東北大学機関リポジトリ
TOUR
Tohoku University Repository

Simultaneous Multiplex Potentiostatic Electroanalysis with Liquid Junction Removed Reference Electrode System using a Closed Bipolar Electrode

Simultaneous multiplex potentiostatic electroanalysis with liquid-junction-removed reference electrode system using a closed bipolar electrode

Kumi Y. Inoue (Prof.)* Miho Ikegawa, Takahiro Ito-Sasaki (Dr.), Shinichiro Takano (Dr.), Hitoshi Shiku (Prof.), Tomokazu Matsue (Prof.)*

Graduate School of Environmental Studies, Tohoku University, 6-6-11 Aoba, Aramaki, Aoba, Sendai 980-8579, Japan

*Corresponding authors:
  Kumi Y. Inoue
    Tel: +81-22-795-6167   Fax: +81-22-795-6167
    E-mail: inoue@bioinfo.che.tohoku.ac.jp
  Tomokazu Matsue
    Tel: +81-22-795-7209   Fax: +81-22-795-7209
    E-mail: matsue@bioinfo.che.tohoku.ac.jp

Abstract

A novel liquid-junction-removed reference electrode system was developed for simultaneous multiplex amperometry using a closed bipolar electrode. The liquid junction between the reference cell and the sample cell was replaced by a bipolar electrode to ensure electron conductivity in lieu of ionic conductivity in conventional systems. Under the condition where the redox reaction of the analyte at the bipolar electrode in the sample cell was a limiting process, voltammetric and amperometric measurements were performed for quantitative detection of the analytes. After characterization of the system, a chip-type device was fabricated with six sample cells and one reference cell. Simultaneous determination of ferrocenemethanol was conducted in six solutions in the 0.1–0.5 mM range. This is the first demonstration of the simultaneous multiplex determination of analytes with a liquid-junction-removed system. Although the potential control problem, alternative to the liquid potential problem of conventional liquid junction, still remains by increase of the solid-liquid interface, this system is expected to solve some problems associated with the liquid junction of the reference electrode, including sample contamination by chloride ion, and the complicated process of fabricating a reference electrode on a chip.

In a conventional electrochemical system with a three-electrode configuration, a liquid junction is required for the reference electrode to complete the electrical circuit by allowing the ions to pass through the reference electrolyte and the sample. All types of liquid junctions, including salt bridges, ceramic plugs, and ground sleeves, cause problems such as sample contamination and stuck of junction. Furthermore, it is very hard to fabricate a liquid junction on an integrated system via microfabrication.[1] Previously, we proposed a galvanic cell system without a liquid junction for the accumulation step of substitutional stripping voltammetry using a closed bipolar electrode system (cBPES), [2] which is one form used in the application of bipolar electrochemistry. [3-5] The cBPES comprises a bipolar electrode connecting two separated electrochemical cells with a driving electrode in each cell. [6-11] By applying an adequate potential between the two driving electrodes, two conjugate electrochemical reactions occur at each end of the bipolar electrode. Under the condition that the target redox reaction proceeds as the rate limiting process of the whole system, quantitative measurements are available. In this paper, we report for the first time, a liquid-junction-removed reference system for potentiostatic multi-electroanalysis by amperometry and voltammetry. Figure 1 shows the schematic comparison between a conventional potentiostatic electroanalytical system that utilizes a reference electrode with a liquid junction (Fig. 1A) and our proposed system without a liquid junction (Fig. 1B). As shown in Fig. 1, the electron flow through the bipolar electrode completes the electric circuit of the measurement system and is an alternative to the movement of ions through the liquid junction. We first characterized our proposed system by cyclic voltammetry. Next, a chip-type device having six sample cells and one reference cell was fabricated to demonstrate quantitative amperometric detection using this liquid-junction-removed system.
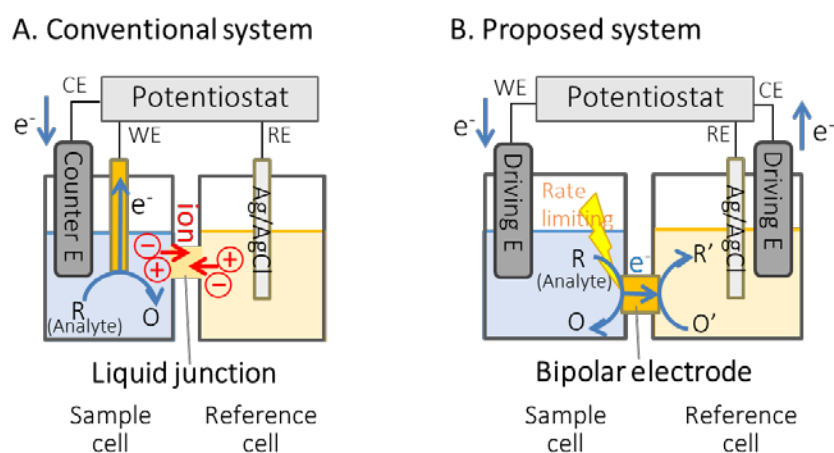

