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An instantly usable screen-printed paper-based Ag/AgCl electrode was fabricated for use as a cost-effective disposable reference electrode. The potential stability of the reference electrode was approximately 75 h. The setup time, which was less than 1 min, was much shorter than those for similar previously reported electrodes.

**Introduction**

Ag/AgCl reference electrodes are the most practical and effective reference electrodes. The fabrication of a cost-effective and disposable Ag/AgCl reference electrode has attracted a great deal of interest over the past decade. Recently, solid-state reference electrodes that have a gel or solid liquid junction have been reported. Screen printing is used for fabricating cost-effective and disposable solid-state Ag/AgCl reference electrodes, since this technique allows for precise control over the product dimensions, is highly reproducible, and can be used for mass production.

In most of the screen-printed solid-state Ag/AgCl reference electrode, a paste containing KCl powder is printed on a Ag/AgCl electrode surface as an inner electrolyte layer. In addition, a polymer liquid junction, which prevents serious leakage of KCl from the inner electrolyte layer into a solution, can be formed on a substrate. The screen-printed solid-state Ag/AgCl reference electrodes previously reported exhibit a good long-term stability. For example, Suzuki et al. fabricated a solid-state reference electrode that have a photocurable hydrophilic polymer liquid junction and a screen-printed electrolyte layer composed of a mixture of KCl powder, poly(vinylpyrrolidone) and 2-propanol. Tymekci et al. developed a fully screen printed solid-state Ag/AgCl reference electrode. Electrolyte layer was formed by printing a UV-cured polymer paste containing KCl powder, and chloroprene rubber was printed as a liquid junction. Recently, we fabricated a screen-printed solid-state Ag/AgCl reference electrode in which a poly(dimethyl siloxane) emulsion was used to form the inner electrolyte layer and the liquid junction. The screen-printed solid-state Ag/AgCl reference electrode exhibited potentials accurately for more than 2 months during continuous measurements in a phosphate buffer solution.

However, one problem encountered with previous solid-state Ag/AgCl reference electrodes is that they require a long setup time prior to use (approximately 1–2 h).

When the reference electrode is immersed in the solution to be tested, the solution penetrates the liquid junction, coming into contact with both the electrode surface and the electrolyte layer. As a result, the KCl powder contained in the inner electrolyte layer dissolves in the solution. Consequently, the Cl− concentration near the Ag/AgCl electrode surface reaches the saturation level, and the potential of the reference electrode becomes stable. As mentioned above, the polymer liquid junction is very useful to prevent leakage of KCl from the inner electrolyte layer. On the other hand, the speed of the penetration of the test solution is slow in the polymer liquid junction. Thus, the setup time becomes long.

If the reference electrode is to be used for in situ monitoring instantaneously, a solid-type reference electrode having a low setup time would be more desirable. The test solution must be supplied quickly to the electrolyte layer, in order to reduce the setup time. A balance between a short setup time and the ability to accurately measure the potential for a long period is very important, if solid-state reference electrodes are to find use in field monitoring pH sensors and biosensors.

We focused on paper-based devices. A paper has good potential to act as a liquid junction. The test solution can be supplied rapidly into an inner electrolyte layer by the phenomenon of self-pumping, leading to the short setup time since the paper has hydrophilic surface and porous structure. In addition, a large amount of KCl powder can be included in the paper layer. Thus, we consider that a screen-printed paper-electrode may have a long stability with a short set-up time.

Figure 1 shows a schematic diagram of the paper-based screen-printed reference electrode. We used a piece of filter paper as a substrate. The reference electrode consisted of a paper liquid junction and a paper-based electrolyte layer containing KCl. The KCl-containing layer and the paper liquid junction were fabricated using a water-repellent ink. The paper liquid junction was attached directly onto the Ag/AgCl electrode.

In the present study, we defined the period before the potential became stable as the setup time. The operational time until the potential drifted by more than 30 mV was defined as the period for which the reference electrode was stable. The electrochemical characteristics of the reference electrode were investigated using cyclic voltammetry. Further, the stability of the reference electrode was examined by continuously measuring its potential shift relative to the potential value measured using a commercial reference electrode.
Experimental

The reference electrode (Fig. 1) was fabricated using the screen-printing technique. The details of the fabrication process and the sizes of the different layers are shown in Supplementary Information (SI). First, a Ag/AgCl ink was used to print a pattern on one side of a water-repellent-treated paper substrate (see SI, size of the paper substrate: 22 mm × 65 mm). The thickness of the paper substrate was 800 μm. Next, a conducting layer of Ag was formed using a Ag ink. The patterns printed using the Ag and Ag/AgCl inks were cured at 120 °C for 20 min. Then, a resist ink was used to print a pattern to prevent inner electrolyte (KCl) from releasing the test solution, which was cured at 100 °C for 20 min. Next, 0.5 mL of a saturated KCl solution, which was produced by dissolving 246 mg of KCl in 1 mL of ultrapure water, was dropped on the opposite side of the water-repellent-treated paper substrate three times. Then, a pattern was printed using a resist ink and dried at 120 °C for 20 min. Finally, the entire reference electrode (without the liquid junction and the Ag lead) was coated again with a water-repellent ink.

