An Electrochemical Cell Coupled with Disposable Screen-Printed Electrodes for Use in Flow Injection Analysis

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An electrochemical cell coupled with disposable screen-printed electrodes (SPEs) that is specifically designed for use in flow injection analysis (FIA) is described in this study. The cell is made of foldable polyoxymethylene (acetal) thick platelets with the bottom portion consisting of a cavity track to drag the SPEs in position and the top portion having pre-drilled T-like holes to arrange the Ag/AgCl reference electrode and stainless steel inlet & outlet. An "O ring" is suitably fixed on the top of the working electrode to form a thin-layer space where the electrochemical reaction can take place. Hydrodynamic characterization was validated by using a benchmark hexacyanoferrate redox couple. The results of practical analysis of glucose in human plasma clearly demonstrate the characteristics and applicability of the proposed wall-jet electrochemical cell in FIA.

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Introduction

Flow injection analysis with electrochemical detection (FIA-ECD) is normally operated under a current–time (*i*–*t*) mode at a fixed applied potential (E_{app}) where the analyte can get oxidized or reduced on the working electrode surface under a constant hydrodynamic flow (H_{f}) of the carrier base electrolyte.[1,2] The advantages of FIA-ECD include remarkable sensitivity, wide linear calibration range, low dead volume (usually < 20 μL), fast response time, and relatively simple instrumentation. It is frequently used in clinical, medicinal, biochemical, environmental, and immunochemical researches with/without the incorporation of high performance liquid chromatography (HPLC) or capillary electrophoresis (CE).[3–14] Furthermore, the bare electrodes can be chemically modified with redox mediator and enzymes for biosensor applications; a cavity type of FIA-ECD is also available for carbon-paste electrode preparation.[15,16] Despite the fact that commercially available FIA-ECD can provide high sensitivity with good resolution, it is not optimally suited for routine analysis. This is because the FIA-ECD's performance often depends on the condition of the electrode after a manual polishing procedure. To get away from the problem, screen-printed electrodes (SPEs) were developed for innovative applications in electroanalytical chemistry.[17] Low manufacturing cost, ease of mass production, and flexibility in design make disposable SPEs especially attractive in practical applications. In this work, we disclose a user-friendly design of an electrochemical cell that is suitable for coupling with SPEs (designated as SPE-ECD) for use in FIA. Note that the proposed button-and-lock-type of FIA-ECD device with special care given to reusability and precision in electroanalysis is different from some attempts in the past.[18–20]

Two kind of SPEs, *i.e.*, screen-printed carbon electrode (SPCE) and copper nanoparticle plated screen-printed carbon electrode (Cu-SPCE), together with various model systems of hexacyanoferrate(II), ascorbic acid, and carbohydrates with/without the linkage to HPLC were used to evaluate the proper function of the proposed FIA system. Finally, using the proposed setup, real sample analysis of glucose in human plasma was validated with a commercial YSI®-accredited glucose analysis instrument.

Experimental

Chemicals and reagents

Carbohydrate, ascorbic acid, and hexacyanoferrate(II) were obtained from Sigma (St. Louis, USA). Disposable SPCEs were purchased from Zensor R&D (Taichung, Taiwan). Polyoxymethylene (acetal) copolymer, a high performance engineering polymer that can resist impact, fatigue, and chemicals with high strength, stiffness, and low moisture absorption, was used to fabricate the electrochemical cell. All compounds (ACS-certified reagent grade) were used without further purification. Aqueous solutions were prepared with deionized water purified using a Millipore-Q purification system.

Apparatus

Cyclic voltammetric and chronoamperometric experiments were carried out with a CHI 900 electrochemical workstation (Austin, USA). The three-electrode system consists of either a SPCE or a Cu-SPCE working electrode (geometric area = 0.196 cm^2), an Ag/AgCl reference electrode, and a platinum auxiliary electrode. The FIA system contains a Cole-Parmer

