An all-solid-state reference electrode based on the layer-by-layer polymer coating

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A solid-state reference electrode (SSRE) was fabricated by layering a silicone rubber (SR) film containing KCl on an AgCl surface, then a perfluorinated ionomer film, and finally a polyurethane-based membrane containing an ionophore, a lipophilic ionic additive, and a plasticizer, respectively. The addition of SiCl4 to the polyurethane-based membrane layer enhanced the strength of the membrane in an aqueous solution. The morphologies of the membranes were studied separately by SEM. The fabrication of the Ag/AgCl electrode through this layer-by-layer polymer coating improved the electrode stability enormously. In addition, the potential drift of the SSRE according to the pH of the medium was minimized by introducing a H+-ion-selective ionophore (tridodecylamine; TDDA) into the outmost polymer membrane. The cyclic voltammetric and potentiometric responses using the SSRE and a conventional reference electrode, respectively, were consistent. The SSRE exhibited little potential variation even in the case of the addition of very high concentrations of various salts, such as Na salicylate, LiCl, KCl, CaCl2, MgCl2, KNO3, NaCl, and NaHCO3. The practicability of the proposed SSRE was tested for the determination of blood pH and pCO2 in a flow cell system. The SSRE fabricated in the present study was stable over two years.

Introduction

The function of a reference electrode in an electrochemical system is to provide a reproducible potential, with respect to which the potential of a working electrode is established. Hence, it is important that the potential of the reference electrode be kept constant and invariant with the solution composition.1–4 Until now, various kinds of reference electrodes have been introduced, which include SCE, SHE, Ag/AgCl, HgO/Hg, etc. Nowadays, most reference electrodes are based on Ag/AgCl, because of its easy fabrication, miniaturization, and freedom from toxicity.3,4 Ag/AgCl reference electrodes are available in two types: one is the conventional liquid-junction type, which has unavoidable drawbacks, such as internal filling solution leakage and the difficulty in fabricating them to a very small size necessary for in vivo experiments;5 the other is the solid-state reference electrode (SSRE), which is free from the storage problem of the internal filling solution because no internal filling solution is used, and it is also easily fabricated in miniature, which makes it useful for in vivo experiments.5,6,7 Thus, SSRE fabrication is an essential recent topic in the research and the development of portable miniaturized sensors in medicinal and biological sciences and technology.5,6,8,9

There have been many reports on the applicability of polymer membranes in the fabrication of SSREs. For example, miniaturized reference electrodes in potentiometric sensors have been fabricated using polymer membranes, such as plasticized polyvinyl chloride, silicone rubber, polyurethane, and others.10–13 Earlier, an aromatic-polyurethane-type membrane-based SSRE for a wide pH range had been reported; however, it lacked long-term stability.

A common method to make a solid-state reference electrode is to cover the Ag/AgCl surface with an electrolyte-doped polymeric material [e.g. polyvinyl acetate–KCl, quaternized polychloromethylstyrene–Cl–, Refex (a KCl-doped vinyl ester resin), and polyvinyl chloride–NaCl, etc.].5,8–11 SSREs have also been made with a cation-exchange polymer (Nafion) membrane by curing at high temperature in order to achieve a more stable Nafion matrix; however, they exhibited lesser durability.5,9 The above-mentioned SSREs have shortcomings in their fabrication: the stability of the potential response over a wide pH range, tolerance for other ions, and long-term reproducibility, among other drawbacks. The wide application of SSREs requires that these problems are overcome.

In the present study, SSREs were fabricated using a layer-by-layer polymer membrane approach on an Ag/AgCl surface in order to obtain long-term usability and stability for all electrochemical experiments in the chemical, biochemical and medical fields. The SSRE was characterized with cyclic voltammetric and potentiometric methods and examined for blood gas analysis in a flow cell.

