Potentiometric ion- and bioselective electrodes based on asymmetric polyurethane membranes

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Abstract

The potentiometric ion responses of ammonium- and proton-selective electrodes prepared by incorporating appropriate neutral carriers within novel asymmetric polyurethane membranes are reported. The membranes are formed by first casting a plasticized polyurethane (PU)/terpoly(vinyl chloride/vinyl acetate/vinyl alcohol) (PVA)-based ion-selective membrane and then applying a thin second layer of a more hydrophilic polyurethane (HPU) containing polylysine. The resulting asymmetric membranes function equivalently to normal PU/PVA membranes and conventional pol(vinyl chloride) type membranes in terms of potentiometric ion selectivity and dynamic response properties. The large amount of amine functional groups from the polylysine within the outer hydrophilic layer can be further activated for direct enzyme immobilization. As examples, adenosine deaminase and urease are immobilized on ammonium- and proton-selective membranes, respectively, to yield adenosine and urea electrodes with good dynamic responses and sensitivities. Advantageous use of this new membrane system for preparation of solid-state microfabricated enzyme-based sensors is also described.

Keywords: Biosensors, Ion-selective electrodes, Potentiometry, Asymmetric membranes, Polyurethane membranes, Solid-state electrodes

Over the last three decades, a variety of bioselective electrodes have been developed using potentiometric gas- and ion-selective membranes as transduction elements [1–4]. Appropriate bioagents (such as enzymes, antibodies, bioreceptors, cells and tissues) are immobilized on such transducers in a manner that enables the detection of the product of the corresponding biological reaction in a thin layer of solution adjacent to the electrode's surface. Immobilization methods used include entrapment within a crosslinked polymer matrix, physical adsorption and covalent attachment through bifunctional crosslinking reagents [5]. Use of simple polymer membrane type ion-selective electrodes [6–10] (e.g., ammonium, carbonate, pH) as the base sensing elements has the advantage of providing biosensors that are easy to fabricate and exhibit rapid response times. In principle, potentiometric biodetection schemes based on these transducers can also be combined with the modern silicon technology to make small, multisensing and disposable solid-state biosensors [11–15] that could serve as flow-through biode-tectors, or single-use biosensing devices. While lack of selectivity over endogenous ions in real
samples has been a problem with ion-selective electrode-based biosensors [7], a variety of new approaches to eliminate these interferences, particularly in flow-through detection systems, have been introduced recently with considerable success [16–18]

Traditionally, enzymes have been deposited on conventional poly(vinyl chloride) (PVC) type ion-selective membranes via glutaraldehyde crosslinking reactions, etc [2,5] Such immobilized protein layers, however, generally have poor adhesion to the very hydrophobic surface of the plasticized PVC membrane While modification of the membranes such as using functionalized polymers (e.g., aminated or carboxylated PVC) has been attempted to covalently attach or adsorb the bioreagents to the hydrophobic PVC membranes [10,19,20], the number of available functional groups for such purposes is often quite low, yielding biosensors with poor bioreagent loading factors

The problem of efficiently attaching enzymes and other bioreagents to polymer membrane electrode surfaces has been addressed previously by Cha and Meyerhoff [9], who developed novel asymmetric cellulose acetate type ion-selective membranes for such purposes These membranes, while useful for preparing conventional electrodes in which the membrane is mounted between the sample and inner reference electrolyte solutions, are not suitable for preparation of solid-state (or solid-contact) type probes This is because the more hydrophilic outer cellulose acetate film used for enzyme immobilization must be coated from the back side with appropriate plasticizer/ionophore/cellulose acetate cocktail to render the resulting dual layer films ion responsive Although Gotoh et al [12] have suggested the use of poly(vinyl butyral) (PVB) membranes to prepare micro-field effect transistor based solid-state biosensors, such PVB films served only as a matrix for the immobilized enzymes and did not need to exhibit electrochemical response and selectivity toward given ions This is because the underlying metal oxide gate of the FET was used as an effective pH transducer to monitor pH changes arising from the enzyme reactions within the PVB layer

