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Design of two electrode system for detection of antioxidant capacity with photoelectrochemical platform

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ABSTRACT

Recently, a flow photoelectrochemical cell has been first developed and applied to assay global antioxidant capacity in our group. Yet, shortcomings of liquid reference electrode such as sample contaminations from the leaking of the reference solution, mechanically fragile, temperature and light sensitivity, *etc.* are significant restrictions for integration and miniaturization of photoelectrochemical sensing instruments, which have greatly limited their practical applications. Bearing these problems, in this work a novel two electrode flow photoelectron–chemical system (two-EPCS) has been developed for detection of antioxidant capacity. It is noteworthy that the electrochemical modulation-free mode (detection at the potential of 0.0 V) is performed, which has greatly simplified the analysis process and will result in significant simplifications of the instrument integrations. During the sample analysis, both standard antioxidants and commercial beverages were detected. Results evaluated from the two-EPCS are well agreed with those of the traditional three-EPCS at low potentials. By unloading of the reference electrode, it is of great convenience to design a novel photoelectrochemical microfluidic chip based on the two-EPCS, which has also been successfully applied for antioxidant capacity assay. It is satisfactory that comparable detection concentration range and sensitivity were accomplished by applying the microfluidic chip technique. Moreover, the two-EPCS is verified to be a universal platform which does not depend on selected optoelectronic materials but pervasive for general photocatalysts. Such a two-EPCS should be considered as a feasible alternative to the three-EPCS, which will become a promising candidate for industrial and commercial photoelectrochemical sensing instrument integrations in the future.

1. Introduction

Photoelectrochemical technique has attracted tremendous attentions since it combined merits of both optical and electrochemical methods, which has been applied as efficient strategy to develop DNA sensor (Liang and Guo, 2007; Li et al., 2014; L. Wang et al., 2014; W. Wang et al., 2014), cytosensing (Qian et al., 2010; W.-W. Zhao et al., 2012a, 2012b; X. Zhao et al., 2012), enzymatic analysis (Yildiz et al., 2008), immunoassay (Zhao et al., 2012a, 2012b, 2012c; W.-W. Zhao et al., 2012a, 2012b; X. Zhao et al., 2012) and many other small molecules sensing (Long et al., 2011; Golub et al., 2009; Pardo-Yissar et al., 2003; Tu et al., 2010) *etc*. However, just as the traditional electrochemical approach, during the photoelectrochemical detections, the work electrode is easily fouled by the productions, which will result in the gradually attenuation of the detection signal. In order to improve the recyclability of the work electrode, a flow photoelectrochemical cell with thin layer structure has been developed to assay global antioxidant capacity in our previous study (Ma et al., 2014). Yet, intrinsic shortcomings of the liquid reference electrode exhibited quite a few restrictions to flourish the flow photoelectrochemical cell system into industrial applications as well as the corresponding commercial instruments. As is known that, sample contaminations from the leaking of the reference solution (Peters, 1997; Zhang et al., 2012), mechanically fragile, temperatures sensitivity (Oijerholm et al., 2009) and light sensitivity (Ansuini and Dimond, 1994), *etc*. are typical inevitable drawbacks of the liquid reference electrode. In addition, another serious defect should be the unavailability of a reliable miniature liquid reference electrode (Suzuki et al., 1998; Vonau et al., 2010; Suzuki et al., 1999), which has significantly

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restricted the design of high throughput integration of electrode array and their biomedical applications. In response to these problems, amount of methods have been developed by researchers, *e.g.*, utilizing of all solid state reference electrode (Vonau et al., 2010; F.-X. Rius-Ruiz et al., 2011; F.X. Rius-Ruiz et al., 2011; Michalska, 2012), introducing of screen printed electrode (F.-X. Rius-Ruiz et al., 2011, F.X. Rius-Ruiz et al., 2011; Liao and Chou, 2006) and bipolar electrodes (Mavre et al., 2010; Zhu et al., 2014) *etc*. Although great progresses were achieved, there are still many problems need to be urgently solved upon the practical applications of the photoelectrochemical technique.

In this research, to face these challenges, a novel two electrode flow photoelectrochemical system (two-EPCS) has been designed for detection of global antioxidant capacity without employment of the reference electrode. It is gratified that results of such a two-EPCS are well agreed with that of the traditional three electrode flow photoelectrochemical system (three-EPCS) at low potentials. Based on this, the technique of the two-EPCS photoelectrochemical microfluidic chips have been designed and successfully applied for antioxidant capacity assay. In addition, several optoelectronic materials were performed to construct the two-EPCS, all of which showed favorable responses. Therefore, the advisable universality of the two-EPCS should make itself a general strategy for miniaturization of photoelectrochemical sensing instruments in industrial and biomedical application.

2. Experimental section

2.1. Reagent

Caffeic acid (CA), (+)-catechin hydrate (CT), L-cysteine (Cys), glucose, melamine and Folin–Ciocalteu (F–C) reagent (10%) were received from Sigma-Aldrich. Titanium trichloride ($TiCl_3$), Gallic acid (GA), Zinc acetate dehydrate (Zn $(AC)_2$ $2H_2O$) and cadmium acetate dehydrate (Cd$(AC)_2$ $2H_2O$) were purchased from Alfa. (−)-Epigallocatechin gallate (EGCG) was gained from J&K Chemical. Other reagents were used as received without purification. The PBS buffer was made from sodium phosphate (NaH_2PO_4/Na_2HPO_4, 81:19 (molar ratio)) and sodium chloride dissolved in deionized water at final concentrations of 10 mmol L^{-1} (pH: 7.4).