Figure 1. Schematic comparison of the (A) conventional potentiostatic electroanalytical system using a reference electrode with a liquid junction and (B) proposed system without a liquid junction.

We first confirmed our strategy using two separate electrochemical cells, as shown in Fig. 2A. In the sample cell, 0.5 mM ferrocenemethanol (FMA) was added as a model analyte. In the reference cell, a mixture solution of 2.5 mM $K_4[Fe(CN)_6]$ and 2.5 mM $K_3[Fe(CN)_6]$ with 100 mM KCl was placed in order to facilitate the redox reaction in the reference cell. To ensure that the redox reaction of FMA at the bipolar electrode in the sample cell (sensing electrode) was the rate-limiting process of entire system, an Au disc electrode (1.6 mm in diameter) was used in the sample cell that was sufficiently small relative to the other electrodes. Figure 2B (a) shows a cyclic voltammogram when the potential of the Pt driving electrode in the sample cell was swept against the Ag/AgCl reference electrode in the reference cell. A clear redox peak pair appeared at 0.102 V and 0.175 V. Note that the peak at 0.102 V corresponded to the oxidation of FMA and the peak at 0.175 V to the reduction of the oxidized form of FMA (FMA$^+$), contrary to the normal voltammogram. When the potential was swept from 0.40 V to the negative direction, the following electrochemical reactions occurred:
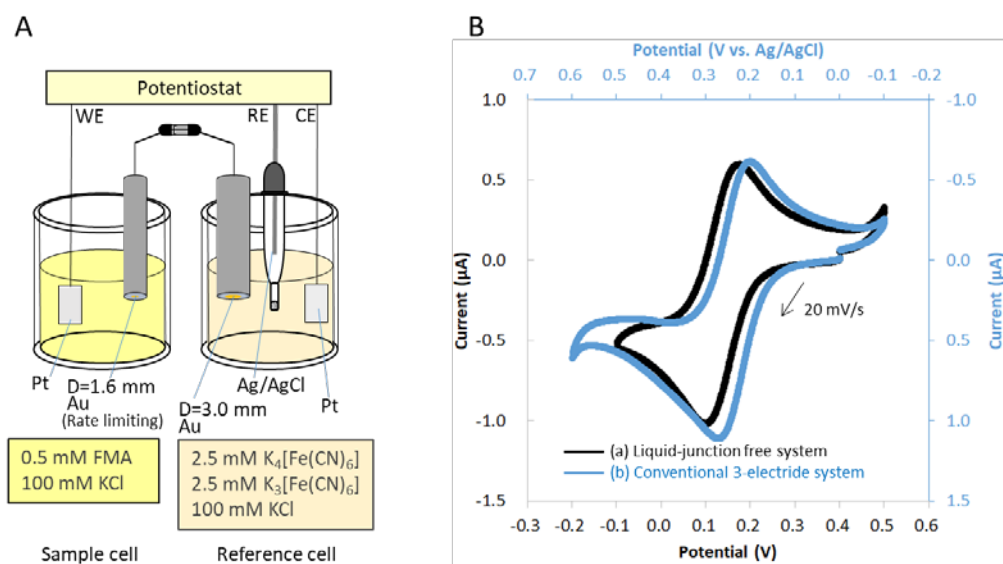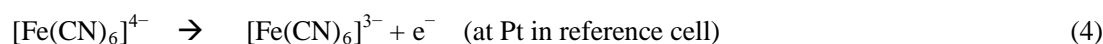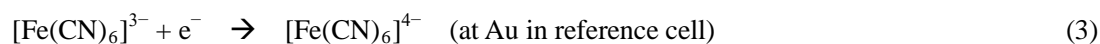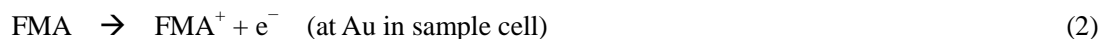
$$O_2 \text{ (dissolved)} + 4H^+ + 4e^- \;\rightarrow\; 2H_2O \quad \text{(at Pt in sample cell)} \tag{1}$$