In this paper a new type of asymmetric ion-selective membrane is described which is potentially more useful for fabrication of solid-state type potentiometric biosensors The proposed membrane system consists of a very thin hydrophilic polyurethane (HPU) membrane possessing a high density of amine functional groups (in the form of polylysine) that is coated and fused to an underlying more hydrophobic plasticized polyurethane (PU)/poly(vinyl chloride/vinyl acetate/vinyl alcohol) (PVA) membrane containing the appropriate ion carrier The potentiometric response of the asymmetric polyurethane membranes is shown to be essentially the same as the base ion-selective membrane (PU/PVA) and the large amount of functional groups within the outer hydrophilic film can be used for the covalent attachment of bioreagents to the membrane’s surface Since the underlying PU/PVA ion-selective film has been shown previously to adhere very tightly to silicon dioxide and silicon nitride surfaces [21], the new asymmetric membrane system is well suited for fabrication of solid-state biosensor devices Indeed, the performance of both conventional and solid-state potentiometric enzyme electrodes prepared with this asymmetric membrane system is presented below using urea and adenosine as model analytes It should be noted that although polyurethane matrices have been used previously as solid-phases for enzyme and protein immobilization [22,23], in such systems, the polyurethanes were never an integral part of a signal transducer, as is reported herein

EXPERIMENTAL

**Apparatus**

For preparation and testing of conventional sensor designs, the asymmetric membranes with and without immobilized enzymes were mounted in Philips electrode bodies (1S-561) (Glasblaserei Moller, Zurich) The external reference electrode was a double-junction Ag/AgCl Fisher electrode The potentiometric measurements of the ion-selective electrodes were monitored through a high impedance amplifier to a Zenith Z-100 PC
computer equipped with a Data Translation A/D converter system (DT2801).

Gel permeation chromatography (GPC) on a Waters ALC 200 system (Milford, MA) was used to determine the molecular weight of the hydrophilic polyurethane. The effluent (in THF) was monitored by a differential refractometer (R401) and the retention time of the sample through a series of three StyrageL columns of pore size 500, 10³ and 10⁴ Å was used for the calculation of molecular weight based on prior calibration with narrow molecular weight polystyrene standards.

Reagents

Tecoflex polyurethane (PU) (SG-80A) was obtained from Thermedics (Woburn, MA). The terpolymer, poly(vinyl chloride/vinyl acetate/vinyl alcohol) (80 5 15 wt %, MW 40,000) was a product of Scientific Polymer Products (Ontario, NY). Nonactin, bis(2-ethylhexyl) sebacate (DOS), bis(2-ethylhexyl) adipate (DOA) and potassium tetrakis(4-chlorophenyl) borate (KTPCIPB) were purchased from Fluka (Ronkonkoma, NY). Adenosine, adenosine deaminase (ADA, Type VII, from calf intestinal mucosa), urease (Type VII, from Jack Beans), glutaraldehyde and polysine (PLS) (hydrobromide, MW 30,000–70,000) were obtained from Sigma (St Louis, MO). Tridodecylamine (TDDA) was obtained from Eastman Kodak (Rochester, NY) and silicon(IV) tetrachloride (SiCl₄, 1.0 M solution in dichloromethane) was from Aldrich (Milwaukee, WI). The hydrophilic PU (HPU) (40% water uptake) was a gift from Mr. Peter Burleigh, Mallinckrodt Sensor Systems (Ann Arbor, MI). It was prepared according to the procedure outlined in Ref 24.

All other chemicals were of analytical-reagent grade. All standard solutions and buffers were prepared with reverse osmosis/deionized water.

Preparation of asymmetric ion-selective membranes

Figure 1 shows a schematic diagram of the asymmetric polyurethane membrane, depicted in this case with an enzyme layer attached to the polysine incorporated within the outer HPU film.

The basic underlying ion-selective membrane was composed of 33 % of PU/PVA (80 % of PU and 20 % of PVA), 66 % of plasticizer and 1 wt % of ionophore. For ammonium ion-selective membranes, DOA was used as plasticizer and nonactin as the ionophore. For proton-selective membranes, DOS and TDDA served as the plasticizer and pH ionophore [25], respectively, and KTPCIPB at 1 wt % was also added to the membrane casting solution. All of the membrane components (total mass = 200 mg) were dissolved in THF and cast into a 22 mm (1 in) glass ring placed on a glass plate. The THF solvent was allowed to evaporate overnight before further coating with the outer HPU/PLS layer.

