparation was from a Millipore Autopure WR600A system (Millipore, Ltd., USA).

Synthesis of graphene oxide (GO) and reduced graphene oxide (rGO)

GO was prepared from natural graphite using the modified Hummers' method [25]. The GO was dispersed in water to obtain a 0.5 mg mL^{-1} yellow-brown aqueous solution under ultrasonication for 3 h. Then, 50 mM KOH was added and the mixture was refluxed at 100 °C for 20 h to obtain rGO solution [24].

Synthesis of Pt₃PdNP/rGO

In a typical experiment, a mixture of 0.15 mL 10 mM K_2PdCl_4 and 0.45 mL 10 mM K_2PtCl_4 was slowly added to 1 mL 0.5 mg mL^{-1} rGO solution under vigorous stirring. Then, the mixture was sealed in a 5 mL autoclave and maintained at 90 °C for 2 h. After reaction, the mixture was centrifuged and washed using 50 mM H_2SO_4 and then pure water four times to remove the remaining reagents. Finally, the sediment was redispersed in 0.5 mL H_2O.

Modification of a glassy carbon electrode

0.5 mL of the Pt₃PdNPs/rGO suspension was dispersed in 0.5 mL of 0.5 % Nafion ethanol solution, then, 5 μL of the mixture was deposited on the polished GC electrode (diameter 3 mm) and dried in the air for 4 h at room temperature.

Instruments

The morphology of the Pt₃PdNPs/rGO was examined using a high resolution transmission electron microscope (HRTEM, FEI Tecnai-F30 FEG, Netherlands). The elemental composition was determined using an energy dispersive X-ray spectrometer (EDS, EDAX Phoenix,USA) that was attached to the HRTEM system. Electrochemical measurements were performed using a CHI 660B Electrochemical Analyzer (CHI Co. Shanghai, China) equipped with a conventional three-electrode system including a GCE coated with Pt₃PdNPs/RGO (5 μL) film as the WE, a Pt line as the auxiliary electrode and a saturated calomel electrode as the reference electrode.

Results and discussion

Characterization of Pt$_3$PdNPs/rGO

The TEM and HRTEM images depicted in Fig. 1a to c are direct morphological observations of the Pt$_3$PdNPs/rGO. As shown in Fig. 1a, the characteristic wrinkles on the sheets indicated the edge of the rGO. The Pt$_3$PdNPs were supported on the rGO sheets at a rather high density, and single NPs with a diameter around 3 nm were homogeneous and well dispersed (Fig. 1b). From the HRTEM image (Fig. 1c), it can be clearly seen that several NPs were assembled together, indicating the structure of the Pt$_3$PdNPs composite. Because Pd was more easily deposited on RGO and the atomic ratio of precursor Pt:Pd was 3:1, it seemed that the PtNPs were assembled around the PdNPs. The interplanar spaces of the composite NPs were 0.229 and 0.222 nm, which agrees well with the (111) lattice spacing of face-centered-cubic Pt (0.227 nm) and Pd (0.224 nm). Fig. 1d shows a typical EDS analysis of the prepared Pt$_3$PdNPs/RGO sheets, in which obvious Pt and Pd peaks were found. Furthermore, an atomic ratio of Pt:Pd at 75.3 : 25.7 was obtained, indicating that K_2PdCl_4 and K_2PtCl_4 were well reduced by RGO and that Pt$_3$PdNPs were successfully attached onto the RGO sheets.