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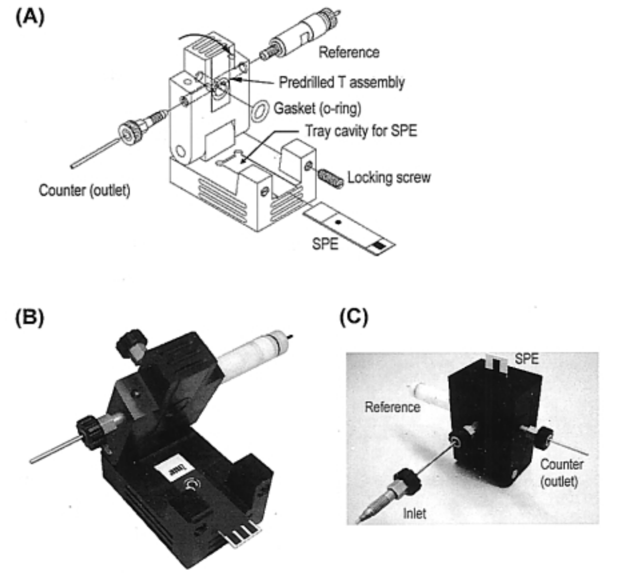

Fig. 1 Pictures of the proposed FIA/ECD device specifically designed for SPEs.

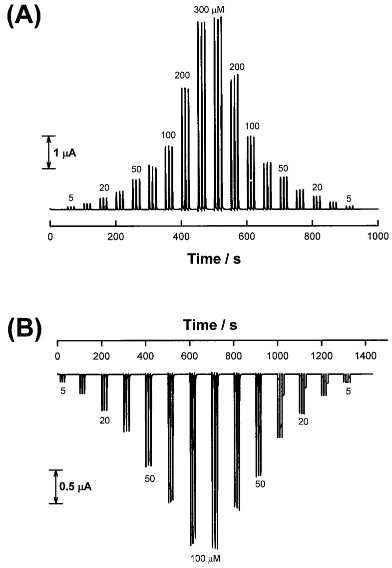

Fig. 2 FIA responses of $Fe(CN)_6^{3-}$ (A) and ascorbic acid (B) on a bare SPCE in 0.1 M KCl carrier solution at H_f = 1 mL/min and E_{app} = 0.0 and 0.6 V *vs*. Ag/AgCl for (A) and (B), respectively.

microprocessor pump drive, a Rehodyne Model 7125 sample injection valve (20 μL loop) with interconnecting Teflon tube, and the proposed SPE-ECD system. Standard glucose analyses were carried out using an YSI® glucose detection instrument (Yellow Springs, USA). An anion-exchange column (Hamilton RCX-10, serial No. 472) was used for the carbohydrate separation.

Procedure and electrode preparation

A Cu layer was electrochemically plated on a SPCE in 200 mg/L $Cu(NO_3)_2$ + 0.1 M HNO_3 solution at –0.7 V *vs*. Ag/AgCl for 300 s under photo-illumination to prepare the Cu-SPCE, as per our perviously reported procedure.[21] The SPCEs were first washed thoroughly with deionized water, and then dipped into working solution for electrochemical experiments. As to FIA, the SPCEs can simply slide into the holding trail and the device was locked as illustrated in Fig. 1. Note that the outlet system also serves as a counter electrode. More details about the design will be discussed in a later section.

Fifteen blood samples were collected from laboratory volunteers for real sample analysis. The plasma sample was prepared as follows: 2 mL of the blood was centrifuged at 2000 rpm for ~10 min in room temperature and the upper solution was then used for analysis in FIA. Samples were diluted by 200 times with 0.1 M NaOH prior to the injection.

Results and Discussion

Figure 1 sketches different structural views of the proposed button-and-lock-type assembly specifically designed for disposable SPEs in FIA. The device contains two major parts: (1) a tray-cavity arrangement to drag the working SPE inside the assembly and (2) a pre-drilled T-like assembly to hold the stainless-steel inlet, outlet, and reference electrode in position. An "O ring" is suitably fixed on the center to cover the SPE inside the working space. The wall-jet design through the center micro-hole can then guide the inlet portion to have a proper function on the SPE. The "O ring" assembly can effectively prevent the leakage of the carrier buffer in the setup. Both parts are connected as a padlock-type arrangement to allow for folding the device tightly with the working electrode. Finally, locking-type screws are also fixed to further tighten the folded device.

