Electrochemical Performance, Biocompatibility, and Adhesion of New Polymer Matrices for Solid-State Ion Sensors

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Ammonium and potassium ion-selective membranes formulated with PVC/hydroxylated PVC, polyurethane/hydroxylated PVC, and moisture-curable silicone rubber matrices are studied in an effort to extend the lifetime of solid-state ion sensors through improved membrane adhesion. The PVC/hydroxylated PVC and polyurethane/hydroxylated PVC membranes exhibit electrochemical performance equivalent to that of conventional PVC membranes in terms of slope, detection limit, and selectivity. The polyurethane- and silicone-rubber-based membranes have better adhesion to silicon nitride than do PVC or hydroxylated PVC matrices. Incorporating a silanizing reagent (silicon tetrachloride) significantly improves the adhesion of the polyurethane matrix. The use of silicon tetrachloride in membrane matrices also enhances the electrochemical stability of the interfacial potential between ion-selective polymer–matrix membranes and silver epoxy inner reference electrodes of solid-state sensors. The biocompatibility of the polymer matrices is examined via radiotracer protein adsorption studies and whole blood clotting time measurements. The polyurethane- and silicone-rubber-based membranes exhibit less overall nonspecific protein adsorption than the PVC or hydroxylated PVC matrices.

In recent years, there has been considerable interest in the development of various types of solid-state electrochemical sensors (i.e., ion, gas, and biosensors), in which polymer membranes are cast on solid surfaces with no internal reference electrolyte solutions (1–5). These devices are usually made on silicon and include integrated electronics. Such devices could offer the advantages of miniaturization (for in vivo measurements), the capability of including multiple sensors on a single chip, integration of signal processing circuitry, and mass fabrication for cost reduction (6–8). Potentiometric solid-state ion sensors have used ionophore-doped polymeric membranes as transducers due to their excellent selectivity toward the ion of interest and the wide range of ions for which ionophores are available. Furthermore, these sensors can borrow from ongoing developments in conventional ion-selective electrode (ISE) technology.

As in conventional ISEs, poly(vinyl chloride) (PVC) is the most commonly used membrane matrix in solid-state ion-selective sensors. However, one of the primary causes of failure in these miniature sensors has been poor adhesion of the PVC membranes to the chip surface, leading to electrolyte shunts around the membrane that render the sensor inoperative (8). This problem has been addressed by others through modification of the PVC matrix for binding to hydroxyl-bearing surfaces (9–11), mechanical attachment of the membranes (12,

13), use of other materials (14–17), and photocuring techniques (18, 19), some of which have improved adhesion but offer inferior electrochemical performance when compared to PVC.

In this paper, we describe three new membrane matrices, PVC/hydroxylated PVC, polyurethane/hydroxylated PVC, and moisture-curable silicone rubber, that offer significant advantages over previously reported matrices for solid-state sensors (see Figure 1 for the polymer structures). The potentiometric properties of ion-selective membranes (K+ and NH4+) prepared with these alternative matrices are compared with those of the conventional PVC membranes. A well-controlled mechanical peel test is used to characterize the membrane adhesion. This new adhesion test yields repeatable, quantitative results for both wet and dry membranes. In addition, incorporating silicon tetrachloride in the polymer matrices is shown to improve membrane adhesion to the inner metallic contact (silver epoxy) and also to reduce the emf drift of the resulting solid-state ion sensors. The membrane matrices are also examined for their biocompatibilities via radiotracer protein adsorption and whole blood clotting time studies.

EXPERIMENTAL SECTION

Reagents. Nonactin, bis(2-ethylhexyl) adipate, *o*-nitrophenyl octyl ether (NPOE), and potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB) were obtained from Fluka (Ronkonkoma, NY), while hydroxylated PVC (a copolymer of vinyl chloride, vinyl acetate, and vinyl alcohol (PVC/Ac/Al), 80/5/15 wt %) was a product of Scientific Polymer Products, Inc. (Ontario, NY). Poly(vinyl chloride) (PVC) powders were purchased from both Fluka and Scientific Polymer Products. Valinomycin, albumin (bovine; fatty acid free), and fibrinogen (Type IV; from bovine plasma) were obtained from Sigma Chemical Co. (St. Louis, MO); silanol-terminated polydimethylsiloxane (Petrarch FF) and 10–12% (cyanopropyl)methyl–88–90% dimethylsiloxane copolymer were from Petrarch Systems (Bristol, PA); silicon tetrachloride (1 mol/L in dichloromethane) was from Aldrich Chemical Co. (Milwaukee, WI); and Tecoflex polyurethane (SG-80A) was from Thermedics, Inc. (Woburn, MA).

All other chemicals used were analytical–reagent grade. Standard solutions and buffers were prepared with deionized water.

Preparation of Ion-Selective Membranes. PVC-based potassium- and ammonium-selective membranes were prepared by incorporating valinomycin and nonactin, respectively, into PVC matrices as described elsewhere (9, 10, 20, 21). The polyurethane and hydroxylated PVC membrane compositions were similar to those of the PVC-based membranes (i.e., 1 wt % neutral carrier, 66 wt % plasticizer (bis(2-ethylhexyl) adipate), and 33 wt % polymeric material, unless otherwise indicated). Silicone rubber matrix membranes were prepared by dissolving appropriate amounts of silicone rubber, neutral carrier, and additives (KT*p*ClPB and/or cyano group derivatized silicone rubber) in THF and casting the mixture in a glass ring placed on a flat Teflon plate.
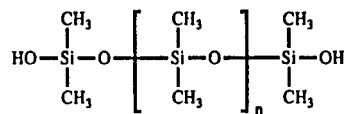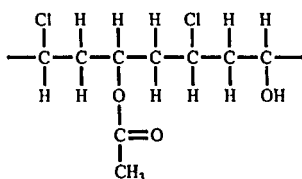
Evaluating Potentiometric Response and Selectivity. Electrochemical properties of the membranes were evaluated in conventional ion-selective electrodes. Smaller disks were punched

