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A Planar pCO_2 Sensor with Enhanced Electrochemical Properties

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To develop planar microchemical pCO_2 sensing devices with improved electrochemical properties, we combined two advanced technologies. One is a differential sensor arrangement to simplify the microfabrication procedure by employing pH-sensitive gas-permeable membranes, and the other is the use of an enzyme (carbonic anhydrase) to shorten total measurement time by accelerating the rate of CO_2 hydration. The adhesion of the polyurethane−matrix gas-permeable membrane is enhanced significantly by incorporating a silanizing reagent (silicon tetrachloride), improving the stability and extending sensor lifetime. The proposed differential pCO_2 microelectrodes exhibited significantly improved performance in their preconditioning period, response and recovery times, stability, response slope, and lifetime.

It has been well recognized that the determination of carbon dioxide in physiological samples is of utmost relevance to understand and control acid−base status and electrolyte balance.[1,2] Therefore, much effort has been devoted to the development of methods for the accurate and reliable measurement of pCO_2 to effectively assess the urgently changing status of critically ill and surgical patients.[3] Typically, most gas measurements have been performed in centralized laboratories remote from the patient using a very expensive instrument.[4,5] However, since the time delay between sampling and measuring can cause serious deterioration of discrete blood samples, in modern health care settings there has been a large demand for point-of-care or decentralized testing devices capable of rapidly and inexpensively monitoring pCO_2 levels at or near the patient's bedside.[6,7] To meet such requirements, there is a need for planar microchemical pCO_2 gas sensor systems with a rapid hydration time (the preconditioning period required by the sensor to achieve the optimal analytical performance) and response time, resulting in practical reduction of total measurement time.

Commercially available blood gas analyzers frequently used in clinical laboratories have employed Severinghaus-type pCO_2 gas sensors consisting of internal pH glass and reference electrodes, a gas-permeable membrane, and an electrolyte solution. Unfortunately, the classical Severinghaus electrode has met with little success in the development of portable pCO_2 analyzers, since the sensor is too expensive and cumbersome for miniaturization and has a relatively slow response, increasing total measurement time. To overcome the problem of fabricating miniature pCO_2 gas sensors, many attempts have been explored, including the application of solvent polymeric membrane electrodes,[8−11] metal/metal oxide electrodes,[12−14] and ion-selective field effect transistors[15−18] as an alternative to the pH glass electrode. However, such devices have inherent drawbacks in construction, cost, and reliability due to the complication that the working pH and reference electrodes should be inside a gas-permeable membrane to complete an electrical measurement circuit. Recently, we demonstrated that a differential arrangement of pCO_2 sensors employing an ionophore-doped low-resistance gas-permeable membrane is much simpler to microfabricate, because the reference electrode can be placed outside the gas-permeable membrane (i.e., within the sample solution).[19,20]

Several approaches to enhance preconditioning and response times of Severinghaus-type pCO_2 electrodes have also been examined previously. For instance, thin-film techniques using

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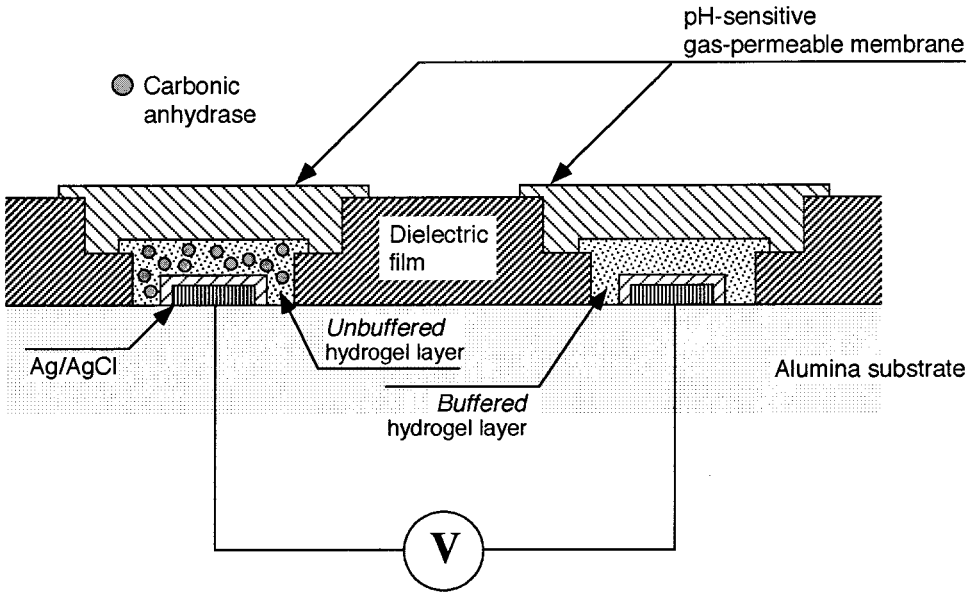