$$FMA \;\rightarrow\; FMA^+ + e^- \quad \text{(at Au in sample cell)} \tag{2}$$

$$[Fe(CN)_6]^{3-} + e^- \;\rightarrow\; [Fe(CN)_6]^{4-} \quad \text{(at Au in reference cell)} \tag{3}$$

$$[Fe(CN)_6]^{4-} \;\rightarrow\; [Fe(CN)_6]^{3-} + e^- \quad \text{(at Pt in reference cell)} \tag{4}$$
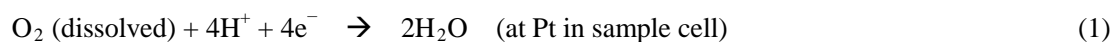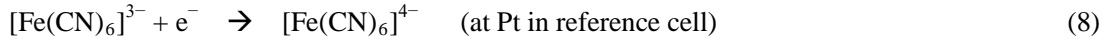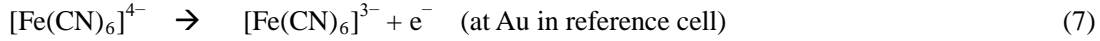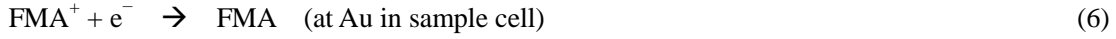

Figure 2. (A) System for experimental verification of a principle characteristic of the proposed potentiostatic electroanalytical system with a liquid-junction-removed reference electrode system. (B) Comparison of the cyclic voltammogram obtained with the system shown in (A) and that obtained with a conventional three-electrode system using a 1.6 mm diameter Au disc electrode as a working electrode. Sample was 0.5 mM FMA solution containing 100 mM KCl as the electrolyte.

As reaction (2) was the rate-limiting reaction, the voltammogram in the forward scan in Fig. 2 shows the oxidation peak of FMA. After the direction of potential sweep switched back to positive at -0.2 V, the following electrochemical reactions took place:

$$\text{FMA} \rightarrow \text{FMA}^+ + e^- \quad \text{(at Pt in sample cell)} \tag{5}$$

$$\text{FMA}^+ + e^- \rightarrow \text{FMA} \quad \text{(at Au in sample cell)} \tag{6}$$

$$[\text{Fe(CN)}_6]^{4-} \rightarrow [\text{Fe(CN)}_6]^{3-} + e^- \quad \text{(at Au in reference cell)} \tag{7}$$

$$[\text{Fe(CN)}_6]^{3-} + e^- \rightarrow [\text{Fe(CN)}_6]^{4-} \quad \text{(at Pt in reference cell)} \tag{8}$$

Therefore, the peak at 0.15 V corresponded to the reduction of FMA^+ (reaction (6)). To confirm the above phenomena, a cyclic voltammetry experiment was conducted with a conventional three-electrode system comprising a 1.6 mm-diameter Au disc electrode as the working electrode. Figure 2B (b) shows the resulting voltammogram with the upper potential-axis and right current-axis, both with inverted scales. As the shapes of the two voltammograms shown in Fig. 2B were nearly identical, it was confirmed that the present system without a liquid junction reproduced the voltammograms of a conventional system.
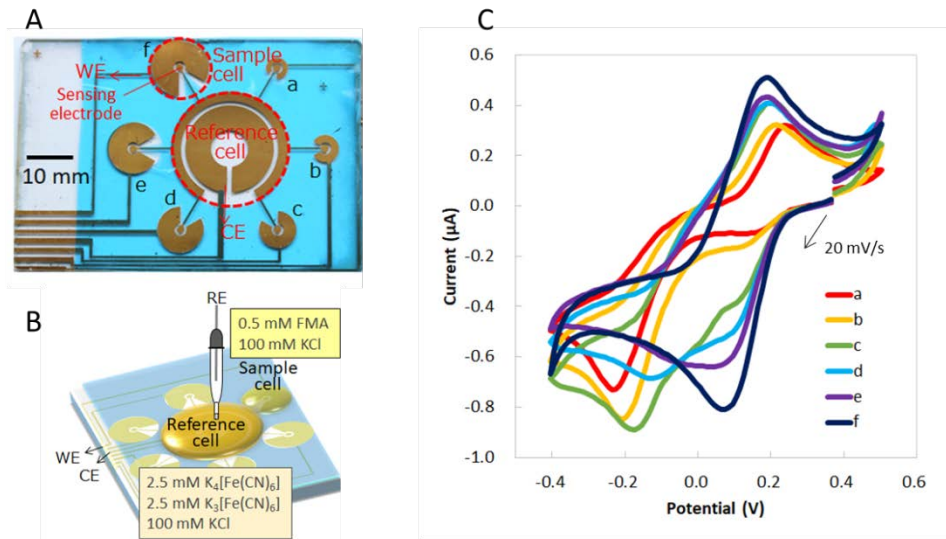