Experimental

Reagents and instruments

A pure silver rod (99.9+%; i.d.: 3.0 mm) and a wire (99.9+%; i.d.: 0.5 mm) were used for fabrication of the SSREs. Potassium tetrakis(p-chlorophenyl)borate (KTPCIPB), bis-(2-ethylhexyl)adipate (DOA), and high-molecular-weight polyvinyl chloride (PVC) were purchased from Fluka (Buchs, Switzerland). For the SR membrane, a silicone resin (Silastic 1021), made by Dow Corning, USA, was used. For the ionophilic polymer, a lipophilic ionic additive, 1,1,1-tris(3-aminopropyl)triethylammonium chloride (TAP) (99%, Sigma-Aldrich Inc, USA) was used. Polyvinyl acetate (PVA), unmodified NaCl, KCl, LiCl, CaCl2, MgCl2, KNO3, NaCl, and NaHCO3 were used as fillers and as supporting electrolytes. Polyvinyl chloride (PVC) was used for the formation of the liquid-junction liquid membrane. Polyethylene glycol (PEG)-600, polyethylene glycol (PEG)-400, diethylhexyl adipate (DOA), anion-exchange polymer (Nafion), tridodecylamine (TDDA), and KCl were used as plasticizers. Reagents were purified to ensure the best possible purity. All waters used were of deionized water quality.
Switzerland); Tecoflex polyurethane (TPU, SG-80A), from Thermedics (Woburn, MA); room-temperature-vulcanizing (RTV)-type silicone rubber (3140 RTV) (SR), from Dow Corning (Midland, MI); tridodecylamine (TDDA), from Eastman Kodak (Rochester, NY); perfluorinated ion-exchange resin (Nafion) and tris-(hydroxymethyl)aminomethane (Tris), from Sigma (St. Louis, MO); and tetrahydrofuran (THF) and silicone tetrachloride (SiCl₄; 1.0 M solution in dichloromethane), from Aldrich (Milwaukee, WI). All other of the chemicals used were of analytical-reagent grade. The aqueous solutions were prepared with deionized water (18.0 MΩ cm).

The pH effect of the medium on the SSRE response was evaluated using an external reference electrode (Ag/AgCl liquid-junction electrode, Orion, USA). The potential differences between the indicator and the reference electrodes were measured employing a 16-channel Multi pH/Ion meter (Model KST101B, Kosentech, S. Korea). The pH values of the test samples were monitored with a glass pH electrode (Model Orion 81-92 ROSS™ combination pH electrode, Orion, USA).

SEM images were obtained using a Cambridge Stereoscan 240 (at KBSI, Busan, S. Korea). Impedance spectra were recorded with the EG&G PAR 273A potentiostat/galvanostat and a lock-in amplifier (PAR EG&G, Model 5210) linked to a personal computer. The frequency was scanned from 100 kHz to 1 Hz at the open circuit voltage, acquiring 5 points per decade. The amplitude of sine voltage of 10 mV was used. The measurements were carried out in a buffered medium (0.01 M PBS, pH 7.0), at a temperature of 25 ± 0.1 °C.

Potentiometric measurements

The pH values of the media were controlled by adding aliquots of LiOH to a background solution of 0.0286 M citric acid–0.0286 M KH₂PO₄–0.0286 M boric acid–0.0286 M diethyl barbituric acid at room temperature. The dynamic potential response curves for the SSREs with various concentrations of salts were obtained by the addition of a standard solution to a background electrolyte (0.1 M Tris-HCl of pH 7.0) at room temperature. The response of the SSREs was also investigated in CO₂ sensing. A calibration plot for the CO₂ analysis was obtained by the addition of the standard NaHCO₃ solution to a blank electrolyte (0.2 M Tris-H₂SO₄ of pH 7.4) with stirring. The potential changes during the pCO₂ measurements using the SSREs coupled with a CO₂ sensor were measured directly by the titration of NaHCO₃.

