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Author(s)	Idegami, Koutarou; Chikae, Miyuki; Nagatani, Naoki; Tamiya, Eiichi; Takamura, Yuzuru
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Description	

Fabrication and characterization of planar screen-printed Ag/AgCl reference electrode for disposable sensor strip

Koutarou Idegami¹, Miyuki Chikae², Naoki Nagatani^{3*}, Eiichi Tamiya⁴, Yuzuru Takamura²

¹*Ishikawa Sunrise Industries Creation Organization, 2-20 Kuratsuki, Kanazawa, Ishikawa 920-8203, Japan*

²*School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan*

³*Department of Applied Chemistry, Graduate School of Engineering, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan*

⁴*Department of Applied Physics, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*

*Corresponding author.

E-mail address: nagatani@dac.ous.ac.jp

An accurate disposable planar Ag/AgCl reference electrode with an internal electrolyte was successfully fabricated by the screen-printing process. The internal electrolyte layer was also printed by using an electrolyte paste of sodium alginate containing KCl. The potential stability of the electrode was investigated at different operation times and Cl⁻ concentrations in test solutions. Results show that the electrode has long-term potential stability (approximately 60 min), and it does not depend on the Cl⁻ concentration. This electrode can be used to provide a promising various applications in sensing technique based on the disposable strips for sensing purposes.

KEYWORDS: reference electrode, Ag/AgCl, sodium alginate, screen-printing process. potential stability

1. Introduction

Recently, electrochemical biosensors based on potentiometric, voltammetric, and amperometric techniques have attracted considerable attention because they are simple, fast, portable, and inexpensive and can be used to detect specific biological interactions.¹⁾ Such sensors are generally fabricated by the screen-printing process or a semiconductor microfabrication process, because these processes are simple, highly reproducible, and inexpensive and can be used for mass production. Screen-printed sensor strips based on the three-electrode system—consisting of a working electrode, counter electrode, and reference electrode—have been widely used as biosensors. Our group has developed a screen-printed sensor strip and various types of biosensors.²⁻⁵⁾

The reference electrode is an essential component of an electrochemical biosensor system. A Ag/AgCl reference electrode needed internal electrolyte for ideal behavior, because the potential of it depending on the ions in test solutions. In the screen-printing process, it is difficult to separate the internal electrolyte of a reference electrode from the test solution, and most of the disposable sensor strips consist of only bare Ag/AgCl without internal electrolyte.³⁻⁵⁾ It is necessary to fabricate accurate reference electrodes so that they can be used to develop highly sensitive biosensor strips.

Many studies demonstrated the separation of the internal electrolyte and test solution by using various types of Ag/AgCl reference electrodes for the semiconductor capable and/or miniaturized sensor chip. In most of them, the Ag/AgCl surface was coated with an electrolyte-doped polymer. For example, Suzuki *et al.* microfabricated a liquid junction Ag/AgCl reference electrode. Its electrolyte layer was screen-printed by using an electrolyte paste, which was a mixture of ground KCl powder, poly(vinylpyrrolidone), and 2-propanol.⁶⁾ Kim *et al.* demonstrated the use of a polyelectrolyte as a salt bridge for fabricating a miniaturized reference electrode. The polyelectrolyte liquid junction was formed via photopolymerization of diallyldimethylammonium chloride in the presence of saturated KCl.⁷⁾ Hashimoto *et al.* produced a needle-type Ag/AgI reference microelectrode with active liquid junctions made from polyvinyl alcohol and starch.⁸⁾ Kakiuchi's group utilized a hydrophobic ionic liquid to fabricate a Ag/AgCl reference electrode consisting of a Ag/AgCl wire coated with a AgCl-saturated ionic liquid, 1-methy-3-octylimidazolium bis-(trifluoromethylsulfonyl)imide, instead of an internal aqueous solution.⁹⁾ Tymecki *et al.* developed screen-printed and all-solid-state Ag/AgCl reference electrodes. The electrolyte film was printed by using a UV-cured protective paste containing finely powdered KCl.¹⁰⁾

Kwon *et al.* fabricated a miniaturized reference electrode on a silicon chip (all-solid-state reference electrode) by coating a AgCl surface with a silicon rubber film containing KCl salt and Nafion.¹¹⁾ In contrast, Simons *et al.* produced a Ag/AgCl quasi-reference electrode without a KCl-containing membrane.¹²⁾ Liao and Chou screen-printed an electrolyte layer using agar gel containing KCl and chloroprene rubber.¹³⁾ It should be noted that the fabrication processes used in the above studies were complicated and inefficient.

In the present study, an accurate disposable planar reference electrode with internal electrolyte was fabricated by the screen-printing process using an internal electrolyte paste. The potential stability of the electrode in test solutions was also investigated.