Figure 3. (A) Photograph of a fabricated chip-type device for size optimization of the driving electrode in the sample cell. The sizes of the driving electrodes in the sample cells were (a) 10 mm^2, (b) 20 mm^2, (c) 40 mm^2, (d) 60 mm^2, (e) 80 mm^2, and (f)100 mm^2. The size of each sensing electrodes was 2 mm^2. (B) Schematic illustration of the measurement setup using the chip-type device shown in (A). (C) Cyclic voltammograms obtained using the chip-type device shown in (A).

To demonstrate the applicability of the present closed bipolar system to quantitative amperometric detection, a chip-type device having six sample cells and one reference cell was fabricated. First, a test device was prepared to optimize the size of the driving electrode in the

sample cell and ensure that reactions (2) and (6) were the rate-limiting processes of the entire system. Figure 3A shows the design of the test device with six driving electrodes with different surface areas (10–100 mm^2) in the sample cells. The area of each sensing electrode was 2.0 mm^2. The other electrodes were larger than 120 mm^2 in size so as to have a sufficiently large area not to limit the process rate. The sample solution (0.5 mM FMA with 100 mM KCl) was put on a sample cell as a droplet and a mixture solution of 2.5 mM $K_4[Fe(CN)_6]$ and 2.5 mM $K_3[Fe(CN)_6]$ with 100 mM KCl was put on the reference cell. The Ag/AgCl reference electrode was externally inserted into the reference cell (Fig. 3B). Figure 3C shows the voltammograms at the sensing electrodes (a) to (f) in Fig. 3A. A voltammogram having a similar shape to that depicted in Fig. 2B(a) was obtained only when the area of the driving electrode was 100 mm^2 (curve f). As the electrode area was reduced, another peak appeared at −0.2 V. This new peak was attributed to the oxygen reduction reaction (1), which became the rate limiting process at the small driving electrode. Based on this result, we subsequently employed 100 mm^2 as the electrode area of all driving electrodes in the sample cells.

Next, simultaneous multiple quantification was demonstrated using the chip-type device having 100 mm^2-driving electrodes in each of the six sample cells (Fig. 4A). In the reference cell on the device, the Ag/AgCl reference electrode was printed with Ag/AgCl ink (BAS, Japan) on an Au electrode. The 0–0.5 mM FMA solutions with 100 mM KCl were placed in the sample cells as droplets and the mixture of 2.5 mM $K_4[Fe(CN)_6]$ and 2.5 mM $K_3[Fe(CN)_6]$ with 100 mM KCl was placed in the reference cell (Fig. 4B). Using an eight-channel potentiostat (Hokuto Denko, Japan),
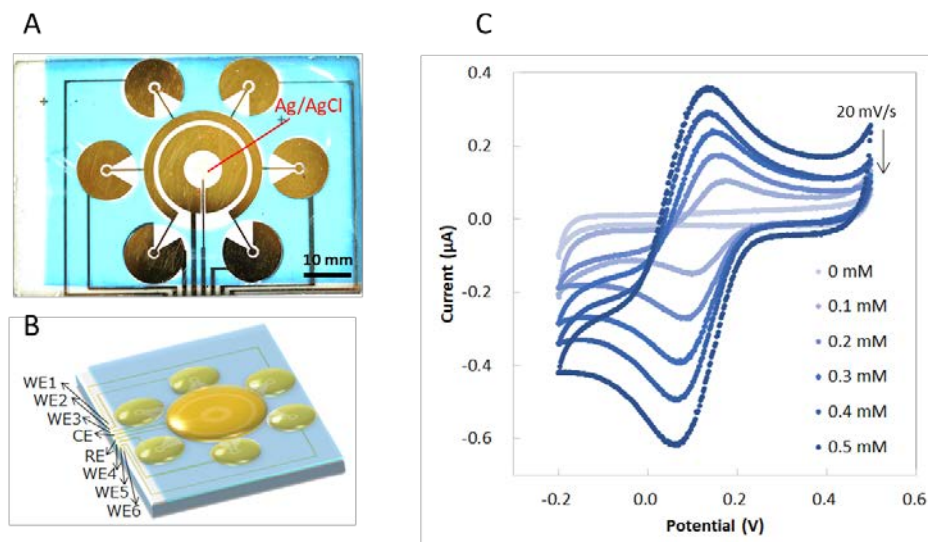