Cyclic voltammetric measurement

The cyclic voltammetric (CV) measurements were performed in a 1.0 mM K₃Fe(CN)₆–0.1 M KNO₃ solution using a potentiostat/galvanostat (Model: KST-CV 104A, Kosentech, S. Korea) with a three-electrode system. The electrodes were a glassy carbon electrode, a Pt counter electrode, and either an SSRE or the liquid-junction reference electrodes (Ag/AgCl/ KCl sat.).

Preparation of the SSREs

An acetal resin (polyoxymethylene) cylindrical holder (i.d.: 3.0 mm; length: 20 mm) was used as the body in constructing the SSREs. As shown in Fig. 1(a), graphite paste was used as a supporting electrical connector between the silver rod and a copper wire connected to the potentiometer, which was made by mixing graphite powder (approx. 10 g) with 2 drops of mineral oil and molding it to the Acetal holder to about a 10 mm thickness. A copper wire (1.0 mm in diameter) was inserted through the Acetal tube into the graphite paste. A silver rod (3.0 mm in diameter) was also inserted into the graphite paste by mechanical pressing, and the silver surface was successively polished with 100, 1000, and 1500 fine grade sandpapers, and finally with 1 and 0.3 μm alumina to ensure a clean surface. A layer of silver chloride was formed on the silver surface by applying +0.5 V for 3 min in a 0.1 M KCl solution. To enhance the stability of the Ag/AgCl layer, the electrode was dried at room temperature for 10 days, and then stored in 3.0 M KCl for 10 days. After the electrode was dried, the Ag/AgCl surface was spin-coated with the immobilizing electrolyte solution. The electrolyte was freshly prepared by saturating 10 ml of THF with KCl at room temperature and then adding 0.1 g of silicone rubber (SR) to the electrolyte. After spin-coating, the electrodes were dried in a desiccator for 48 h to evaporate the solvent. After that, the electrodes were dried and spin-coated with a protective polymer solution (5% Nafion, 6 μl) to prevent the leakage of chloride ions. After the second spin-coating, the electrodes were dried in a desiccator for 48 h and then cured at 100 °C for 1 h. Thermal curing was performed to remove the solvent and also to lower
table 1 compositions of polyurethane-based membranes tested in this study

<table>
<thead>
<tr>
<th>Membrane</th>
<th>TPU SG-80A (wt%)</th>
<th>PVC (wt%)</th>
<th>3140 RTV (wt%)</th>
<th>KTpClPB (wt%)</th>
<th>TDDA (wt%)</th>
<th>DOA (wt%)</th>
<th>SiCl4 (wt%)</th>
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<tr>
<td>PUM 1</td>
<td>42.0</td>
<td>11.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>40.0</td>
<td>10</td>
</tr>
<tr>
<td>PUM 2</td>
<td>42.0</td>
<td>13.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>40.0</td>
<td>10</td>
</tr>
<tr>
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<td>42.0</td>
<td>11.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>40.0</td>
<td>10</td>
</tr>
<tr>
<td>PUM 4</td>
<td>42.0</td>
<td>13.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>40.0</td>
<td>10</td>
</tr>
<tr>
<td>PUM 5</td>
<td>42.0</td>
<td>11.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>44.0</td>
<td>10</td>
</tr>
</tbody>
</table>

* a: Polymer: Tecoflex polyurethane. b: High-molecular-weight polyvinyl chloride. c: Room-temperature-vulcanizing (RTV)-type silicone rubber. 