2. Experimental Methods

Graphite- and Ag/AgCl⁻-based polymeric pastes were obtained from Dupont (USA), and an insulating paste was obtained from Taiyo Ink Mfg. Co., Ltd. (Japan). Sodium chloride, calcium chloride, and sodium alginate were provided by Wako (Japan). Other reagents were of analytical grade, and all solutions were prepared and diluted using ultrapure water (18.3 MΩ-cm) from a Millipore Milli-Q system.

Figure 1A shows a screen-printed sensor strip consisting of a carbon working electrode, a carbon counter electrode, and a Ag/AgCl reference electrode with a total length of 18.6 mm and width of 7 mm. The structure of the Ag/AgCl reference electrode is shown in Figure 1B. This strip was printed by using a semi-automatic screen-printing machine NT-150TVM-J (Neo Techno Japan Co., Ltd., Japan). The graphite paste was printed on the glass-epoxy substrate to produce conductive tracks (working and counter electrodes) and cured at 130°C for 10 min. Then, the Ag/AgCl paste was printed and cured at 120°C for 10 min. The internal electrolyte paste was prepared as a saturated KCl in 2% sodium alginate. After printing the internal electrolyte paste, the surface of the alginate layer was gelled by applying 3 wt% CaCl₂ solution; then, the alginate layer was dried at room temperature for 1 day. Two slits were made in the insulating layer; one slit was made to allow the test solution to come into contact with the inner component of the reference electrode (left side of **e** in Figure 1B), and the other was made to allow air to escape from this component (right side of **e** in Figure 1B). The insulating layer was cured at 100°C for 5 min. Finally, the reference electrode was uniformly covered with a hydrophilic polymer-coated polyester film, provided by Takara Bio Inc. (Japan).

The potential of the Ag/AgCl reference electrode was monitored against a commercial

Ag/AgCl reference electrode in 3 M KCl solution RE-1B (BAS Inc., Japan) and was measured in the unstirred condition at room temperature.

3 Results and Discussion

As the preliminary examination for preparing the screen-printable internal electrolyte paste, various types of commercially available polymers, saturated with KCl, were performed, and we selected an alginate because of its unique properties, such as water-absorbable, gel-casting, non-toxicity, and low production cost. Alginate is a linear copolymer consisting of two polymers, β -D-mannuronic acid and α -L-guluronic acid, joined by glycosidic links β -1,4 and α -1,4. An aqueous solution of sodium alginate can be screen-printed because it is viscous and can dissolve in KCl easily. Additionally, a printed layer of sodium alginate could be immobilized completely onto the Ag/AgCl paste by gelation using Ca^{2+} , for the cross-linking of the alginate polymer.

When 80 mL of the test solution was applied to the three electrodes of the fabricated strip, the test solution was induced through the slit of the insulation layer. Figure 2 shows a plot of the potential stability versus the operation time of the reference electrode in 0.1 M KCl solution measured against a commercial Ag/AgCl reference electrode with 3 M KCl as the internal electrolyte. The initial potential of the bare Ag/AgCl reference electrode on the screen-printed strip was 65 mV; then, the potential shifted to the negative side depending on the operation time due to the dissolution of AgCl from the Ag/AgCl element (Figure 2). In the case of the Ag/AgCl reference electrode fabricated in this study, the dried layer of alginate became gel after the introduction of the test solution, and it served as a stable internal electrolyte after 4 min. The potential was constant for 60 min, and then, it gradually shifted to the negative side (Figure 2), because of the infusion of water into compartment and diffusion of the internal electrolyte. It should be noted that the width of the slit of the insulation layer should be optimized; in this study, the slit width was 0.3 mm. If the slit is very narrow, the test solution penetrates through the layer slowly or incompletely. However, if the slit is very wide, the potential stability of the electrode decreases due to the infusion of water into the compartment and diffusion of the internal electrolyte (data not shown). Our results show that the Ag/AgCl reference electrode has long-term potential stability; therefore, it can be used as a disposable electrode.

Figure 3 shows the dependence of the potential on the Cl^- concentration. The potentials of screen-printed Ag/AgCl reference electrodes at different Cl^- concentrations (pH 7.0) were

measured against a commercial Ag/AgCl reference electrode with 3 M KCl as the internal electrolyte. When a Ag/AgCl electrode is immersed in an aqueous solution, AgCl is found to be in reversible equilibrium with solid Ag and Cl⁻. In the steady state, the equilibrium potential (E) associated with this reversible reaction can be expressed by the Nernst equation (1):

$$E(\text{Ag}/\text{AgCl}) = E^0(\text{Ag}/\text{AgCl}) - (RT/F)\ln a_{\text{Cl}^-} \quad (1)$$

a : ion activity

Consequently, the potential of the bare Ag/AgCl reference electrode was influenced by the Cl⁻ concentration of the test solution. In contrast, the electrode produced in this study contained an internal electrolyte layer, and therefore, its potential was constant (approximately 30 mV) versus a commercial Ag/AgCl reference electrode with 3 M KCl as the internal electrolyte. The variation in the potential of our electrode was small (10 mV) at test solution concentrations between 1 mM and 1 M.