A benchmark redox couple, $Fe(CN)_6^{3-/4-}$, was first taken as a model system to validate the performance of the electrochemical cell coupled with a bare SPCE in FIA. Figure 2A shows typical responses for continuous injection (20 μL) of 5 – 300 μM $Fe(CN)_6^{3-}$ under experimental conditions of E_{app} = 0.0 V *vs*. Ag/AgCl and H_f = 1 mL/min using 0.1 M KCl as a carrier solution. As can be seen, well-defined FIA responses corresponding to the reduction of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ were observed. The same electrochemical setup was further applied to the oxidation of ascorbic acid (5 – 100 μM) at E_{app} = 0.6 V. As shown in Fig. 2B, well-defined oxidative FIA responses were once again obtained. The facts that the FIA signals are regular and that they systematically responded in both cases clearly indicates the appropriate hydrodynamic function of the proposed electrochemical cell. Meanwhile, continuous flow of 0.1 M KCl overnight also shows no solution leakage with virtually the same base current values as those found when using a bare SPCE. Overall, the performance of the proposed SPE-ECD is comparable to the commercial FIA-ECD using a classical glassy carbon electrode.[2,22,23] Most important of all, this observation validates the applicability of the SPE-ECD to electroanalysis by FIA.

As mentioned earlier, the screen printing fabrication technology is commonly used for large-scale production of inexpensive and yet highly reproducible electrochemical sensors. A particular advantage of the proposed SPE-ECD is the ability to readily exchange the sensing electrode, which should be very valuable for many practical applications. A broad range of important analytes can thus be exploited for detection owing to the variety of electrode materials and electrochemical processes. The application of Cu-SPCE as an electrochemical detector for the determination of sugars was demonstrated next. Previously, it has been found that cuprous oxide is an effective electrode modifier for the detection of sugars in FIA, giving a high sensitivity and selectivity.[24,25] The

Table 1 Results of determination of glucose in fifteen human plasma samples[a]

Sample	Linear equation	R^2	Detected glucose/mM		Net error, %
			Proposed method[b]	YSI®	
#1	$y = 2.219 \times 10^{-8}x + 1.189 \times 10^{-6}$	1.000	5.36	5.48	–2.21%
#2	$y = 2.261 \times 10^{-8}x + 1.502 \times 10^{-6}$	1.000	6.64	6.94	–4.29%
#3	$y = 1.359 \times 10^{-8}x + 1.298 \times 10^{-6}$	0.999	9.56	9.70	–1.42%
#4	$y = 1.449 \times 10^{-8}x + 6.847 \times 10^{-7}$	1.000	4.73	4.91	–3.83%
#5	$y = 2.789 \times 10^{-8}x + 1.519 \times 10^{-6}$	1.000	5.45	5.37	1.45%
#6	$y = 2.836 \times 10^{-8}x + 2.677 \times 10^{-6}$	1.000	9.44	9.85	–4.14%
#7	$y = 3.014 \times 10^{-8}x + 1.554 \times 10^{-6}$	1.000	5.16	5.17	–0.17%
#8	$y = 2.780 \times 10^{-8}x + 1.278 \times 10^{-6}$	1.000	4.60	4.64	–0.97%
#9	$y = 2.835 \times 10^{-8}x + 1.634 \times 10^{-6}$	0.999	5.76	5.69	1.34%
#10	$y = 1.734 \times 10^{-8}x + 1.024 \times 10^{-6}$	0.996	5.91	5.83	1.29%
#11	$y = 1.652 \times 10^{-8}x + 8.674 \times 10^{-7}$	0.999	5.25	5.26	–0.13%
#12	$y = 1.594 \times 10^{-8}x + 7.525 \times 10^{-7}$	0.999	4.72	4.53	4.10%
#13	$y = 1.572 \times 10^{-8}x + 1.049 \times 10^{-6}$	0.999	6.67	6.80	–1.84%
#14	$y = 1.477 \times 10^{-8}x + 9.720 \times 10^{-7}$	0.999	6.58	6.79	–3.14%
#15	$y = 2.491 \times 10^{-8}x + 1.134 \times 10^{-6}$	1.000	4.55	4.49	1.34%

a. Diluted by 200 times. b. Cu-SPCE as detector in 0.1 M NaOH by standard addition method.

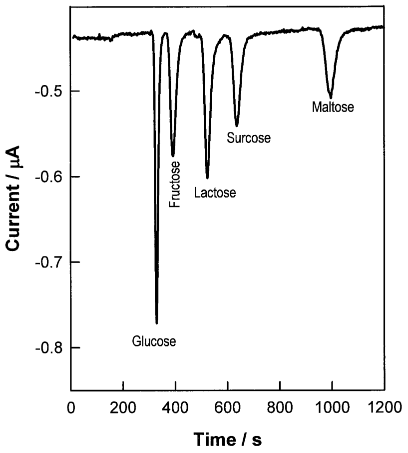

Fig. 3 Typical FIA responses of carbohydrate mixture (20 μM each) at a Cu-SPCE coupled with anion-exchange HPLC column using 0.1 M NaOH as carrier solution.