Table 1 Compositions of polyurethane-based membranes tested in this study

Preparation of polyurethane-based membranes (PUMs)
The polyurethane-based membranes (PUMs), as the final protective layer of SSREs, were prepared by dissolving TPU, PVC, and/or SR in THF, along with the given amounts of the other active membrane components. Table 1 summarizes the compositions of PMUs examined in this study. All of the membranes were prepared by dissolving components of 10 wt% in the THF solvent, and then 500 μl of the 10 wt% polymer membrane solution was used to prepare the polymer film in glass rings (i.d.: 2.0 cm) on a flat Teflon plate. The solvent was allowed to evaporate at room temperature in a dust-free environment for 72 h. The PUM membranes were prepared with or without the addition of a particular amount of SiCl4 to the membranes, by mixing them with the solution listed in Table 1.16,17

Results and discussion
Characterization of the layers
The SSREs were fabricated by layering the SR-mixed electrolyte (SR-KCl) on an AgCl-coated surface, by protecting it with a perfluorinated ionomer (Nafion) coating, and by casting the PUMs. The SR-mixed electrolyte layer was added in order to increase the stability of the AgCl layer and maintain a constant Cl⁻ concentration. SR was chosen as a binder due to its high polarity and effective binding to the AgCl surface, which might also enhance ion transfer in the AgCl layer. In order to prevent the leaching of the Cl⁻ ion, the Ag/AgCl/SR-KCl layer was coated with Nafion. The Nafion coating on the Ag/AgCl/SR-KCl layer impedes the dissolution of Ag/AgCl. Thereafter, to enhance the stability of the electrolyte containing the AgCl layer, control the cation transfer, and enhance the sensor performance, the polyurethane membranes (PUMs) were cast onto the Nafion layer. The PUMs used as the final layers of the SSREs were composed of polymer matrices of a TPU/PVC/SR mixture, silicon tetrachloride (SiCl4), an ionophore (TDDA), a lipophilic ionic additive (KTpClPB), and a plasticizer (DOA). The addition of an ionophore (TDDA) to the PUMs effectively assisted the transport of cations into the Nafion film, rendering an added advantage to the SSREs. In this study, the five different polymeric materials shown in Table 1 were examined as matrices in fabricating the PUMs. Only PVC was found to be an inadequate material for the preparation of a hardly responsive membrane, which requires a large quantity of plasticizer due to a high glass transition temperature (Tg). In spite of the excellent potentiometric performance of the PVC membrane, it has a limited lifetime. TPU is one of the most successful alternatives to PVC as the ion-selective electrode (ISE) matrices, and being a family of block copolymers consisting of alternating hard and soft segment-units have attracted particular interest, especially in biomedical applications.16,18,19 Besides these polymers, SR increased the stability of the membrane due to its self-assembling property, which also increased the polarity of the membrane. Thus, we used a hybrid mixture of TPU/PVC/SR as the PUMs, which could provide a constant response over a wide pH range, a high resistance to interference ions, and a long lifetime.

Surface morphology of the polymer layers
The steps involved in the fabrication of the SSREs were separately confirmed by taking SEM pictures of the surface of each layer. As described in Fig. 2, the surfaces were: (a) AgCl only, (b) AgCl/SR-KCl, (c) AgCl/SR-KCl/Nafion, (d) AgCl/SR-KCl/Nafion/PUM3, (e) AgCl without immobilized electrolyte (SR-KCl), and (f) AgCl with immobilized electrolyte (SR-KCl). The surface morphology of the AgCl layer, shown in Fig. 2(a), exhibited plain and uniform AgCl particles having diameters of ca. 100–900 nm. When the electrolyte (SR-KCl) was immobilized onto the AgCl layer by spin-coating [Fig. 2(b)], the layer was found to be of a different morphology compared with that shown in Fig. 2(a). The image shown in Fig. 2(c) was obtained after the application of the protective polymer (Nafion) coating, which exhibited a smooth surface with only some roughness. Fig. 2(d) shows an image of Ag/AgCl/SR-KCl/Nafion covered with PUM, and it was found to be a very smooth surface. A high-resolution SEM image of the AgCl surface (the electrolyte layer without SR) did not exhibit any particles [Fig. 2(e)]. In the case of the SR-mixed electrolyte (SR-KCl) coated on the AgCl layer [Fig. 2(f)], the nano-sized particles (diameter: ca. 10–30 nm) of KCl, which increased the concentration of Cl⁻ to facilitate the ionic equilibrium of the Ag/AgCl layer, were clearly observed. The Nyquist plot obtained for the final polymer layer shows a well defined semicircle, indicating that it is in a single layer (not shown).
Enhancement stability of the PUM