The second layer of the asymmetric membrane was prepared by first treating the surface of the
base PU/PVA ion-selective membrane with 150 μl of 0.02 M NaCl in CH₂Cl₂ for 2–3 min. Then, 1 mg of PLS with 10 mg of HPU in a mixed solvent (250 μl of methyl alcohol and 50 μl of THF) was applied on top of the base PU/PVA membrane. Several hours were required for the outer film to cure. In case of proton-selective asymmetric membranes, only 1 mg of PLS and 5 mg of HPU in the same amount of mixed solvent were applied to the surface of the base PU/PVA membrane.

Electrodes were prepared by punching small disks from the above membranes and mounting them in the Philips electrode body with HPU/PLS layer facing out toward the sample side. The internal reference electrolyte was 0.1 M of NH₄Cl for NH₄⁺-selective electrodes and 0.02 M NaH₂PO₄–0.03 M Na₂HPO₄–0.015 M NaCl, pH 7.0, for the proton-selective electrodes. All potentiometric measurements were made at room temperature with a sample volume of 100 ml.

**Immobilization of adenosine deaminase / urease on the asymmetric ion-selective membranes**

Adenosine deaminase and urease were attached to the surface of the asymmetric membranes using the glutaraldehyde crosslinking method described in Ref. 9 with minor modifications. For the two-step glutaraldehyde method, the asymmetric ion-selective membrane mounted in the electrode body was immersed in 2.5% of glutaraldehyde solution for 5 min. After brief washing of the membrane with cold water, 10 μl of ADA (19 units) or urease (286 units) (prepared by dissolving 0.5 mg ADA/urease in 10 μl of 0.05 M phosphate buffer, pH 7.0) was applied onto the outer surface of the membrane (surface area of 12.6 mm²).

For the one-step glutaraldehyde method, 3.5 μl of glutaraldehyde and 10 μl of enzyme solution were sequentially applied on the surface of the asymmetric membrane mounted within the electrode body. The coupling reaction was allowed to proceed for 12 h at 4°C. The membrane was then washed with Tris–HCl buffer (0.05 M, pH 7.2) and stored in buffer at 4°C before use.

**Evaluating potentiometric responses of conventional ion- and bioselective electrodes**

The potentiometric responses of NH₄⁺/H⁺-selective electrodes and the corresponding ADA/urease enzyme electrodes were evaluated by addition of standard solutions of inorganic salt or adenosine/urea into 100 ml of well stirred buffer solution at room temperature. The background electrolyte used depended on the ions and substrates being examined (0.05 M Tris–HCl, pH 7.2, for NH₄⁺, adenosine, and urea response with NH₄⁺-selective membrane electrodes, 11.4 mM boric acid–6.7 mM citric acid–10 mM NaH₂PO₄ for pH response, and 0.001 M Tris–HCl–0.1 M NaCl, pH 7.0, for urea response with H⁺-selective membrane). For evaluating the pH response of the TDDA-doped membranes, the pH of the boric acid/citric acid/phosphate universal buffer was varied by addition of either NaOH or HCl while simultaneously measuring the pH of the solution with a calibrated glass electrode. For the ammonium-selective membranes, ion selectivity coefficient data were obtained by the separate solution method [26].

**Preparation of solid-state enzyme electrodes**

The solid-state biosensor design examined in these studies is illustrated in Fig. 2. Aluminum conductor leads were patterned on silicon wafers, and insulated (except at the sensing site) with silicon nitride using standard microelectronic fabrication procedures. A layer of silver epoxy was
then screen printed over the exposed aluminum at the sensing site. A thin (100–300 μm) layer of the plasticized PU/PVA polymer casting solution containing either TDDA or nonactin was then cast onto the silver epoxy layer. This membrane adhered tightly to the silver epoxy and the surrounding silicon nitride coating of the wafer. A very thin layer of HPU containing polysulfone was then coated over the ion-selective layer using a SiCl₄ pretreatment step as described above for the regular asymmetric membranes. Urease and adenosine deaminase were then immobilized by the one- or two-step glutaraldehyde procedures outlined above.