4. Conclusions

In summary, we successfully fabricated a reliable disposable planar Ag/AgCl reference electrode with an internal electrolyte by a screen-printing process. A fabrication of one sensor strip both the proposed reference electrode and working electrode and counter electrode will promises the high potential for mass production in low cost. The potential stability of the Ag/AgCl reference electrode formed on a sensor strip was investigated; it was found that the electrode has long-term potential stability that is independent of the Cl⁻ concentration. The proposed Ag/AgCl reference electrode can be used to fabricate disposable strips for biosensing purposes.

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Legends

Fig. 1. (A) Screen-printed sensor strip consisting of a carbon working electrode, carbon counter electrode, and Ag/AgCl reference electrode with a total length of 18.6 mm and width of 7 mm. (B) Structure of the reference electrode: (a) glass-epoxy substrate, (b) conductive track, (c) Ag/AgCl layer, (d) internal electrolyte layer, (e) insulating layer with two slits, and (f) hydrophilic polymer-coated polyester film.

Fig. 2. Potential stability versus operation time of the screen-printed reference electrode in 0.1 M KCl solution, bare Ag/AgCl (○) and Ag/AgCl with an internal electrolyte (●), measured against a commercial Ag/AgCl reference electrode with 3 M KCl as the internal electrolyte.

Fig. 3. Potentials of screen-printed reference electrodes at different Cl^- concentrations (pH 7.0), bare Ag/AgCl (○) and Ag/AgCl with an internal electrolyte (●), measured against a commercial Ag/AgCl reference electrode with 3 M KCl as the internal electrolyte.

Fig. 1

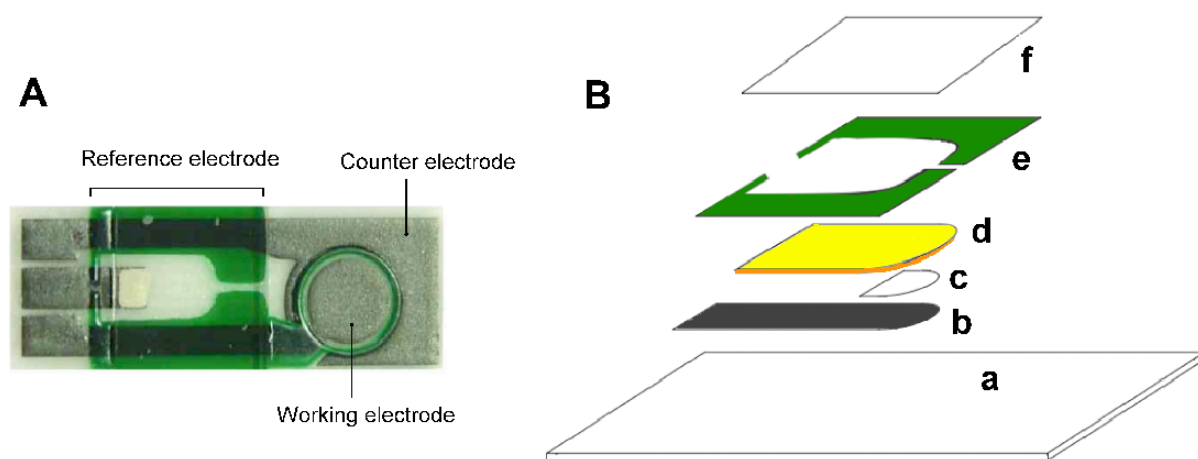


Fig. 2

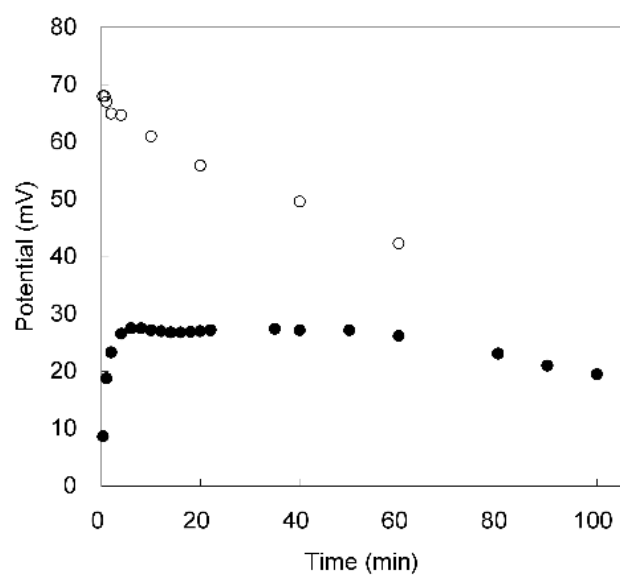


Fig. 3