Polydimethylsiloxane, Silanol Terminated

Vinyl Chloride/Vinyl Acetate/Vinyl Alcohol Copolymer
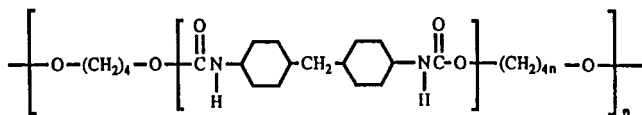
Tecoflex Polyurethane

Figure 1. Structures of polymer matrices examined for preparing potentiometric ion sensors.

from the membranes formed on Teflon or glass plates and mounted in Phillips electrode bodies (IS-561) (Glasblaserei Möller, Zurich). The inner filling solution varied, depending on which ion-selective membrane was being evaluated: 0.1 mol/L KCl for K^+-selective membranes and 0.1 mol/L NH_4Cl for NH_4^+-selective membranes. The external reference electrode was an Orion sleeve-type double junction Ag/AgCl electrode (Model 90-02). Electrodes were connected through a high-impedance amplifier to a Zenith Z-100 PC computer equipped with an analog-to-digital converter (DT 2801, Data Translations, Inc., Malborough, MA). The calibration plots were obtained from additions of standard solutions to 250 mL of background electrolyte (0.05 mol/L Tris–HCl, pH 7.2) and measurements of emf values taken every 10 s at room temperature. Selectivity coefficients were determined by the separate solution method (22).

Radiotracer Protein Adsorption Studies. Fibrinogen and albumin were radioiodinated by using the chloramine-T method (23). The ^{125}I-labeled proteins were mixed with native proteins to a specific activity of 3500 cpm/μg (cpm $\equiv$ counts per minute) and used at a concentration of 100 μg/mL.

A piece (0.8 × 1.25 cm) of each membrane matrix, incorporating valinomycin for potassium selectivity, was prehydrated in a tube containing 3 mL of phosphate-buffered saline (0.01 mol/L phosphate, 0.05 mol/L EDTA, 0.14 mol/L NaCl, 0.2 g/L thimerosol, pH 7.0) with care to ensure the absence of bubbles at the membrane/solution interface. After the membranes were soaked overnight and equilibrated to room temperature, 1 mL of the radiolabeled protein solution was pipetted rapidly into the tubes. After a 60-min incubation period, the protein solution was removed from the tube, and the membrane was rinsed with phosphate-buffered saline to terminate protein adsorption. For this, a test tube cap was fitted with two 14-gauge hypodermic needles, which served as entrance and exit ports. Vacuum was applied to one of the needles, and rinsing buffer entered through the other. The rinse buffer was pulled through the tube for 1 min at a rate of 100 mL/min. After rinsing, the membranes were placed in a counting tube containing 2 mL of buffer, and the retained radioactivity was measured in an automatic γ counter (Model 1290 GammaTrac, Tm Analytic, Elk Grove Village, IL). In this study, each membrane matrix was tested in triplicate.

Whole Blood Clotting Time Measurements. Polymer matrices were coated on the inner walls of glass test tubes (10 × 75 mm) by adding 1 mL of each membrane casting solution to the tubes, tilting 90°, and rotating the tube until a thin film formed. After the coating had been allowed to dry overnight, the whole blood clotting times were measured by the method described previously (24). Briefly, 1 mL of fresh human blood withdrawn
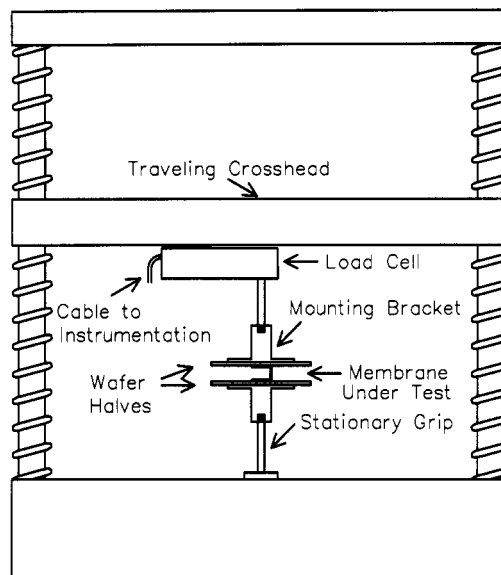
Figure 2. Schematic drawing of adhesion tester.

from volunteers was placed in each tube, and the tubes were gently tilted every 60 s until the blood was clotted. The clotting time for each matrix type was determined as a mean of 14 determinations ± standard deviation.

Evaluating Membrane Adhesion. For testing the adhesion of membranes to silicon nitride surfaces, 3600 Å of silicon nitride was first deposited on 7.62-cm silicon wafers by low-pressure chemical vapor deposition. To evaluate the adhesion to silver-loaded epoxy surfaces, a screen printing technique was utilized to deposit a smooth layer of silver epoxy (Epo-Tek H20E; Epoxy Technology Inc., Billerica, MA) on 7.62-cm silicon wafers. A programmable diamond saw (Model M-1006, Micro Automation Inc., Fremont, CA) was then used to cut a 200 μm deep by 75 μm wide scribe lane across the center of the 375 μm thick wafers on the backside. Acetone, isopropyl alcohol, deionized water, and nitrogen were used to clean and dry the wafers.

The membrane casting solutions were prepared by completely dissolving the polymer membrane components in THF without adding ionophore. Silicon tetrachloride, when used, was mixed with the casting solution immediately before the mixture was applied. Glass rings (22-mm i.d.) were placed on the front of the silicon wafer, centered over the backside scribe lane. These glass rings were filled with casting solution, and the solvent was allowed to evaporate for 30 min until the membranes were partially cured. The glass rings were then removed, and the membranes were dried for 24 h. It should be noted that the adhesion results can be affected by the relative humidity when the membranes are cast. In the case of silicone rubber membranes, the membranes failed cohesively before peeling at a force roughly 15 times that of PVC (with no silanizing reagent added) on a silicon nitride surface. To increase the cohesive strength of this membrane for the adhesion test, a nylon mesh was embedded in the matrix when it was cast.