2.2. Instruments

All electrochemical experiments were performed with a CHI660A Electrochemical Workstation (CHI). A thin layer three-EPCS, comprising ITO or modified ITO as the working electrode, a platinum wire as the auxiliary electrode, and an Ag/AgCl electrode (3 mol L^{-1} KCl) as reference electrode was used in the photoelctrochemical measurement. The structure of the two-EPCS is similar with that of the three-EPCS except without an Ag/AgCl electrode. 0.1 mol L^{-1} PBS solution containing 10 mmol L^{-1} NaCl was applied as supporting electrolyte and bubbled with N_2 for 15 min before each experiment. LED light (420 nm or 545 nm, Beijing Perfectlight Technology) was employed as light source for photoelectrochemical sensor. The peristaltic pump was bought from longerpump (BT100-2J). The microfluidic chip was designed through photolithographic technique (MDA-400M, Positive photoresist RZJ-390G, Suzhou Ruihong Electronic Chemical Co., LTD). The microfluidic chip was sputtered with thin layer gold via the vacuum sputter coater (SCD 050, 3Δ-TEC). The UV–visible spectrum was performed on a Hitachi U-3900 spectrophotometer at 765 nm. X-ray photoelectron spectroscopy (XPS) was recorded with an ESCALAB-MKII250 photoelectron spectrometer with Al Kα X-ray radiation as the X-ray source for excitation.

2.3. Preparation of the optoelectronic materials

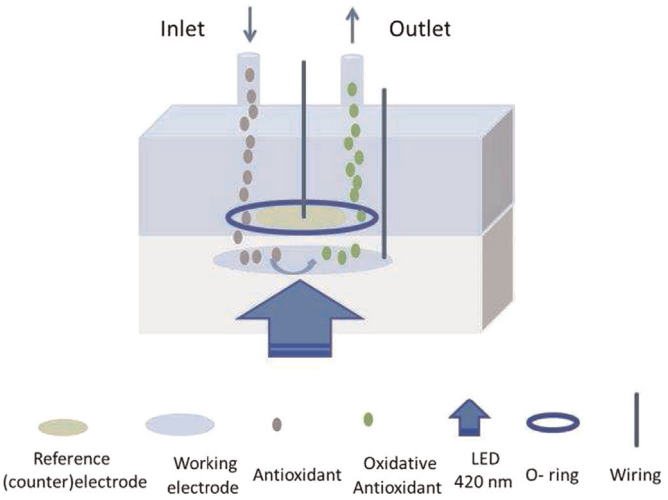
First, ultrathin graphitic carbon nitride (utg-C_3N_4)/TiO_2 were synthesized by our previous method (Ma et al., 2014). To prepare ZnO/utg-C_3N_4 directly from utg-C_3N_4, utg-C_3N_4 (40 mg) and Zn $(AC)_2$ $2H_2O$ (87.3 mg) were dispersed in DMSO (40 mL). After vigorous stirring, the solution was transferred into a Teflon-lined stainless steel autoclave (50 mL) and reacted under 180 °C for 12 h. The obtained solution was then washed extensively with acetone and alcohol in a sonication washer to remove non-reacted reactants. Finally, the product was centrifuged at 5000 rpm, and dried in a vacuum drier at 60 °C. To obtain CdS nanoparticles, 106 mg Cd $(AC)_2$ $2H_2O$ was dissolved in 40 mL DMSO and then transferred into a Teflon-lined stainless steel autoclave (50 mL) and reacted under 180 °C for 12 h. Other steps are similar with the synthesis of ZnO/utg-C_3N_4. The evidences for the structural properties of the ZnO/utg-C_3N_4 and CdS nanoparticles composites from XPS were presented in Fig. S1 (in Supporting information).

2.4. Preparation of the work electrode

After an ITO electrode was cleaned with NaOH (1 mol L^{-1}) and H_2O_2 (30%), washed with acetone and twice-distilled water and dried at room temperature, a certain amount of photocatalyst suspension, for example 100 μL of utg-C_3N_4/TiO_2 (1 mg mL^{-1}), was cast onto the ITO electrode and dried at room temperature to obtain the photocatalyst modified ITO electrode. Then, the working electrode was ulteriorly dried at 70 °C for 12 h to improve the adhesion. The utg-C_3N_4/ZnO or CdS modified ITO electrode was prepared analogously.

2.5. Assay of antioxidant capacity

The measurement process of the two-EPCS was illustrated in (Scheme 1). After the two-EPCS was successfully fixed, the buffer or sample solution was injected into the flow cell with assistant of the peristaltic pump at 2 rev min^{-1}. During the detections, the light irritated from the backside of the modified ITO electrode (LED, 420 nm), which can effectively prevent interference from the colored samples. Each sample was detected for three times and the average value was recorded. The photoelectrocurrent was collected following the principle: $I=I_{smpale}-I_{blank}$ (I_{smpale}: the photoelectrochemical current with sample, I_{blank}: the photoelectrochemical current without sample). In addition, CdS modified ITO electrode was irritated by the 545 nm LED. Other conditions



Scheme 1. Schematic of the two-EPCS for detection of antioxidant capacity.