SiCl4-doped polyurethane-based membranes exhibited much better adhesion and stability, and a much longer lifetime. Recently, we have shown that the addition of SiCl4 to TPU-based matrices enhances the potentiometric response and stability, due to the chemical bonding between Si and C = O in urethane groups, in which the cross-linking was confirmed by DSC, FT-IR, and XPS experiments. Thus, the stabilities of the SSREs using PUMs with and without SiCl4 were tested by dipping them in a 1.0 M KCl solution at room temperature for two weeks. After that, their surface morphologies were observed by SEM (Fig. 3). The surface image of the SSREs containing PUM1 (without SiCl4) shows some swelling of the polymer layer [Fig. 3(a)]. In addition, the polymer layer was much damaged and exhibited numerous cracks, indicating mechanical failure and the loss of the protective polymer. Some parts of the membrane revealed degraded patches, associated with the region where the polymer layer was diminished. Fig. 3(b), however, shows that a representative SEM image of the surfaces of the SSREs containing PUM3 (with SiCl4) exhibits a very good, clean, and smooth surface without any swelling or defect. The surface appeared to be totally intact, and no cracks could be seen. In addition, the results for PUM2 and PUM4 were found to be the same as those for PUM1 and PUM3, respectively (not shown). From the above results it was inferred that the addition of SiCl4 to PUM enhanced the stability of the SSRE over a long period.

**Fig. 3** SEM images of SSREs coated by (a) PUM1 (without SiCl4) and (b) PUM3 (with SiCl4). Stability tests were performed on these electrodes while they were stored in a 1.0 M KCl solution at room temperature for 2 weeks.

**Fig. 4** pH responses of SSREs having different compositions of the polymer layers: (a) Ag/AgCl/SR-KCl/Nafion, (b) Ag/AgCl/SR-KCl/Nafion/PUM3, (c) Ag/AgCl/SR-KCl/Nafion/PUM4, and (d) Ag/AgCl/SR-KCl/Nafion/PUM5.

Emf variations according to compositions of polymer layers

The pH dependencies of the potential drift for differently composed SSREs, such as (a) Ag/AgCl/SR-KCl/Nafion, (b) Ag/AgCl/SR-KCl/Nafion/PUM3, (c) Ag/AgCl/SR-KCl/Nafion/PUM4, and (d) Ag/AgCl/SR-KCl/Nafion/PUM5, were studied, and the results are shown in Fig. 4. Fig. 4(a), obtained for composition (a), shows a potential drift below pH 7; however, the constancy in the potential was observed above pH 7 due to the low concentration of H+ ions compared to the sufficient exchange capacity through the Nafion layer. In contrast, near-constancy in the potential (showing a slope of 1.5 mV/pH) over a wide pH range (pH 2–12) was observed in the case of composition (b). This was due to the inclusion of the ionophore (TDDA) in PUM3, which assists the transport of H+ ions in a controlled manner even for lower pHs. In the case of composition (c), the SSRE with PUM4 shows a small potential change over pH 3–4 compared with composition (b) [Fig. 4(b)]. The same response, however, was obtained above pH 5. This trend was possibly due to the high polarity of the SR. Fig. 4(d) shows a higher deviation in the potential according to the pH; this was due to the absence of the ionophore in this composition. From the above results, it was concluded that the use of PUM3 was more effective than that of the others.
Comparison of the reference electrodes