Mounting brackets were attached with epoxy to the backside of the wafer on either side of the scribe lane. After the epoxy was dry, the wafer was scribed down the precut lane and cleaved. This was done by carefully folding the halves, held together by the membrane, forward, so that the membrane halves were touching. The cleaved wafer sample was mounted on an Instron 1131 mechanical tester (Instron Corp., Canton, MA) with one half attached to the load cell and the other to a stationary grip (see Figure 2).

This mounting method leaves the membrane free of deformation from mounting fixtures or grips since the membrane only contacts the substrate. The tester has a traveling crosshead which moves at 1.27 cm/min, inducing a pulling force at both membrane/substrate interfaces. The membrane peels from both interfaces at approximately the same rate, maintaining a constant peel angle of 90°. The load cell measures the membrane pulling force and converts it to an electronic signal that is read by the instrumentation, controlled by an IBM-XT microcomputer with
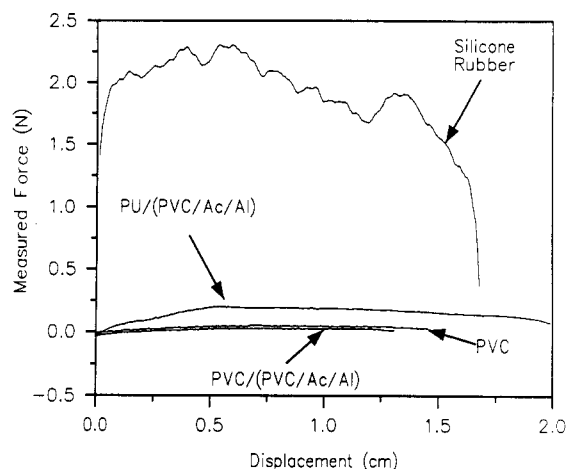
Figure 3. Typical peel-force curves for the four membrane matrices evaluated in this study. The membranes were dry and had no silanizing reagent added to the matrix.

a programmable data acquisition/control adapter card (IBM part No. 6451502). Typical peel-force curves for the polymer matrices are shown in Figure 3. The processed data were scanned by the microcomputer for the maximum force value, which was taken to represent the adhesion of the membrane. In our experience, the maximum force that the interfacial bond can withstand before yielding is the best representation of the adhesive strength of the membrane material (25).

Evaluating Stability of Solid-State Sensors. For the preliminary interface stability studies, silver epoxy-based solid-state ion sensors were fabricated by following a modification of the method previously described by Lima and Machado (26). Silver-loaded epoxy resin (Eop-Tek 410LV) was applied to one end of a glass tube (12-cm length; 0.95-cm i.d.) to form a layer of silver epoxy about 1 cm thick. A copper wire was inserted through the tube and into the silver epoxy layer, and the tube assembly was left in an oven at 100 °C for 1 h to cure the epoxy resin. The silver epoxy glass tube was fitted into a piece of Tygon B-44-3 tubing (VWR Scientific, Francisco, CA) to form a cavity on the silver epoxy surface. Potassium- and ammonium-selective sensors were prepared by inverting the silver epoxy electrode, applying 0.3 mL of membrane casting solutions (i.e., 2 mg of neutral carrier, 143 μL of bis(2-ethylhexyl) adipate, and 66 mg of polymeric material in 1.2 mL of THF) dropwise into the cavity, and evaporating the solvent overnight (see Figure 4). In cases in which silicon tetrachloride was incorporated in the membranes, 25 μL of 1.0 mol/L SiCl$_4$ was applied to the silver epoxy surface prior to the addition of the casting solution.

The potential drift of the resulting solid-state sensors was evaluated in 250 mL of 0.05 M Tris-HCl buffer, pH 7.2, containing 0.1 mmol/L KCl (for the potassium sensors) or NH$_4$Cl (for the ammonium sensors). The emf values were recorded every 5 min for 92 h at room temperature, using the high-impedance amplifier/analog-to-digital converter setup described in the previous section. The membranes were not conditioned prior to the measurements.

RESULTS AND DISCUSSION

Hydroxylated PVC has been used previously with silanizing reagents, such as silicon tetrachloride, to enhance membrane adhesion to silica substrates (9–11). The hydroxyl groups were incorporated into PVC matrices either by chemically reducing carboxylated PVC (9, 11) or by incorporating a copolymer of vinyl chloride/vinyl acetate/vinyl alcohol (PVC/Ac/Al, 91/3/6 wt %) into PVC (10). However, in these previous studies with PVC/hydroxylated PVC copolymer membranes, only a small number of hydroxyl groups were actually incorporated, because a higher content of the copolymer resulted in membranes with insufficient mechanical strength (10). In contrast, in preliminary studies we found the use of higher molecular weight PVC (MW = 275 000) increased the me-
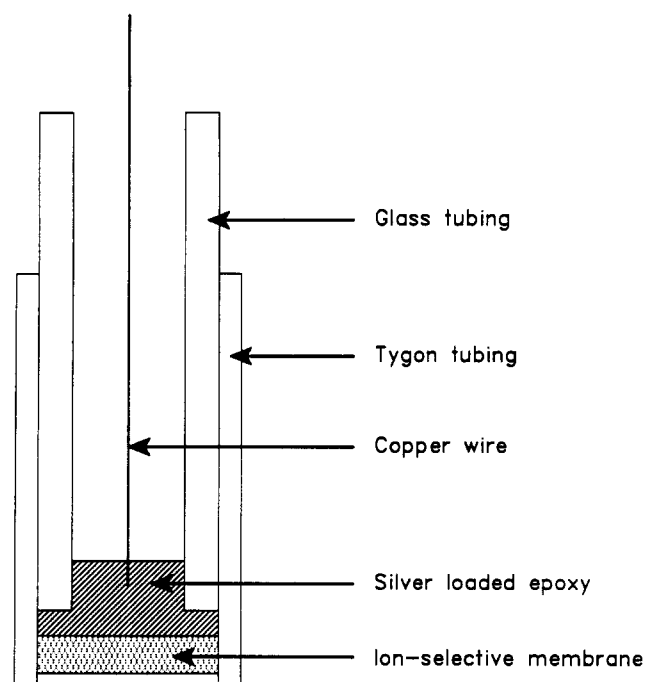