Electrocatalytic oxidation and the ASV detection of glucose

The result from the cyclic voltammetry (CV) of Pt$_3$PdNPs/RGO in 0.5 M H_2SO_4 is presented in Fig. 2a. Hydrogen adsorption at the potential region from −0.23 to 0.08 V was obviously observed, indicating the large electrochemically active surface area of the Pt$_3$PdNPs/RGO. The oxidation and reduction current peaks around 0.90 and 0.50 V showed the typical features of a Pt/Pd based composite modified electrode [26]; while in the phosphate buffer blank, an additional reduction current peak was observed at −0.20 V (Fig. 2b), which was attributed to the reduction of the oxygenated groups on the RGO sheets [27]. The CV response became complicated when 10 mM glucose was added into the phosphate buffer, since the adsorption of glucose on Pt$_3$PdNPs generated an anodic current peak around −0.4 V; the peak at 0.08 V was attributed to electrocatalytic oxidation of the absorbed glucose; while the anodic peak at 0.70 V was caused by the direct oxidation of bulk glucose solution on the Pt$_3$PdNPs. These results suggested that Pt$_3$PdNPs had excellent electrocatalytic ability in the oxidation of glucose, and the electrochemical behavior was quite consistent with previous studies [26, 27]. Thus, the ASV method was considered for the determination of glucose.

Figure 3a shows the potential-time waveform of ASV. In the first step, Pt$_3$PdNPs/RGO modified GCE was maintained at 0.67 V for 15 s. At this potential, high catalytically active

PtO formed to electrooxidation the absorbents and generated a clean surface of the Pt$_3$PdNPs/RGO when the potential was low enough to reduce the PtO formed [20]. Next, the potential was set to −0.45 V to concentrate glucose on the surface of the Pt$_3$PdNPs/RGO. In order to accelerate the diffusion progress, the solution was kept stirred. In this step, dehydrogenation occurred on the C1 of the glucose molecule. After being concentrated for 30 s, the solution was then kept static to insure the equilibration of glucose at both the bulk solution and electrode surface. Finally, in the stripping step, the preconcentrated glucose was electrocatalytically oxidized and this resulted in an obvious anodic peak around 0.1 V. The CV and LSV experimental results at the same solution conditions were compared and, as shown in Fig. 3b, without preconcentration, no current peak was observed in LSV, while comparing to that of ASV, only a rather small CV peak was found. These results indicated that the glucose was largely absorbed on the Pt$_3$PdNPs and that the preconcentration of glucose effectively improved the response sensitivity.

Figure 4a shows the ASV responses in a glucose range concentration from 0 to 200 μM. Each measurement was repeated three to four cycles until a stable response was achieved. For convenience, only the potential region from −0.30 to 0.25 V is shown. It is obvious that the current intensity around 0.06 V increased with the increasing concentration of glucose. As shown in Fig. 4b, current response was observed even at a glucose concentration of 2 nM, indicating the very high sensitivity of the developed ASV method compared to the amperometric i-t type of electrochemical glucose sensor [8, 9]. Figure 4c and d present the relationship between the amperometric responses $(I - I_0)$ at 0.06 V and the glucose concentrations corresponding to the results of Fig. 4a and b. As shown in Fig. 4c, it was found that the sensitivity at higher concentration glucose was lower than the sensitivity at low concentration since only lower electrocatalytic activity OH_{ads} was generated on the surface of Pt$_3$PdNPs at the potential around 0.1 V [19]. Once the concentration of glucose reached a high level, it was unable to electrocatalytically oxidize the absorbed glucose completely, resulting in a block of active sites and loss of sensitivity. As shown in Fig. 4d, the peak current was linearly depended on the logarithm of glucose concentration in the range of 2 nM to 5 μM with a correlation coefficient of 0.9908 and a linear equation $(I - I_0)/\mu A = 0.8592 + 1.3061 \log(C/nM)$. Comparisons with some previously reported non-enzymatic glucose sensing methods and materials were made and the results are given in Table S1 (Electronic Supplementary Material). The advantage of the proposed glucose sensor was obvious and it showed ultrasensitive performance.