Figure 4. (A) Photograph of a fabricated device for six simultaneous measurements with a liquid-junction-removed reference electrode system. (B) Schematic illustration showing the measurement setup using the device shown in (A). (C) Simultaneously obtained cyclic voltammograms using the chip-type device shown in Fig. 3(A) connected to an eight channel potentiostat.

six voltammograms were obtained simultaneously. The resulting voltammograms of the downward peaks at 0.1 V increased with the increasing FMA concentrations in the sample cells (Fig. 4C).

We also conducted chronoamperometry for FMA quantification with a step potential of the driving electrodes from open circuit to –0.15 V. Figure 5A shows typical amperograms for 0–0.5 mM FMA in the sample cells. Data are consistent with Cottrellian characteristics at short times (~2 s) after the potential step, then the slope of the Cottrell plot became moderate due to the convection. This is similar to the conventional 3-electrode measurement, showing that our liquid-junction-removed system successfully conducted amperometric measurement equivalent to the conventional 3-electrode measurement. The amperometric currents increased with increasing FMA concentrations. The averaged currents during 22–25 s after the potential step are plotted against the FMA concentration and are shown in Fig. 5B. The circles and error bars represent the averages and standard deviations for five replicated experiments, respectively. The current responses increased linearly with the FMA concentration. The relatively large error bar (0.007–0.051 µA) was a result of the non-uniformity of the sizes of the six sensing electrodes depending on the use of the inaccurate photomask made by inkjet printing.
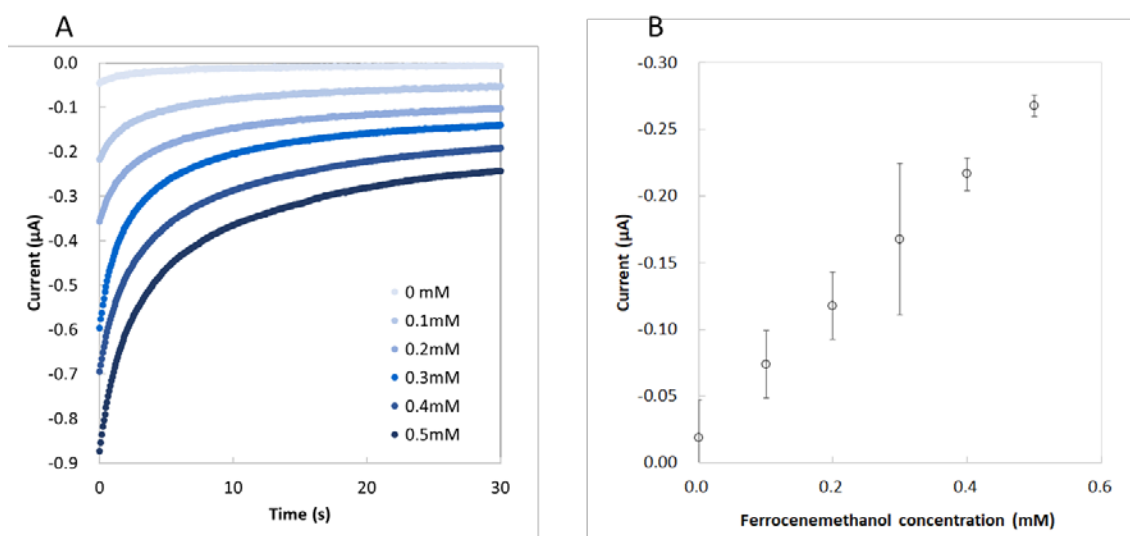

Figure 5. (A) Typical amperograms obtained with the device shown in Fig. 4(A). (B) Calibration plots for the quantitative detection of FMA using the device shown in Fig. 4(A). The averaged currents of 22–25 s in Fig 5(A) were plotted against FMA concentrations. Five repetitive experiments were conducted for six simultaneous sample measurements (0–5 mM) with exchange to prevent those having the same concentration from occupying the same cell. Circles and error bars represent the average values and standard deviations for five experiments, respectively.