Figure 4. Schematic drawing of polymer-coated solid-state ISE.

chanical strength of the final plasticized membranes. Thus, in this study, a more highly hydroxylated PVC copolymer (vinyl chloride/vinyl acetate/vinyl alcohol, PVC/Ac/Al, 80/5/15 wt %) could be used to increase the hydroxyl content of the resulting membranes. The mechanical strength of the PVC/hydroxylated PVC membranes was further improved, without significant loss of potentiometric slope and selectivity, by reducing the amount of plasticizer (59 wt % for (PVC)/ (PVC/Ac/Al) membranes vs 66 wt % for the standard PVC-matrix membranes). The resulting optimized membranes consist of 20 wt % PVC, 20 wt % PVC/Ac/Al, 59 wt % plasticizer, and 1 wt % neutral carrier (see Table I), and the polymer matrix is 2.7 wt % hydroxylated, 3–12 times more than in membranes previously reported (9, 10). It was thought that a hydroxylated PVC matrix with good mechanical strength as well as an increased hydroxyl content would result in membranes with adhesion better than what had been previously reported (9, 10).

Polyurethane has received attention as a potential outer membrane for chemical sensors because of its excellent biocompatibility (27–29). In this study, we used Tecoflex polyurethane (SG-80A), which is a thermoplastic, aliphatic resin specially formulated for medical applications. This polyurethane material is a reaction product synthesized from methylene bis(cyclohexyl)diisocyanate, poly(tetramethylene ether glycol), and 1,4-butanediol chain extender (see Figure 1 for the structure). It can be dissolved in several organic solvents including THF, and develops good adhesion to silica surfaces. Unfortunately, the response slope and selectivity of potassium-selective membranes prepared with pure polyurethane matrix were inferior to those of the standard PVC matrix membranes (tested in conventional ISE arrangements); for the polyurethane membrane, the slope was 53.6 mV/ decade for 10^{-5} to 10^{-1} mol/L K$^+$ and selectivity (log k_{K^+,Na^+}^{pot}) was −3.5, compared to a slope of 57.1 mV/decade and selectivity of −4.3 for the PVC membranes. The potentiometric performance of the polyurethane membranes can be improved, however, by adding PVC or hydroxylated PVC to the membrane formulation. Polyurethane membranes incorporating hydroxylated PVC have better adhesion to glass and silicon nitride than do those containing added PVC. As can be seen in Table I, polyurethane membranes incorporating 6.6 wt %

Table I. Compositions and Electrochemical Characteristics of Four Different Membrane Matrices Selective to Potassium and Ammonium

no.	comp of matrix, wt %[a]	slope, mV/decade		det limit, μM		selectivity log $k_{i,j}^{pot}$		
						$i = K^+$	$i = NH_4^+$	
		K^+	NH_4^+	K^+	NH_4^+	$j = Na^+$	$j = Na^+$	$j = K^+$
1	PVC, 33	57.1	55.8	0.58	0.98	−4.28	−2.82	−0.85
2	PVC, 20 PVC/Ac/Al, 20	57.3	56.0	0.52	0.91	−4.22	−2.77	−0.82
3	PU, 26.4 PVC/Ac/Al, 6.6	57.2	56.8	0.59	0.99	−4.21	−2.89	−0.89
4	P–FF, 78 Si–CN, 21	56.5	50.0	1.0	1.4	−4.16	−2.64	−0.68

[a] Potassium- and ammonium-selective membranes are doped with 1 wt % valinomycin and nonactin, respectivity. The balance of the membrane is DOA (bis(2-ethylhexyl) adipate) plasticizer. The silicone rubber membrane contains 0.34 wt % KTpClPB and no plasticizer; PU = polyurethane, P–FF = polydimethylsiloxane, silanol terminated, Si–CN = 10–12% (cyanopropyl)methyl and 88–90% dimethylsiloxane copolymer.

hydroxylated PVC exhibited electrochemical performance comparable to the conventional PVC matrix membrane.

Various silicone rubber compositions have been previously used as membranes for ISEs and ISFETs (30–32). The electrical resistance of silicone rubber membranes is typically very high and can be as much as 10–100 times the resistance of a conventional PVC membrane. The increased resistance can degrade the electrochemical performance as well as increase the signal noise of the ISE. The silicone rubber matrix employed in this study is easily cured at room temperature by moisture activation, which causes a condensation of silicone prepolymers to high molecular weight rubber with acetic acid as a byproduct. It exhibits high bond strength to a wide range of substrates including silicon nitride. In addition, reduced protein adsorption and blood clotting have been reported for this type of material (29, 33, 34). However, the electrical resistance of this silanol-terminated polydimethylsiloxane membrane is very high. The signal noise associated with high resistance was improved by incorporating the lipophilic additive (potassium tetrakis(p-chlorophenyl)borate) and cyano group derivatized silicone rubber (10–12% (cyanopropyl)-methyl–88–90% dimethylsiloxane copolymer).

These optimized membrane compositions were evaluated in terms of their electrochemical performance, biocompatibility, and adhesion characteristics. Table I summarizes the electrochemical properties of PVC and the three new membrane compositions incorporating valinomycin and nonactin for potassium and ammonium selectivity, respectively. These data ($n = 3$) were taken with membranes mounted in conventional ion-selective electrode bodies. All of the new membranes exhibited electrochemical performance comparable to that of PVC, except for the ammonium-selective silicone rubber membrane, which had slightly inferior slope and selectivity.