Pt based electrocatalysts are easily poisoned in physiological environments due to a high concentration of Cl^-, resulting in a low sensitivity in analytical applications [26]. In our experiment, the performance of Pt$_3$PdNPs/RGO was further

Fig. 1 **a** and **b** TEM images, **c** HRTEM image and **d** EDX spectrum of Pt$_3$PdNPs/RGO.
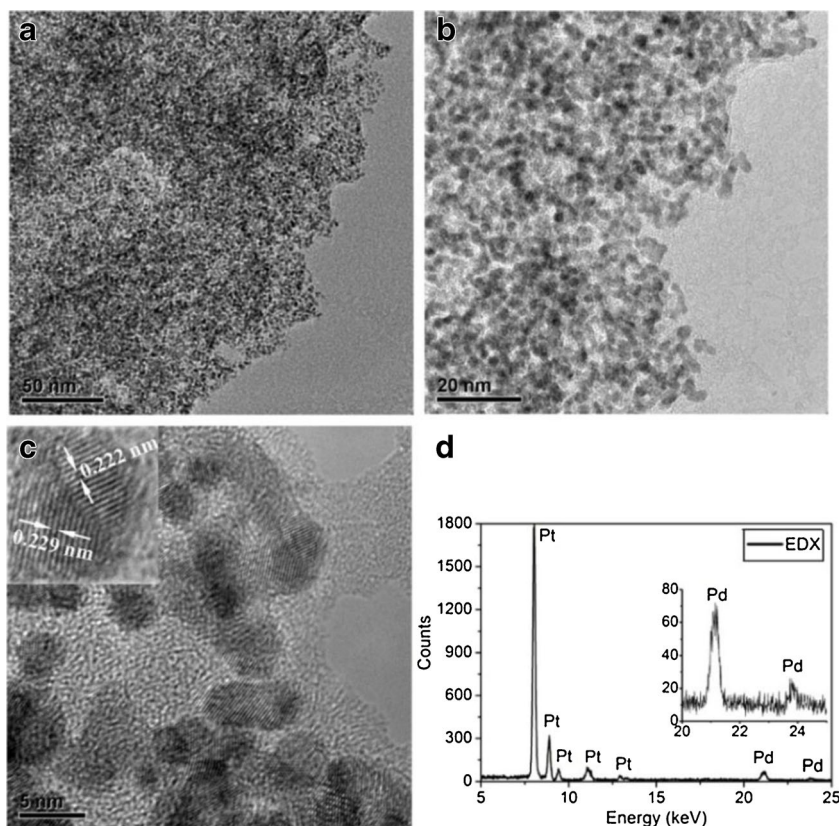
investigated in the presence of 0.1 M NaCl. As shown in Fig. S1a, an interference anodic peak appeared around 0.0 V, however, an obvious anodic peak from the oxidation of glucose can still be observed at a low concentration of 500 nM. Generally, the normal physiological level of glucose is around 3 to 8 mM. For the linearity experiment, a glucose concentration ranging from 0.2 to 9 mM was selected. Fig. S1c presents the relationship between the amperometric responses $(I-I_0)$ at 0.60 V and the glucose concentrations. The linear equation $I/\mu A=5.0915+5.5782(C/mM)$ and a linear coefficient of 0.9833 were obtained. These results indicated that the non-enzymatic sensing method for glucose developed was of high sensitivity, stability and with a wide linear range. An amperometric i-t experiment was also conducted for comparison. As

shown in Fig.S2a and S2b, obvious current responses were observed when the concentration of glucose reached 5 and 25 μM in the absence and presence of 0.1 M NaCl, respectively. The lowest detectable concentration of glucose was much better than that obtained using the ASV method under the same conditions. The linear range was 30 μM to 3.0 mM in the presence of 0.1 M NaCl, and the linear equation was $I/\mu A=0.8188+1.5187(C/mM)$ with a linear coefficient of 0.9964. The sensitivity was 1.52 μA mM^{-1}, which was only 1/5 that of the ASV method.