Figure 1. Schematic drawing of the differential pCO_2 microelectrode employing carbonic anhydrase in the hydrogel layer.

photolithographic methods for creating a hydrogel layer and a gas-permeable membrane were employed to reduce the practical diffusion pathway of CO_2 gas, thereby improving the response time of pCO_2 electrodes.[21,22] Cosofret et al. reported that all-solid-state microchemical sensors stored in containers with highly humid atmospheres could exhibit reduced hydration and response times in blood sample measurements.[23] In another approach, an additional outer layer of hygroscopic materials on the gas-permeable membrane, capable of efficiently absorbing water vapor from the ambient atmosphere to the electrolyte layer, could rapidly activate gas sensing devices and enhance the electrode response time.[24] The addition of enzyme carbonic anhydrase (CA) to an internal bicarbonate solution in electrochemical and optical pCO_2 sensing systems has also been proposed to enhance the response time, since this biocatalyst accelerates the rate of the CO_2 hydration reaction.[25−28]

In our work, we combined two advanced technologies in an attempt to obtain a planar pCO_2 microsensing device with faster preconditioning and response characteristics for dissolved CO_2 measurement in physiological samples: one is a differential sensing arrangement to facilitate the microfabrication of potentiometric pCO_2 electrodes, and the other is the use of CA to shorten total measurement time (Figure 1). The pH-sensitive polymeric membranes adapted for use in constructing a differential pCO_2 sensor system in this work function as both a gas-permeable membrane and an internal pH sensing element. In the differential configuration, the pCO_2 electrode is made with an *unbuffered* recipient layer including CA; hence the pH changes are promoted and detected. The reference electrode, on the other hand, employs a strongly *buffered* hydrogel layer; therefore, diffused CO_2 cannot change the pH in the recipient layer. In addition, the pH and ion response signals of the outer membrane surfaces (on the sample side) at both the pCO_2 and the reference electrodes are identical; therefore they cancel out.

In this work, the compositions of silicone rubber− and polyurethane−matrix membranes doped with H^+-selective ionophore, tridodecylamine (TDDA), are optimized for use in the fabrication of the solid-state differential pCO_2 electrodes. The effect of the enzyme on the performance (e.g., preconditioning and response times, stability, slope, and lifetime) of the resulting pCO_2 electrode is also discussed. Finally, the practical analytical utility of the proposed microchip-based pCO_2 sensor system is demonstrated by determining pCO_2 levels in human whole blood specimens.

EXPERIMENTAL SECTION

Reagents. The sources of reagents used were as follows: bis-(2-ethylhexyl) sebacate (DOS), 2-nitrophenyl octyl ether (NPOE), potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB), poly(vinyl chloride) (PVC), and poly(vinyl alcohol) (PVA; MW 22 000) from Fluka (Buchs, Switzerland); carbonic anhydrase from bovine erythrocytes (CA; 3240 W-A units/mg of protein), 2-(*N*-morpholino)ethanesulfonic acid (MES), and tris(hydroxymethyl)aminomethane (Tris) from Sigma (St. Louis, MO); tridodecylamine (TDDA) from Eastman Kodak (Rochester, NY); Tecoflex polyurethane (TPU; SG-80A) from Thermedics (Woburn, MA); one-component room-temperature vulcanizing (RTV)-type silicone rubber (3140 RTV) from Dow Corning (Midland, MI); silicon tetrachloride ($SiCl_4$; 1.0 M solution in dichloromethane) from Aldrich (Milwaukee, WI); and blood gas/electrolyte control samples from Alko Diagnostic (Holliston, MA) and Nova Biomedical (Waltham, MA). Whole blood samples were obtained from a local blood bank. All other chemicals used were of analytical reagent grade. Standard solutions and buffers were prepared with deionized water.