For future use of the proposed membrane formulations in biomedical sensors (e.g., in vivo measurements), it is important to document the biocompatibility of such materials. It is known that two major determinants of membrane biocompatibility are the amount of nonspecific protein adsorption which occurs on the surface and the type of protein bound (35, 36). In general, the greater the amount of fibrinogen bound, the greater the chance for thrombogenesis and inflammatory reactions, while bound albumin decreases the chance for thrombogenesis and inflammatory reactions. To assess the relative adsorption of proteins to the membranes, albumin and fibrinogen were radioiodinated and incubated with the various membrane matrices containing valinomycin for potassium selectivity (as described in the Experimental Section). The time of adsorption was strictly controlled (at 60 min) since the adsorption of proteins at the solid surfaces is time-dependent. After the membranes were washed with
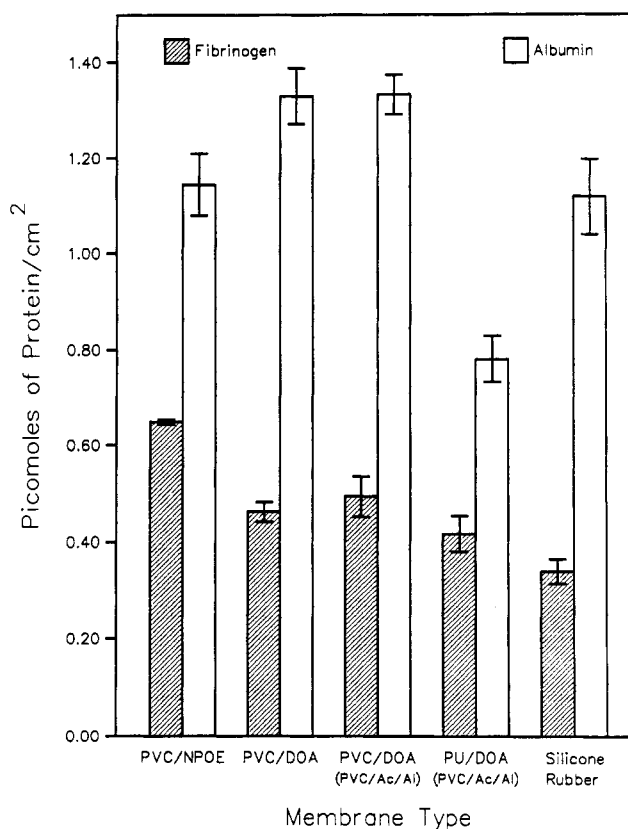

Figure 5. Nonspecific protein adsorption for various membrane matrices. Each membrane matrix was tested in triplicate.

buffer, the amount of radiolabeled proteins bound per unit area of the membranes (pmol/cm^2) was determined. For purposes of calculation, the molecular weight of albumin was taken to be 60 000 and that of fibrinogen to be 400 000. In order to examine the effect of iodination on protein-binding characteristics, radiolabeled proteins were incubated with the membrane matrices in the presence of varying concentrations of native, nonlabeled albumin and fibrinogen. Resulting competitive adsorption isotherms revealed the half-maximal binding of each protein, when competing with itself, to be very close to the 1:1 ratio of unlabeled to labeled, indicating that iodination had little effect on the binding of the protein. From this study, it was found that the polyurethane- and silicone-rubber-based membranes exhibited less overall nonspecific protein adsorption than the PVC or hydroxylated PVC matrices as well as an albumin to fibrinogen molar ratio (see Figure 5) favorable for biocompatibility. Note that the PVC membrane prepared with the plasticizer DOA exhibited

Table II. Whole Blood Clotting Time Measurements for Various Polymer-Coated Test Tubes

no.	membrane comp, wt %	av clotting time, min	std dev, min
1	glass (no polymer coating)	8.75	1.97
2[a]	PVC, 20.2 PVC/Ac/Al, 20.2	20.43	4.30
3[b]	P-FF, 78.5 Si–CN, 21.1	22.14	5.19
4[a]	PVC, 33.3	24.11	4.17
5	PU, 100	24.63	4.79
6	PU, 80 PVC/Ac/Al, 20	24.95	3.97
7[a]	PU, 26.7 PVC/Ac/Al, 6.7	25.50	5.00

[a] Balance of the membrane is DOA (bis(2-ethylhexyl) adipate) plasticizer. [b] The silicone rubber membrane contains 0.34 wt % KTpClPB.

Table III. Comparison of Normalized Pulling Force Required To Peel Different Membranes from a Surface

matrix type	normalized pulling force[a]		
	dry		wet[b]
	no SiCl₄	with SiCl₄	
a. Si₃N₄ Surface[b]			
PVC	1.00	1.77	1.01
PVC/(PVC/Ac/Al)	0.54	1.04	1.01
PU/(PVC/Ac/Al)	4.15	54.99	30.84
silicone rubber	39.29		36.16
b. Silver Epoxy Surface[c]			
PVC	0.89	0.89	0.55
PVC/(PVC/Ac/Al)	0.86	1.75	1.62
PU/(PVC/Ac/Al)	5.37	71.22	53.20
silicone rubber	29.88		30.34

[a] All data are normalized to dry PVC on a Si₃N₄ surface with no SiCl₄ added to the matrix. [b] The PVC, PVC/(PVC/Ac/Al), and PU/(PVC/Ac/Al) membranes had 10 wt % SiCl₄ added to the matrix.

nonspecific adsorption properties (i.e., less fibrinogen and more albumin bound) different from those of the same PVC matrix prepared with a higher dielectric plasticizer, NPOE. These data on protein adsorption may also relate directly to the magnitude of the protein asymmetry potential (37) displayed by each membrane when exposed to blood, but such measurements were not carried out in this study.

A whole blood clotting time test was also performed to evaluate the blood compatibility of each membrane composition. For this, the inner walls of glass tubes were coated with PVC and the alternative membrane compositions, without any ionophore. The mean clotting time and standard deviation ($n = 14$) for each of the membrane compositons is presented in Table II. The effects of plasticizer and hydroxylated PVC on the blood compatibility of polyurethane were evaluated with membranes 5–7. All polymer-coated tubes showed longer clotting times than the plain glass tubes. In this experiment, the polyurethane-based membranes appeared to show the best blood compatibility. However, application of the single-factor ANOVA test (analysis of variance at a 95% confidence level) (38) to this data indicates that only membranes 2 and 7 from Table II show a statistically significant difference in clotting times.