In nonenzymatic glucose ASV detection, the interfering is still a major challenge. The co-existing oxidizable compound such as ascorbic acid, uric acid, fructose or p-acetamidophenol may generate electrochemical signal. Since high active surface

Fig. 2 CVs of Pt$_3$PdNPs/RGO modified GCE **a** in 0.5 M H$_2$SO$_4$ at a potential scan rate of 50 mV s^{-1}, **b** in 0.05 M phosphate buffer at pH 7.4 containing 10 mM glucose at a scan rate of 20 mV s^{-1}
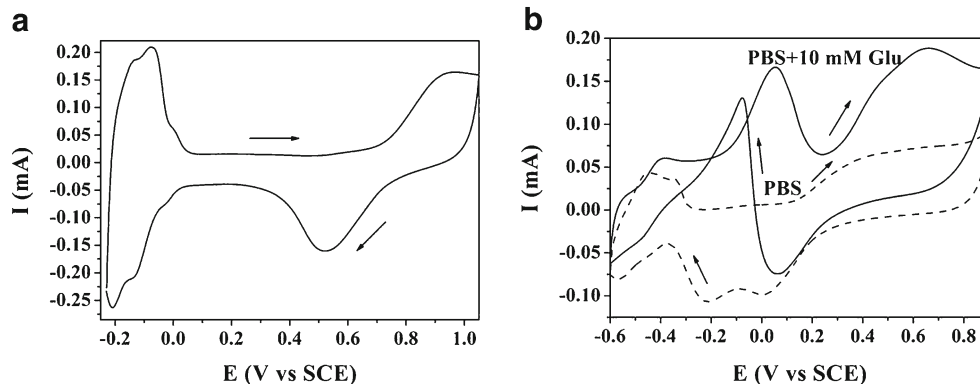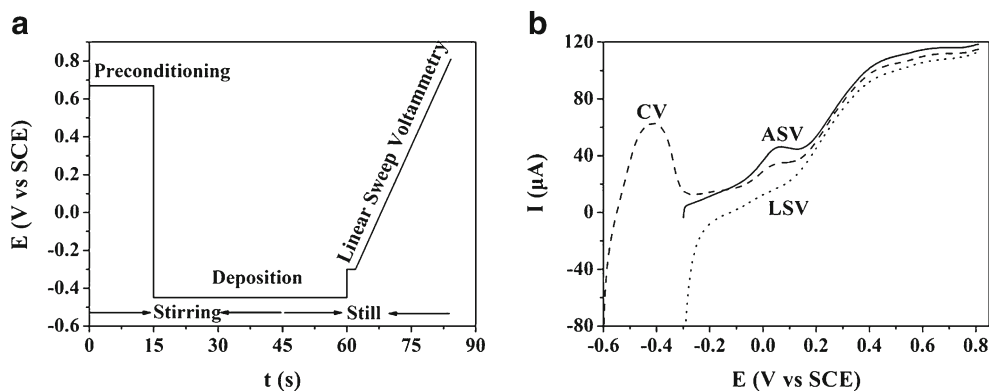
area favors faradic currents associated with kinetically controlled sluggish reactions such as the oxidation of glucose to a greater extent than a diffusion controlled reactions as the oxidation of interfering species [28], our previous study revealed that nanostructure electrodes can be applied to conquer the poor selective problem met with in nonenzymatic glucose sensing [29]. In the experiments, glucose produced remarkable ASV signals comparing to the other four interfering species. Taken the current density of glucose as 100 %, the interference current density of ascorbic acid, uric acid, fructose or p-acetamidophenol at the same concentraction of glucose was found to be 13.8, 5.0, 8.5, and 4.5 %, respectively. In addition, in a normal physiological sample, glucose concentration (3–8 mM) is generally much higher than those of ascorbic acid

(0.1 mM), uric acid (0.02 mM) and p-acetamidophenol (0.1 mM), and their inference can be further reduced by the dilution of samples.