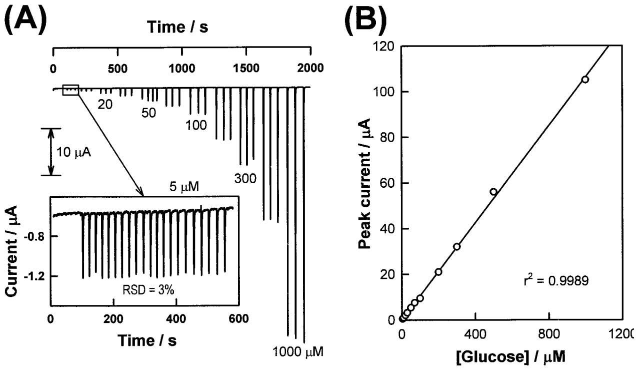

Fig. 4 FIA responses (A) and calibration plot (B) for the increasing glucose concentration on a Cu-SPCE in 0.1 M NaOH carrier solution at E_{app} = 0.6 V and H_f = 800 μL/min. Insert figure shows continuous injection of 5 μM glucose (n = 22).

employment of Cu-SPCE as an electrochemical detector for the determination of sugars is described in this study. Our group previously noticed profound electrochemical activity during detection assays of H_2O_2, *o*-diphenols, dissolved oxygen, and amino acids at this disposable Cu-SPCE.[26-29] The Cu-SPCE can be used for direct detection of sugars without the need of pre-derivatization processes, which are usually required in the spectroscopic detection method.[30,31] Note that another approach that does also not require derivatization and can be used with all HPCE systems equipped with a simple UV detector is indirect UV detection.[32,33] These methods include the use of a highly alkaline electrolyte to ionize the carbohydrates and to make them suitable for indirect UV detection. In this regard, the requirement of electrocatalysis in basic solutions thus presents no difficulty on separation. To demonstrate this, we tested the SPE-ECD coupled with a HPLC column with a mixture of sugar molecules. Figure 3 shows the response for the separation and detection of glucose, fructose, lactose, sucrose, and maltose using a Cu-SPCE. Note that the obtained results were comparable to those from a previous FIA study using a Cu-based electrode.[34] Furthermore, since the polyoxymethylene (acetal) material is highly stable in common organic solvents like methanol, ethanol, and acetonitrile, the cell can be operated not only in strong alkaline solution but also in organic solvents.

Finally, to further improve the accuracy and precision of the proposed system, we performed the FIA detection assay of glucose. Figure 4 shows the FIA responses and calibration plot for increasing concentration of glucose on a Cu-SPCE in 0.1 M NaOH carrier solution at E_{app} = 0.6 V. As can be seen, well-defined FIA responses corresponding to the oxidation of glucose were observed. The figure insert shows continuous injection (n = 22) of 5 μM glucose and the result of RSD = 3% again proves the applicability of the SPE-ECD to electroanalysis by FIA. As to real sample analysis, the detection of glucose in human plasma was also validated with an accredited YSI glucose detection instrument. Responses of standard addition method of the plasma sample (case #11 in Table 1) using the proposed system coupled with HPLC resulted in a value of 5.25 mM; such a result was very close to that of 5.26 mM as determined by a YSI glucose instrument. The analyses were then extended for 15 laboratory volunteers. Fresh prepared Cu-SPCEs were used for each set of experiments. Table 1 compares the observed data with those of YSI measurements. As can be seen, accurate and promising results were obtained from the SPE/ECD specifically designed for use in FIA.

Conclusion

In this report, an electrochemical cell specifically designed for disposable screen-printed electrodes for use in flow injection analysis is introduced. Quantitative analysis of benchmark electrochemical systems using SPCE and Cu-SPCE was successfully demonstrated with promising results. Real sample analysis for glucose in plasma also shows satisfactory results in comparison with those of a YSI-accredited instrument. The electrochemical setup coupled with chemically modified screen-printed electrodes can be applied to the environmental, biochemical, clinical and medicinal research fields. New electrode designs, like the ring-disc screen-printed electrode (RDSPE) and the all-in-one three-electrode SPE, can also be useful in electroanalysis. Numerous applications can readily be imagined from this study.

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