Though adhesion of membranes to the sensor surface is only one of the factors determining sensor lifetime, it has been the limiting factor in most miniature solid-state sensors reported to date. Methods used for characterizing this important parameter can be hard to reproduce and may be subject to systematic and operator errors. For instance, the simple tape test, previously used for chemical membrane adhesion (9), is capable of yielding qualitatively valid results but has a number of potential drawbacks. Adhesion of the tape itself varies with membrane material and some polymers have very poor adhesion to the tape. This variation can invalidate comparisons between different membrane matrices. Furthermore, results of the tape test are binary for all membranes except those having adhesion to the substrate that is similar to their adhesion to the tape. For example, tape test results for conventional PVC membranes and the three new membrane formulations reported here were not very enlightening: all of the PVC and PVC/hydroxylated PVC films pulled off of the silicon nitride stustrate, while none of the polyurethane and silicone rubber films came off. In addition, the tape test cannot be used on wet membranes (the condition of greatest interest).

The mechanical peel test (described in the Experimental Section) for measuring adhesion of thin polymeric membranes to solid surfaces, works for both dry and wet membranes. The method involves straightforward sample preparation, gives valid comparisons between different membrane types, and

produces very repeatable results. Using the method described, we compared the pulling force required to peel membranes of each matrix type from two different surfaces, silicon nitride and silver-loaded epoxy (Table III). The silver epoxy, which can be screen printed, was included for the adhesion test since it can be used as the inner metallic electrode of solid-state sensors. However, it should be noted that the membrane adhesion toward the silicon nitride surface (rather than the silver epoxy surface) determines the sensor lifetime when used in integrated solid-state microsensors (not described here). The peel-force values ($n = 3$) presented in Table III are normalized to the value obtained for the dry PVC matrix on the silicon nitride surface with no silanizing reagent added. The polyurethane/hydroxylated PVC and silicon rubber membranes exhibited the best adhesion to the silicon nitride surfaces when no silanizing reagent was used. When the silanizing reagent (10 wt % silicon tetrachloride) was used, the polyurethane-based matrices exhibited the most dramatic adhesion improvement to the surfaces of both silicon nitride and silver epoxy (see Table III). The silanizing reagent was not used for the silicone rubber membrane since it developed strong adhesion to both substrates without the use of adhesion promoter. As expected, the adhesion of all of these membranes decreased after soaking in room-temperature water. However, the polyurethane and silicone rubber matrix membranes still maintained strong bond strength after a 24-h soak. It should be noted that the adhesion of the PVC membrane to the silver epoxy surface was significantly reduced after the soaking period.

The potential at the membrane/solid interface of solid-state sensors is known to drift over time. This drift is usually attributed to lack of a thermodynamically defined interfacial potential between the sensing membrane and the inner metal layer. It is believed that poor membrane adhesion to the inner solid contact can cause further potential drift at the membrane/solid interface due to osmotic transport of water into this ill-defined layer. Several methods have been reported for improving the stability of this interface. For example, a layer of poly(hydroxylethyl methacrylate) (32), poly(vinyl alcohol) (39), or dextran (40) containing potassium chloride was placed between the sensing membrane and the Ag/AgCl contact. A double-membrane ion-selective electrode has also been reported (41). This latter modified coated-wire electrode was prepared with an internal conducting membrane made of tetrabutylammonium bromide in PVC or naphthalene and an external active membrane containing an ionophore, on a silver-amalgamated electrode. However, in this study, it was found that simple incorporation of silicon tetrachloride into
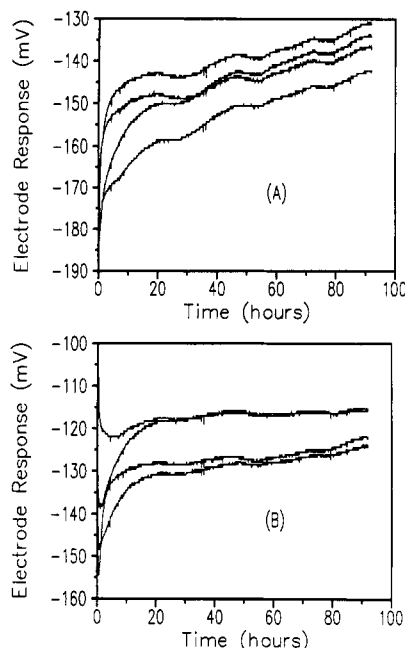
Figure 6. Stability of silver-loaded epoxy electrodes coated with potassium-selective membrane matrices: (A) PVC; (B) polyurethane/hydroxylated PVC/SiCl$_4$.

the polyurethane matrix ion-selective membranes significantly improved the emf stability of the resulting solid-state sensors. As seen in Figure 6, silver epoxy electrodes coated with potassium-selective polyurethane/hydroxylated PVC membranes containing 10 wt % silicon tetrachloride exhibit significantly reduced potential drift over 90 h (in 0.1 mmol/L KCl, 0.50 mol/L Tris-HCl, pH 7.2), compared to that of conventional PVC membranes without silicon tetrachloride. A similar improvement was observed when nonactin was used as the ammonium-selective ionophore. This stability enhancement may be in part due to the improved wet membrane adhesion of the silanized polyurethane matrix membranes toward silver-loaded epoxy (see Table IIIb). It should be noted that in this preliminary study the membrane components in the perimeter region intermix with the Tygon tubing (plasticized PVC) components (see Figure 4), which could add some instability to the electrode response. However, if the membranes were cast directly on integrated solid-state microsensors, as in the intended application, even better stability may result.