In addition, the storage stability of the modified GCEs was also investigated by comparing the changes of current response of the electrode, stored in a dry state at room temperature, before and after 2 weeks storage with additions of 100 μmol of glucose in phosphate buffer (pH 7.4) containing 0.1 M NaCl. It retained 87.6 % of its initial response current after the 2 weeks of storage. Concerning the reproducibility, the modified GCEs were evaluated by comparing their current responses in different batches. The responses of six different batches of electrodes to 1 μM glucose solution were independently tested. The result was satisfactory for the electrode-to-

$$(I-I_0)/\mu A = 0.8592 + 1.3061 \lg(C/nM)$$
$$R^2 = 0.9908$$
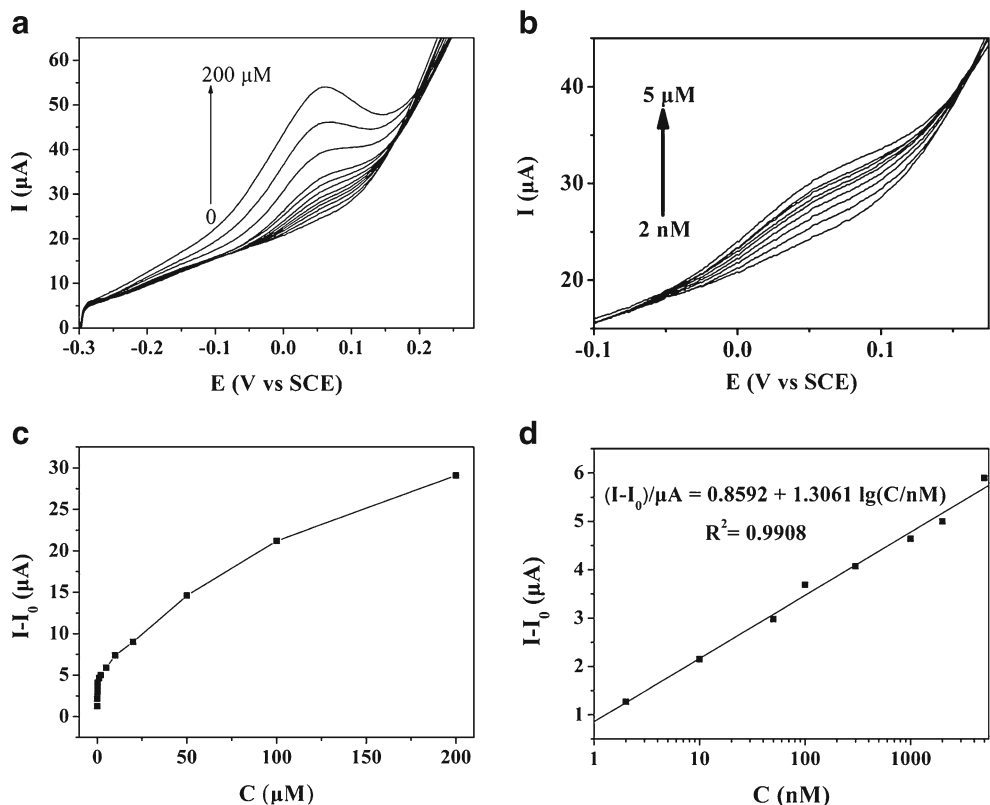
electrode reproducibility with a relative standard deviation (RSD) value of 6.6 %.

Conclusions

Pt$_3$PdNPs/RGO were synthesized using spontaneous redox between GO and PdCl$_4^{2-}$/PtCl$_4^{2-}$, without additional reductant or surfactant. Based on the electrocatalytic mechanism for glucose oxidation using Pt based electrodes, we present an ultra-sensitive non-enzymatic sensing approach for glucose using ASV. For the first time, an ASV type glucose sensor has been reported, in which a Pt based electrode (the Pt$_3$PdNPs/RGO modified electrode) was used as the WE in ASV. Because of the pre-concentration of glucose and the large surface of Pt$_3$PdNPs/rGO, the detectable concentration of glucose at 2 nM is much lower than those reported for other electrochemical glucose sensors, which is much lower than that previously reported for amperometric i-t type sensors. In addition, the non-enzymatic sensing approach usingPt$_3$PdNPs/RGO presented high sensitivity in the presence of 0.1 M NaCl. It is believed that this platform and concept were extended to other electrooxidation systems for the sensitive non-enzymatic sensing of small molecules.

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