RESULTS AND DISCUSSION

Characterization of asymmetric membranes

A key component of the asymmetric ion-selective membrane system is the outer hydrophilic polyurethane (HPU) material which can be loaded with polysulfone for subsequent bioreagent immobilization. This membrane must be hydrophilic enough to readily pass ions liberated from any biocatalytic reaction occurring at the surface. Preliminary characterization of the HPU film consisted of measuring its water uptake as well as determining its elemental composition (C, H and N analysis) and molecular weight (by GPC), and comparing these values to the supposedly more hydrophobic Tecoflex material which comprises 80 wt% of the polymer material used to formulate the underlying ion-selective layer. The average molecular weight of the HPU material was found to be 45,000, which is much lower than that found for the hydrophobic Tecoflex material (MW 190,000). Water uptake experiments indicated that the HPU material absorbs water to a far greater extent than the hydrophobic Tecoflex material (40 wt% vs 1 wt%). Elemental analysis of the two polymers yielded the following results: C 66.48%, H 11.13%, N 2.29% for the hydrophobic Tecoflex material, C 61.30%, H 9.99%, N 3.70% for the HPU. This elemental analysis data correlates with the fact that more disocyanate reagent, and thus more urethane linkages are present within the HPU polymer and thus leads to a more hydrophilic polymer with enhanced water uptake [24].

A scanning electron micrograph of the cross-section of a dry asymmetric ion-selective polyurethane membrane is shown in Fig. 3. The thicker layer is the plasticized PU/PVA ion-selective membrane (approximately 300 μm in thickness), while the upper thin layer is formed from the HPU mixed with polysulfone (HPU/PLS) (approximately 9 μm in thickness). It can be seen that the textures of two layers are quite different. The hydrophobic plasticized bulk layer is more dense while the upper layer appears somewhat porous, apparently due to its more hydrophilic character.

The adhesion of the hydrophilic HPU/PLS layer to the underlying hydrophobic PU/PVA membrane is greatly enhanced by applying 0.02 M of SiCl₄ to the surface of the thicker PU/PVA layer containing the ion-selective reagents (e.g., nonactin or TDDA). Indeed, using this proce-
dure, the two layers remain fused for extended periods even when continuously soaked for 30 days in aqueous solution. The addition of the SrCl$_4$ apparently helps crosslink the terminal hydroxyl groups on the HPU with the surface hydroxyl groups from the vinyl alcohol portion of the PVA terpolymer and the terminal hydroxyl groups of the Tecoflex PU. An energy dispersive x-ray spectrum (EDS) of the cross-section of the asymmetric polyurethane membrane suggests that the SrCl$_4$ is mostly distributed at the interface of the two layers and penetrates into the underlying bulk ion-selective membrane to a depth of about 10 $\mu$m. Only low intensity Si or Cl bands were observed near the surface of the asymmetric HPU/PLS membrane, suggesting that the amine groups of the polylysine at the surface are not greatly affected by the added SrCl$_4$ and are free to be used for enzyme immobilization.

It is believed that the potentiometric ion response of the asymmetric membrane occurs at the interface of the HPU layer and the plasticized PU/PVA film. That is, the HPU/polylysine layer acts as a thin hydrophilic sponge in which ions readily move up to the hydrophobic PU/PVA/ionophore film where they participate in an equilibrium phase extraction creating the interfacial charge separation or phase boundary potential [27] which changes as a function of ion activities.

The ability of the outer HPU film to retain the added polylysine after prolonged exposure to aqueous solution was also investigated. Since the polylysine is not chemically attached to the outer HPU, it was initially thought that this very hydrophilic catonic material would leach out quickly with time. To investigate this possibility, the outer surface of the asymmetric membranes was treated with trinitrobenzenesulfonate (TNBS), a reagent that reacts rapidly with the primary amine groups of polylysine to yield a color change from yellow to orange-red [28]. This test was carried out both before and after extended soaking and washing sequences. It was found that the degree of color formation did not change appreciably even after repeatedly soaking the membranes in Tris–HCl buffer solution for 7 days, with frequent changes of the buffer each day (5 times per day). Thus, it appears that the polylysine is well entrapped/entangled within the hydrophilic polyurethane membrane and reactive amine groups remain at the surface for extended periods of time. It should be noted that polylysine has been used previously for the non-covalent immobilization of anionic charged biomolecules, such as heparin, by taking advantage of its polycationic character to yield strong charge–charge interaction [29]. However, the amine groups of anchored polylysine can also be used for covalent enzyme immobilization via classical crosslinking agents (e.g., glutaraldehyde). Since the covalent binding is irreversible, more stable immobilization can be achieved.