The effects of using silicon tetrachloride on the electrochemical properties of ion-selective membranes were also examined. Others have reported adverse effects on potentiometric slope and selectivity when silicon tetrachloride is used in excess (i.e., more than 5.0 wt %, referenced to the membrane weight) (10). Excess silanol groups over hydroxyl groups in the matrix were thought to cause the degradation in the electrochemical performance. However, it was found that polyurethane/hydroxylated PVC membranes containing up to 10 wt % silicon tetrachloride maintained excellent electrochemical properties. The potentiometric slope and selectivity of potassium-selective sensors with silver epoxy contacts (n = 4) were as follows: for membranes without silicon tetrachloride, slope = 57.0 mV/decade for 10^{-5} to 10^{-1} mol/L K$^+$, selectivity (log k_{K^+,Na^+}^{pot}) = -4.3; for polyurethane/hydroxylated PVC membranes with 10 wt % silicon tetrachloride, slope = 56.3 mV/decade and selectivity = -4.1.

In summary, three alternative ion-selective membrane matrices have been examined for use in the fabrication of solid-state ion sensors. A well-controlled peel test, which works for both wet and dry membranes, was used to evaluate membrane adhesion. The membrane matrices were also examined for their biocompatibilities. The polyurethane/hy-

droxylated PVC membranes have electrochemical performance equivalent to those of PVC. The silicone-rubber- and polyurethane-based membranes have much better adhesion to the silicon nitride surfaces than do PVC membranes. Particularly exciting is the enhancement in membrane adhesion and the elctrochemical stability of polyurethane/hydroxylated PVC membranes cast with silicon tetrachloride. This could lead to the development of truly useful miniaturized polymer-membrane-based solid-state ion sensors for a wide range of applications, including in vivo measurements.

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LITERATURE CITED

(1) Moss, S. D.; Janata, J.; Johnson, C. C. *Anal. Chem.* **1975**, *47*, 2238-2243.
(2) McBride, P. T.; Janata, J.; Comte, P. A.; Moss, S. D.; Johnson, C. C. *Anal. Chim. Acta* **1978**, *101*, 239-245.
(3) Oesch, U.; Caras, S.; Janata, J. *Anal. Chem.* **1981**, *53*, 1983-1986.
(4) McKinley, B. A.; Houtchens, B. A.; Janata, J. *Ion-Sel. Electrode Rev.* **1984**, *6*, 173-208.
(5) Janata, J.; Bezegh, A. *Anal. Chem.* **1988**, *60*, 62R-74R.
(6) Brown, R. B.; Huber, R. J.; Petelenz, D.; Janata, J. In *Proceedings of the 3rd International Conference on Solid-State Sensors and Actuators*, Philadelphia, PA, June 1985; IEEE: Piscataway, NJ, 1985; pp 125-127.
(7) Lauks, I.; Van der Spiegel, J.; Sansen, W.; Steyaert, M. In *Proceedings of the 3rd International Conference on Solid-State Sensors and actuators*: Philadelphia, PA, June 1985; IEEE: Piscataway, NJ, 1985; pp 122-124.
(8) Brown, R. B. Ph. D. Dissertation, University of Utah, June 1985.
(9) Satchwill, T.; Harrison, D. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1986**, *202*, 75-81.
(10) Moody, G. J.; Thomas, J. D. R.; Slater, J. M. *Analyst* **1988**, *113*, 1703-1707.
(11) Harrison, D. J.; Cunningham, L. L.; Xizhong, L.; Teclemariam, A.; Permann, D. J. *Electrochem. Soc.* **1988**, *135*, 2473-2478.
(12) Blackburn, G.; JHanata, J. *J. Electrochem. Soc.* **1982**, *129*, 2580-2584.
(13) Covington, A. K.; Whalley, P. D. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 1209-1215.
(14) Fujihara, M.; Fukui, M.; Osa, T. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *106*, 413-418.
(15) Moody, G. J.; Slater, J. M.; Thomas, J. D. R. *Analyst* **1988**, *113*, 103-108.
(16) Wen, C. C.; Lauks, I.; Zemel, J. N. *Thin Solid Films* **1980**, *70*, 333-340.
(17) Cha, G. S.; Brown, R. B. *Sens. Actuators* **1990**, *B1*, 281-285.
(18) Cattrall, R. W.; Iles, P. J.; Hamilton, I. C. *Anal. Chim. Acta* **1985**, *169*, 403-406.
(19) Cardwell, T. J.; Cattrall, R. W.; Iles, P. J.; Hamilton, I. C. *Anal. Chim. Acta* **1985**, *177*, 239-242.
(20) Ammann, D.; Morf, W. E.; Anker, P.; Meiser, P. C.; Pretsch, E.; Simon, W. *Ion-Sel. Electrode Rev.* **1983**, *5*, 3-92.
(21) Craggs, A.; Moody, G. J.; Thomas, J. D. R. *J. Chem. Educ.* **1974**, *51*, 541-544.
(22) Moody, G. J.; Thomas, J. D. R. *Selective Ion-Sensitive Electrodes*; Merrow: Watford, U.K., 1971; pp 4-32.
(23) Greenwood, F. C.; Hunter, W. M.; Glover, J. S. *Biochem. J.* **1963**, *89*, 114-123.
(24) Lee, R. I.; White, P. D. *Am. J. Med. Sci.* **1913**, *145*, 495-503.
(25) Goldberg, H. D.; Cha, G. S.; Brown, R. B. In *Technical Digest of IEEE Sensor and Actuator Workshop*, Hilton Head Island, SC, June 1990; IEEE Catalog No. 90CH2783-9; IEEE: Piscataway, NJ, 1990; pp 169-172.
(26) Moody, G. J.; Thomas, J. D. R.; Lima, J. L. F. C.; Machado, A. A. S. C. *Analyst* **1988**, *113*, 1023-1027.
(27) Coury, A. J.; Slaikeu, P. C.; Cahalan, P. T.; Stokes, K. B.; Hobot, C. M. *J. Biomater. Appl.* **1988**, *3*, 130-179.
(28) Lelah, M. D.; Cooper, S. L. *Polyurethane in Medicine*; CRC Press: Boca Raton, FL, 1986.
(29) Kambic, H. E.; Kiraly, R. J.; Nose Y. *J. Biomed. Mater. Res.* **1976**, *10*, 561-570.
(30) Fiedler, U.; Růžička, J. *Anal. Chim. Acta* **1973**, *67*, 179-193.
(31) Pick, J.; Tóth, K.; Pungor, E.; Vasák, M.; Simon, W. *Anal. Chim. Acta* **1973**, *64*, 477-480.
(32) Sudholter, E. J. R.; van der Wal, P. D.; Skowronska-Ptasinska, M.; van den Berg, A.; Bergveld, P.; Reinhoudt, D. N. *Anal. Chim. Acta* **1990**, *230*, 59-65.