In conclusion, a novel liquid-junction-removed reference electrode system was developed for amperometry using a closed bipolar electrode. This strategy was first confirmed by connecting

the sample and reference cells with a bipolar electrode instead of a liquid junction using separate electrochemical cells. By optimizing the conditions to ensure that the redox reaction at the bipolar electrode in the sample cell was the rate-limiting process of the entire system, we obtained a cyclic voltammogram with almost exactly the same shape (but rotated 180°) as that obtained in the conventional three-electrode system. This result clearly showed that the present liquid-junction-removed reference system is applicable to electrochemical measurements. Next, a chip-type device with six sample cells and one reference cell was fabricated, and simultaneous determination of FMA in six different solutions was conducted successfully using the device. Although the potential control problem still remains by increase of the solid-liquid interface alternative to the liquid potential problem of conventional liquid junction, it is expected that the present system will address the current problems in electrochemical measurements arising from the liquid junction of the reference electrode, including sample contamination by chloride ion, and the complicated fabrication process of the reference electrode on a chip. Currently, we are investigating the potential calibration of the sensing electrode in order to use this system with a stable reference potential. The driving potential of the redox reaction at the sensing electrode may be predicted by subtracting the potential difference between the bipolar electrode and the RE electrode in the reference cell from the voltage applied by the potentiostat (c.f. Kirchhoff's law), in case that enough electrolyte is contained in the sample cell and the sensing electrode size is enough small compared to other electrodes. [11] The relevant results will be discussed in a forthcoming paper.

Conflict of interest

The authors declare no conflict of interest.

Keywords: closed bipolar electrode system, electrode chip, electroanalysis, electrochemical sensor, liquid junction,

REFERENCES

[1] H. Suzuki, H. Shiroishi, *Anal. Chem.* **1999**, *70*, 5069-5075.

[2] S. Takano, K. Y. Inoue, M. Ikegawa, Y. Takahashi, K. Ino, H. Shiku, T. Matsue, *Electrochem. Commun.* **2016**, *66*, 34-37.

[3] S. E. Fosdick, K. N. Knust, K. Scida, P. Richard M. Crooks, *Angew. Chem. Int. Ed.* **2013**, *52*, 10438–10456.

[4] F. Mavre, R. K. Anand, D. R. Laws, K. F. Chow, B. Y. Chang, J. A. Crooks, R. M. Crooks, *Anal. Chem.* **2010**, *82*, 8766–8774.

[5] L. Koefoed, S. U. Pedersen, K. Daasbjerg, *Curr. Opin. Electrochem.* **2017**, *2*, 13–17.

[6] S. E. Fosdick, R. M. Crooks, *J. Am. Chem. Soc.* **2012**, *134*, 863-866.

[7] J. T. Cox, J. P. Guerrette, B. Zhang, *Anal. Chem.* **2012**, *84*, 8797-8804.

[8] J. P. Guerrette, S. M. Oja, B. Zhang, *Anal. Chem.* **2012**, *84*, 1609-1616.

[9] H. Hotta, N. Akagi, T. Sugihara, S. Ichikawa, T. Osakai, *Electrochem. Commun.* **2002**, *4*, 472-477.

[10] D. Plana, F. G. E. Jones, R. A. W. Dryfe, *J. Electroanal. Chem.* **2010**, *646*, 107-113.

[11] J. D. Zhang, W. W. Zhao, J. J. Xu, H. Y. Chen, *J. Electroanal. Chem.* **2016**, *781*, 56-61.

Graphic Abstract
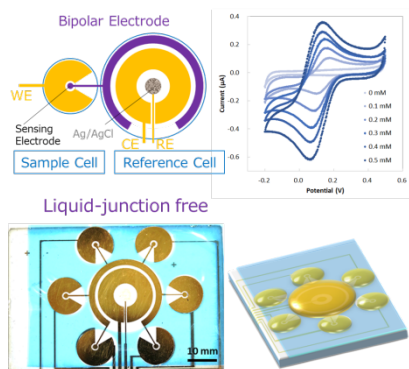
Reference electrode integrated in six-simultaneous amperometric sensor chip without liquid junction: The electron flow through the bipolar electrode completes the electric circuit of the measurement system alternative to the movement of ions through the liquid junction.