To compare the SSRE and the commercial reference electrode (Ag/AgCl/KCl sat.), pH measurements were performed using a glass pH electrode as an indicator electrode. As shown in Fig. 5(A), the slopes for (a) the SSRE and (b) the commercial reference electrode were measured to be 58.5 ± 1.0 and 58.9 ± 1.0 mV/pH, respectively, over a wide pH range (from 2 to 12). Experiments were carried out by measuring the pH of standard buffer solutions (pH 4, 7, and 10), (i) with a glass pH electrode as an indicator electrode and the commercial reference electrode as a reference electrode [Fig. 5(B)(a)], and (ii) with an SSRE as an indicator electrode and a commercial reference electrode as a reference electrode [Fig. 5(B)(b)], and both results were compared. Whereas a dynamic potential response according to pH was observed for the glass pH electrode as shown in Fig. 5(B)(a) (58.6 mV/pH), in the case of using both the SSRE and the commercial reference electrodes, no change in the potential was observed over a wide pH range. This indicates that the performances of the SSREs and commercial reference electrodes are consistent in all aspects.

Interference effect

As shown in Fig. 6, the interference characteristic of the SSRE with the PUM3 layer was further demonstrated in metal salt-containing solutions. As can be seen, its response was similar to that of all of the added salts (Na salicylate, LiCl, KCl, CaCl₂, MgCl₂, KNO₃, NaCl, and NaHCO₃), and the emf signals from these salts were negligible, showing small variations in the potential at the relatively high concentration of 0.1 M. In all of the cases, the potential remained constant up to 10⁻¹ M except with LiCl, which showed a small change in the potential and also attained equilibrium within ±3 mV of the dynamic potential value. The insignificant interference from various salts at high concentrations indicates the superiority of the SSRE compared with conventional reference electrodes.

Application of a cyclic voltammetric measurement

The authenticity of this SSRE was examined as a reference electrode in voltammetric experiments using a three-electrode system, comprising a platinum wire as a counter electrode and a glassy carbon as a working electrode, for which was performed a cyclic voltammetric measurement in a 1.0 mM K₃Fe(CN)₆³⁻/1.0 M KNO₃ solution (at 25 ± 0.1 °C). The experiments were carried out at different scan rates (ν = 25, 50, 100, 150 and 200 mV s⁻¹), and the CVs, represented in Fig. 7(a) and (b), were obtained for the liquid-junction reference electrode and the SSRE, respectively. The scan rate dependency of the redox currents of both of the reference electrode systems are shown in the insert of Fig. 7. The redox peak currents were linearly proportional to the square root of the scan rate. The equations of the scan rate dependency of the currents for both of the reference electrodes [data from Fig. 7(a) and (b)] were $I_{pc} = 4.69 \times 10^{-5} \nu^{1/2} + 8.76 \times 10^{-7}$ and $I_{pc} = 4.57 \times 10^{-5} \nu^{1/2} + 8.70 \times 10^{-6}$, respectively. The diffusion coefficients calculated from the redox peaks were 0.61 × 10⁻⁵ and 0.58 × 10⁻⁵ cm² s⁻¹. These values over
25 mV s\(^{-1}\) are slightly lower than that in a literature report,\(^4\) due to the incomplete reversibility and uncompensated IR drop at the higher scan rates. However, the values below 25 mV s\(^{-1}\) are almost the same as that of 0.76 × 10\(^{-5}\) cm\(^2\) s\(^{-1}\) in the literature. In addition, the values are close to each conventional reference electrode and SSRE, indicating no difference in the function of the reference electrode. This result indicates the successful application of the SSRE without any shortcomings in electrochemical experiments.