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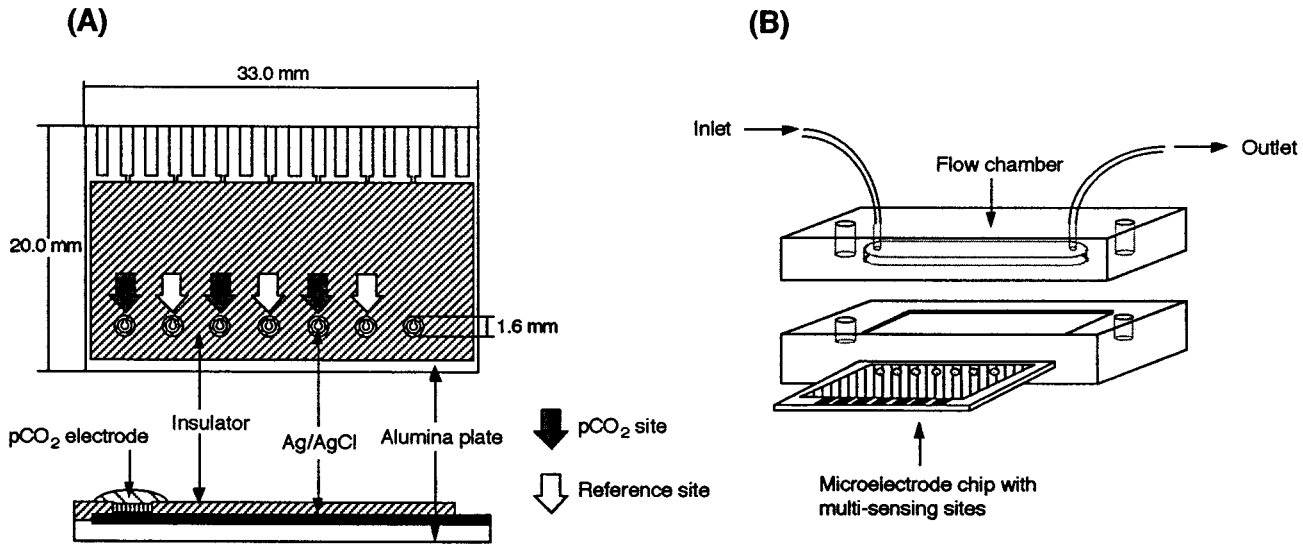

Figure 2. (A) Planar-type solid-state microelectrode with multisensing sites; (B) flow cell cartridge for the electrode chip.

Preparation and Evaluation of pH-Sensitive Membranes. Various silicone rubber− or polyurethane−matrix pH-sensitive membranes were prepared and evaluated for their pH response using conventional ion-selective electrodes as described in previous articles.[29,30] The silicone membrane compositions examined in this study were as follows: in addition to 3140 RTV, (a) 2.9 wt % TDDA; (b) 2.9 wt % TDDA and 1.4 wt % KT*p*ClPB; and (c) 1.9 wt % TDDA, 0.9 wt % KT*p*ClPB, and 35.0 wt % DOS. The polyurethane−matrix membranes were prepared by casting following mixtures: (d) 44.5 wt % TPU/PVC (75 wt % TPU and 25 wt % PVC), 4.0 wt % TDDA, 1.0 wt % KT*p*ClPB, and 50.5 wt % NPOE; and (e) an additional incorporation of $SiCl_4$ into the composition d. An aliquot amount (50 μL) of 0.02 M $SiCl_4$ (in CH_2Cl_2), when used, was mixed with the casting solution for TPU membranes immediately before the mixture was applied. An aqueous mixture of 0.1 M NaH_2PO_4/0.1 M Na_2HPO_4/0.01 M NaCl was employed as the internal filling solution. Potentiometric responses of the membrane electrodes to pH changes were evaluated using an external reference electrode [model 90-02 sleeve-type double-junction Ag/AgCl electrode (Orion, Cambridge, MA)]. The potential differences between the working and the reference electrodes were measured using an IBM-compatible computer equipped with a custom-built high-impedance input 16-channel analog-to-digital converter. Calibration curves were obtained by adding aliquots of LiOH to a background solution of 11.4 mM boric acid/6.7 mM citric acid/10.0 mM NaH_2PO_4 at room temperature.