The shifts in the potential of the reference electrode were estimated by performing open-circuit potential measurements using a two-electrode system. The fabricated Ag/AgCl paper electrode was connected to a connector of a potentiostat for a working electrode and a commercial Ag/AgCl reference electrode was connected to a connector for a reference electrode, respectively. A 0.1 M solution of Na2SO4 was used as the test solution. The potential signal outputs between the electrodes were measured in the test solution.

The efflux of Cl− ions from the electrolyte layer of the reference electrode was also investigated. The synthesized electrode and the commercial reference electrode were kept immersed in ultrapure water, and the total number of effluxing Cl− ions was determined through potentiometric measurements performed using a two-electrode system. A bare screen-printed Ag/AgCl electrode was used as the Cl− ion sensor (i.e., the working electrode), while a commercial saturated KCl/Ag/AgCl electrode was used as the reference electrode. The calibration curve was prepared by plotting the potential differences against the Cl− concentrations of aqueous solutions containing KCl in different concentrations.

Cyclic voltammetry (CV) measurements were performed at a scanning rate of 100 mV s⁻¹ to investigate the suitability of the reference electrode for electrochemical measurements. The CV curves were measured in a 10 mM Na2SO4 solution containing 10 mM K[Fe(CN)6] and 10 mM K3[Fe(CN)6] using a potentiostat (iVium Compactstat); a three-electrode system was employed for the purpose. A glassy carbon disk (diameter of 3 mm) and a Pt wire were used as the working and counter electrodes, respectively.

Results and discussion

Figure 2 shows the variations in the open-circuit potential of the reference electrode in a 0.1 M Na2SO4 solution as measured against a commercial saturated KCl/Ag/AgCl reference electrode. The initial potential of the reference electrode after immersion in the solution was approximately -4 mV. The potential subsequently shifted to the negative side, depending on the operation time. The open-circuit potential eventually became stable within 1 min. This result means that the paper liquid junction supplied enough volume of the test solution into the electrolyte layer within 1 min as expected. The stability of the reference electrode was approximately 75 h. Further, potential drift to the positive side was observed after 75 h.

Fig. 2 Time variation in the open-circuit potential of the fabricated Ag/AgCl reference electrode measured for a commercial Ag/AgCl reference electrode. The experiment was performed using a 0.1 M Na2SO4 solution. The inset figure is the open-circuit potential of the reference electrode for 0 to 10 min.

Next, we investigated the efflux of Cl− ions from the reference electrode as well as from the commercial reference electrode (RE-1C, BAS Inc., Japan), which had a porous ceramic liquid junction (2 mm in diameter, 4 mm in height). The total number of Cl− ions effluxing was measured by using the bare Ag/AgCl electrode as a Cl− ion sensor, in keeping with a previously reported procedure. The synthesized electrode as well as the commercial reference electrode was immersed continuously in ultrapure water. During the immersion process, the total numbers of Cl− ions effluxing were determined at 1-day intervals. This was accomplished by making potentiometric measurements using a two-electrode system. The average change in the number of Cl− ions effluxing from the reference electrode was 5.75 × 10⁻⁶ mol h⁻¹, which was slightly higher than the change in the number of ions effluxing from the commercial reference electrode (4.80 × 10⁻⁶ mol h⁻¹). However, the effect of the effluxing of the Cl− ions can be considered to be limited; the exceptions are cases where the...
volume of the sample solution is very small. If the efflux of the Cl-ion from the reference electrode is 5.75×10⁻⁵ mol h⁻¹, all KCl coated on the electrode (369 mg = 4.95 mmol) is released by about 90 h. The reference electrode cannot keep the stable potential continuously until 90 h since Cl⁻ in the electrolyte layer is gradually diluted when the potential drifts to the positive side (after 75 h in Fig. 2). Thus, the stability of the reference electrode (75 h) is reasonable considering from the amount of the KCl in electrolyte layer. As mentioned above, the one reason why the reference electrode show the good long-term stability is that a large amount of KCl powder (369 mg) is included by using the paper as electrolyte layer.

CV measurements were performed in a 10 mM Na₂SO₄ solution containing 10 mM K₃[Fe(CN)₆] and 10 mM K₄[Fe(CN)₆] using the synthesized and commercial reference electrodes. Figure 3 shows the CV curves obtained at a scanning rate of 100 mV s⁻¹. No significant difference was observed in the peak currents. Further, there was a shift in the CV graph in the x-axis direction; this was owing to the redox reaction of [Fe(CN)]⁶³⁻/[Fe(CN)]⁶⁴⁻, indicating that the reference electrode can be used as a reliable Ag/AgCl electrode.

The stability and setup time of the reference electrode were compared with those of screen-printed solid-state Ag/AgCl reference electrodes exhibiting good stability (SI Table 1).⁹, ¹⁰, ¹⁴. The stability of the reference electrode was lower than that of the screen-printed solid-state Ag/AgCl reference electrodes exhibiting good stability (SI Table 1).⁹, ¹⁰, ¹⁴. The stability and setup time of the reference electrode were 75 h, and its setup time could be reduced to less than 1 min. The reference electrode should be applicable in many technological applications, including as a disposable biosensor and a pH sensor.

**Notes and references**

*Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278-8510, Japan Fax: +81 47123 9890; Tel: +81 4 7124 1501; E-mail: shitandai@rs.noda.tus.ac.jp

*Research Institute for Science and Technology, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan.

†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. S. DOI: 10.1039/b000000x/