**Application of SSRE to a CO\(_2\) measurement**

The present SSRE was examined as a reference electrode connected to a home-built CO\(_2\) sensor in all solid states, and it showed a good emf response, thereby yielding a calibration plot with a slope of 53.0 mV/dec, as shown in Fig. 8. The CO\(_2\) measurement was based on the observation that it formed a weakly dissociated carbonic acid, when CO\(_2\) was dissolved in water, which subsequently formed free hydrogen ions and bicarbonate ions due to the chemical reaction. As a result of this chemical reaction, the pH of the solution was changed. This change generated a potential between the pH and the reference electrode (SSRE) that was proportional to the negative logarithm of the pCO\(_2\). The experiments were performed by adding NaHCO\(_3\) at a concentration range from 0 to 50 mM to a 0.2 M Tris-H\(_2\)SO\(_4\) buffer (pH 7.4) solution. The inset of Fig. 8 shows a calibration plot with a regression coefficient (\(r^2\)) of 0.999. The response time required to reach 90% of equilibrium was within 30 s, which is notably fast. This result also indicates the feasibility of the SSRE in real situations.

**Application to a blood gas analysis**

The present SSRE was also examined as a reference electrode in a home-built shut-tight-type flow cell for an analysis of blood gases. As shown in Fig. 1(b), the flow cell, which was designed to protect against the coagulation of blood due to the direct contact of air, was used for the determination of pH and pCO\(_2\) in human blood samples employing a two-point calibration method. The pH and pCO\(_2\) of whole blood samples, obtained from a local hospital, were measured by using a home-built miniature pH and pCO\(_2\) sensor coupled with the SSRE, and these results were compared with those obtained from the commercial blood analyzer, Nova Systems. The results are given in Table 2, and it was inferred that the pH and pCO\(_2\) levels of all of the blood samples were the same in all aspects. These results showed the practicability of SSRE in the portable blood gas analysis systems.

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>This study(^a)</th>
<th>Commercial analyzer(^b)</th>
<th>This study(^c)</th>
<th>Commercial analyzer(^b)</th>
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<tr>
<td>1</td>
<td>7.51 ± 0.03</td>
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<td>2</td>
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<td>3</td>
<td>7.47 ± 0.02</td>
<td>7.48 ± 0.01</td>
<td>40.3 ± 2.1</td>
<td>41.2 ± 1.8</td>
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</table>

\(^a\) Home made. \(^b\) Reference electrode: SSRE of this study. \(^c\) Units: mm Hg. \(^d\) Sample obtained from a local hospital blood bank. \(^e\) Data are mean values for five times measurement. \(^f\) Biofrofile-200 from Nova biomedical corp.
temperature; the pH was adjusted using 0.1 M LiOH.)

0.0286 M boric acid–0.0286 M diethyl barbituric acid at room temperature; the pH was adjusted using 0.1 M LiOH.)

The stability and usability of the reference electrode are important factors in any electroanalytical procedure, since any variation can affect the response of the working electrode. However, most miniaturized electrodes based on a polymer membrane have short lifetimes ranging from one day to as long as a few months. In this regard, we investigated the long-term stability of SSRs employing PUM3 by checking the calibration curve of the pH over a significantly long time period. Fig. 9 shows the variation in the response slope in the pH range from 2 to 12 using the SSRs, a constant slope (56 ± 3 mV/pH), were found to be very stable for around two years. Furthermore, no considerable decrease in the response or the recovery time was observed. The almost two-year stability of the SSRs suggests their applicability to continuous in vivo experiments or in on-line quality control processes.

**Conclusions**

The lifetime and selectivity of the SSRE was enormously improved by the electrode surface modification performed in this work. The fabricated SSRE coupled with H⁺ ISEs was stable and feasible over a full available range of pH. Cyclic voltammetric and potentiometric measurements using the SSRE and the conventional reference electrode were compared, and the performances were the same. The addition of various salts at high concentrations up to a 0.1 M level did not result in any change in the potential response. The SSRE as the reference electrode was very stable up to a two year period.

The feasibility of the proposed SSRE in a flow cell was also demonstrated for the analysis of blood pH and pCO₂. This study demonstrates that the stability of the SSRE in blood has a significant impact on the lifetime of the reference electrode.

**Acknowledgements**

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**References**