(33) Mason, R. G.; Shermer, R. W.; Zucker, W. H.; Elston, R. C.; Black-welder, W. C. *J. Biomed. Mater. Res.* **1974**, *8*, 341–356.
(34) Maloney, J. V., Jr.; Rher, D.; Roth, E.; Latta, W. A. *Surgery (St. Louis)* **1969**, *66*, 275–283.
(35) Andrade, J. D.; Hlady, V. *Adv. Polym. Sci.* **1986**, *79*, 1–63.
(36) *Hydrogels in Medicine and Pharmacy*; Pappas, N. A., Ed.; CRC Press: Boca Raton, FL, 1986, 1987; Vols. I–III.
(37) Dürselen, L. F. J.; Wegmann, D.; May, K.; Oesch, U.; Simon, W. *Anal. Chem.* **1988**, *60*, 1455–1458.
(38) *Probability and Statistics for Engineering and the Sciences*; Devore, J. L., Ed.; Brooks-Cole Publishing: Pacific Grove, CA, 1991.
(39) Smith, M. D.; Genshaw, M. A.; Greyson, J. *Anal. Chem.* **1973**, *45*, 1782–1784.
(40) Van den Vlekkert, H.; Francis, C.; Grisel, A.; de Rooij, N. *Analyst* **1988**, *113*, 1029–1033.
(41) Diaz, C.; Vidal, J. C.; Galban, J.; Urarte, M. L.; Lanaja, J. *Microchem. J.* **1989**, *39*, 289–297.

Flow-Through Sensor for the Direct Determination of Pesticide Mixtures without Chromatographic Separation

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A flow-through sensor for determination of carbamate compounds (carbofuran, propoxur, and carbaryl) based on retention of the products on C_{18} bonded phase beads packed in a flow-cell was developed. The associated determinative procedure relies on the hydrolysis of the analytes and coupling to diazotized sulfanilic acid to yield the corresponding dyes. A conventional flow injection (FI) method was also developed for comparison. A diode array spectrophotometer was used in both cases to monitor the reaction products at nine wavelengths, resulting in maximal differences between the absorption spectra. Linear calibration curves over the ng/mL to μg/mL range (determination limit 50 times lower than that of the conventional FI method) and excellent results on application to mixture resolution in water samples from different sources testify to the usefulness of the proposed sensor.

INTRODUCTION

Pesticides of the carbamate family have become increasingly popular in recent years on account of their broad biological activity spectrum. They are used as insecticides, miticides, fungicides, nematocides, and molluscicides (*1*). Owing to their usually toxic and frequently chronic character, assessment of water and soil pollution calls for the concentration of carbamates to be determined. Their different toxicity requires discrimination capabilities for them and their metabolites, which are also usually toxic. A host of methods have been developed in the last few years for determination of carbamates; most of them are based on a separation by gas or high-performance liquid chromatography (*2*), if two or more analytes are to be determined, and on simpler techniques if individual carbamates (plus their metabolites) need to be assayed. Such is the case with the determination of carbaryl and its hydrolysis product by stopped-flow (*3*) or by synchronous first- or second-derivative fluorometry (*4*). Also, two carbamates used as insecticides (carbofuran and propoxur)

were recently assayed by a straightforward photometric method (*5*). However, no simple automatic flow methods for the simultaneous determination of carbamates requiring no separation technique has so far been reported.

In this work we developed two straightforward automatic methods for the simultaneous determination of three carbamates used as insecticides, namely carbofuran (CBF), propoxur (PPX), and carbaryl (RYL). Both are based on the use of a flow injection (*6, 7*) manifold where these three compounds are hydrolyzed to their corresponding phenols (carbofuran-phenol, 2-isopropoxyphenol, and 1-naphthol, respectively) and subsequently coupled to diazotized sulfanilic acid. The reaction products can be monitored in two ways, which result in two methods with different features:

(a) The dyes formed can be directly driven to the flow-cell of a diode array spectrophotometer (DAS) to be simultaneously monitored at several wavelengths, which also allows the simultaneous determination of the analytes. This determinative method has moderately good features.

(b) A special flow cell packed with suitable support (e.g. C_{18} bonded silica beads) can be used to integrate retention (concentration) and detection by using the same detector (DAS). The simultaneous in situ concentration and detection thereby achieved endows the resulting method with excellent features.

EXPERIMENTAL SECTION

Instruments and Apparatus. A Hewlett-Packard 8415 diode-array spectrophotometer equipped with an HP 9121 floppy disk drive, an HP 98155 keyboard, and an HP 7470 plotter was used. A Gilson Minipuls-2 and an Ismatec S-840 peristaltic pump, two Rheodyne 5041 injection valves (one of which acted as a selecting valve), and Hellma 178.52QS (10-mm optical path) and 138 OS (1-mm optical path) flow cells were also used. The 138 OS cell was packed with the support by aspirating it in an ethanol suspension with the aid of a peristaltic pump.

Reagents. Standards solutions of carbofuran, propoxur, and carbaryl (from Chemical Service) were prepared in 1:4 dioxane. Working strength solutions were prepared from these by diluting with distilled water. Aqueous solutions of 0.2% $NaNO_2$ and 0.2% sulfanilic acid in 30% acetic acid were used. The carrier solution was a 1:9 ethanol–water mixture. The eluent was a 1:1 ethanol–HNO_3 (2 M) solution. The solid support in the flow cell was