**Potentiometric ion response and selectivity of the asymmetric membranes**

Before using the asymmetric membranes to prepare biosensors, it is important to document that such membranes do indeed respond potentiometrically to ions, and with response characteristics and selectivities nearly equivalent to conventional polymer membrane ISEs. Figure 4 illustrates the typical equilibrium potentiometric responses observed toward ammonium ions in a Tris–HCl buffer background electrolyte for electrodes fabricated with an asymmetric membrane, a plain PU/PVA membrane (no outer HPU/PLS layer), and a conventional PVC membrane, all containing nonactin as the membrane active ionophore. As shown, the potentiometric re-
response to ammonium ions, in terms of slope (typically 56 mV/decade in the range of $10^{-5}$ to $10^{-1}$ M of $\text{NH}_4^+$) and detection limits, is essentially equivalent for the three different membranes. Response times were also quite similar (typically within 10 s), indicating that the diffusion of ammonium ions through the outer HPU/PLS thin film is rather rapid, at least relative to ion-extraction reactions at the PU/PVA/ionic-phore-HPU interface.

Figure 5 shows the potentiometric pH response of TDDA-doped asymmetric and plain PU/PVA membranes. As can be seen, the addition of the HPU/polylysine outer layer does not affect significantly the response to protons. Typically, slopes of 57 mV/pH are observed for the asymmetric membranes in the pH range of 3–10. Again, no noticeable elongation in response time is observed when comparing the dynamic pH response of the asymmetric membranes to plain

PU/PVA films doped with TDDA. It should be noted that these equivalent responses can only be achieved by reducing by one-half of the amount of polylysine loaded into the outer HPU layer (compared to the ammonium-selective membrane system).

To determine whether the presence of the outer HPU layer alone, or an outer HPU/PLS layer influences ion selectivities, the selectivity coefficients of the ammonium membranes were determined via the separate solution method. As shown in Table 1, for the most part, little difference in ammonium ion selectivity is observed for the asymmetric ammonium membranes (PU/PVA–HPU/PLS) when compared to plain PU/PVA, PU/PVA coated with HPU (no polylysine) (PU/PVA–HPU) and conventional PVC membranes. Some increased response to protons is observed, although practical selectivity for measurements of ammonium at or near neutral pH values is still quite acceptable.

**Conventional biosensors based on the asymmetric ion-selective membranes**

Urease and adenosine deaminase served as model enzyme systems to examine the utility of using the asymmetric polyurethane-based membranes for preparation of potentiometric biosensors. These enzymes were coupled to the surfaces of pH and ammonium-selective asymmetric membranes, respectively. Adenosine deaminase catalyzes the following reaction:

$$\text{Adenosine} + \text{H}_2\text{O} \rightarrow \text{Inosine} + \text{NH}_4^+$$

Urease catalyzes the hydrolysis of urea as follows:

$$(\text{NH}_2)_2\text{CO} + 2\text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{NH}_4^+ + \text{HCO}_3^-$$

**TABLE 1**

Selectivity coefficients of nonactin-based membranes prepared with various polyurethane matrices

<table>
<thead>
<tr>
<th>Membrane matrix</th>
<th>$\log K_{\text{NH}_4^+}$&lt;sub&gt;ion&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{Li}^+$</td>
</tr>
<tr>
<td>PU/PVA</td>
<td>-48</td>
</tr>
<tr>
<td>HPU–PU/PVA</td>
<td>-41</td>
</tr>
<tr>
<td>HPU/PLS–PU/PVA</td>
<td>-41</td>
</tr>
<tr>
<td>PVC</td>
<td>-45</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by the separate solution method [26]  
<sup>b</sup> From Ref 19
In practice, both reactions can be detected by either monitoring pH changes or NH₄⁺ ions generated. In the present studies, the use of the proton-selective membrane was restricted to the urea/urease system, while both ADA and urease were immobilized on the ammonium selective asymmetric membranes.

Enzyme immobilization was achieved by covalently attaching the enzymes to the amine groups available on the surface of the membrane through glutaraldehyde crosslinking reactions. With two-step glutaraldehyde method, a monolayer of enzyme is immobilized, while one-step glutaraldehyde method results in multilayers of enzymes due to the crosslinking of enzyme molecules. This usually yields greater enzyme loading [5].

A typical calibration curve for an adenosine electrode prepared by immobilizing ADA on the asymmetric NH₄⁺-selective membrane using the one-step glutaraldehyde method is shown in Fig 6. The linear response range is from 10⁻⁵ to 3 × 10⁻² M of adenosine with a slope of 48 mV per decade. Response times are on the order of 60 s to reach steady-state potential. It should be noted that electrodes in which the asymmetric membrane was treated with enzyme, but no glutaraldehyde, also yield significant adenosine response (not shown). This observed response is due to non-specifically adsorbed ADA, presumably via ion-exchange reactions with protonated amino groups of the polylysine within the outer HPU layer. While the response of these latter electrodes can be eliminated by soaking the electrode in 4 M of MgCl₂ (to dissociate the adsorbed enzyme), the response of the glutaraldehyde treated enzyme electrodes remains the same after such treatment. Indeed, adenosine electrodes prepared in this way can last for at least one month, with little loss in response slope or detection limits.

Urea sensors based on pH-selective asymmetric membranes were also tested. For such biosensors, the thickness of the outer HPU layer was found to be critically important with regard to the magnitude of the potentiometric response to urea observed. By decreasing the total amount of the outer HPU/PLS layer (to 5 μm), optimum response in terms of sensitivity is observed. It is believed that the thinner outer membrane keeps the enzyme layer closer to the hydrophobic ion-selective detector layer (inner PU/PVA) so that rapid diffusion of protons into the bulk solution does not diminish surface steady-state pH changes. A typical calibration plot for a urea sensor based on the proton-selective asymmetric membranes is also presented in Fig 6. The slope is 29.8 mV per decade with the dynamic range of 10⁻⁴ to 10⁻² M of urea. Naturally, as with any enzyme electrode based on pH detection, sensitivity is highly dependent on the buffer capacity of the sample medium [30]. The response time to reach 95% of the steady-state potential is about 3 min. Urea sensors based on the NH₄⁺-selective asymmetric membranes exhibit much higher response slope (56 mV per decade) and shorter response time (in seconds) when compared to those based on proton-selective membranes (see below).

Response of microfabricated enzyme-electrodes

When urease is immobilized on the surface of the asymmetric ammonium selective membrane cast onto the solid-state device shown in Fig 2...
(using two-step glutaraldehyde), the resulting biosensor responds rapidly and selectively toward urea (see Fig 7) Typical calibration curves for this device show a linear relation between the e m f and the logarithm of urea concentrations in the range of $10^{-5}$–$10^{-2}$ M with slopes of 55–56 mV per decade This near-Nernstian slope and wide dynamic measuring range suggests that high enzyme loading has been achieved on the surface of the asymmetric membranes [5] In the case of solid-state adenosine sensors prepared in the same manner (with immobilized ADA), the high slopes and detection limits remain essentially constant during one month of continuous operation These results support previous claims regarding the very tight adhesion of the plasticized PU/PVA/ionophore membranes to silicon nitride and silver epoxy surfaces [21] Indeed, had the membrane lifted around the edges, leakage of electrolyte would have shorted the membrane and eliminated any potentiometric ion or substrate response

It should be noted that each of the films used to construct the solid-state biosensor, starting with the silver epoxy layer, can be screen printed for mass fabrication purposes In addition, the size and number of sensing regions per unit area of the silicon wafer can be varied While the above results have been obtained with single sensing sites on relatively large chips (0.8 cm × 10 cm, with polymer membrane sensing area of 0.2 cm²), it is envisioned that an array of biosensing sites on the same size chip can be readily achieved using screen printed asymmetric membranes and site-directed photomobilization of enzymes onto the highly aminated HPU/PLS layers

In summary, a new asymmetric membrane system with improved surface properties for development of conventional and solid-state type potentiometric biosensors has been described The potentiometric ion response of this new asymmetric membrane is essentially the same as the base PU/PVA membrane as well as the conventional PVC membranes At the same time, the amine functional groups on the membrane surface can be used advantageously for covalently attachment of bioreagents While used here to construct enzyme electrodes for adenosine and urea, it is likely that other bioreagents including intact cells, antibodies, etc., could be incorporated/attached to the outer PU/PLS layer to fabricate a variety of other biosensors (including single use devices) With the advantage of good adhesion to silicon wafer surfaces, this polyurethane asymmetric

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Fig 7 Dynamic potentiometric response of urea solid-state sensor based on NH$_4^+$-selective asymmetric membrane
membrane is a very promising candidate for use in constructing various solid-state biosensing devices.

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