Preparation and Evaluation of Differential pCO_2 Gas Sensors. The planar-type solid-state microelectrodes with multiple sensing sites were fabricated by screen-printing silver and dielectric pastes on an alumina plate with the pattern shown in Figure 2A.[31] The pCO_2 electrode was prepared by covering the internal Ag/AgCl electrode with an *unbuffered* hydrogel layer (20 μm in thickness; 1.0 mm in diameter) and a gas-permeable membrane (30 μm in thickness; 1.8 mm in diameter). The *unbuffered* hydrogel layer was deposited by dispensing 3 μL of an aqueous mixture consisting of 4 wt % PVA, 5 mM $NaHCO_3$, 0.5 mM NaCl, and varying amounts of CA (i.e., 0, 0.1, 0.3, 1.0, and 2.0 mg/mL**)** and then drying the film for 5 min. The gas-permeable membrane was formed by applying 10 μL of the optimized membrane mixtures (compositions c and e) dissolved in THF (400 μL for 200 mg of 3140 RTV and 900 μL for 66 mg of TPU/PVC), evaporating the solvent and curing the membrane overnight. In the case of the reference electrode, a *buffered* solution containing 4 wt % PVA, 0.5 mM NaCl, and 0.2 M MES−NaOH (pH 5.5) was employed as an internal hydrogel layer, and a membrane with the same composition used for preparing the pCO_2 electrode was used as a gas-permeable membrane. Both hydrogel layers and gas-permeable membranes were cast with a pneumatic dispenser (EFD model 1000XL, Providence, RI). Response and calibration curves for CO_2 were obtained through the addition of standard solutions ($NaHCO_3$ solution) to 200 mL of a background electrolyte (0.2 M Tris−H_2SO_4, pH 7.4) with stirring. The emf differences between the pCO_2 and the reference electrodes were directly measured at room temperature using the aforementioned 16-channel potentiometric A/D converter.

Determination of CO_2 Levels in Gas/Electrolyte Control Samples and Whole Blood Specimens. To evaluate the analytical usefulness of the differential-type pCO_2 sensor system for the determination of CO_2 levels in blood gas/electrolyte control and human blood samples, its potentiometric response was measured with the flow cell cartridge shown in Figure 2B.[31] In this experiment, the pCO_2 and the reference electrodes were created on the same chip. The pCO_2 sensing device employed a two-point calibration to define the linear relationship between the sensor output and CO_2 concentration. The performance of the microchip-based pCO_2 gas sensor system was compared to that of a commercial blood gas/electrolyte analyzer (Stat Profile Ultra M, Nova Biomedical) as the laboratory reference.

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RESULTS AND DISCUSSION

Since the introduction of the first potentiometric pCO_2 electrode in the late 1950s,[32,33] some polymer membranes, e.g., poly(tetrafluoroethylene) (PTFE), PVC, and polysiloxane (silicone rubber), have been utilized as a gas-permeable membrane of pCO_2 measurement devices.[14,34−36] Among these hydrophobic membranes, the RTV-type silicone rubbers have received attention as the most attractive choice for constructing solid-state pCO_2 miniature electrodes, because they have strong adhesion to a wide range of substrates while providing high CO_2 permeability.[16−18] Recently, one-component RTV-type silicone rubbers, which can simplify the manufacturing procedure of the membranes by eliminating the need for a cross-linking agent, have been successfully employed in the fabrication of valinomycin-doped gas-permeable membranes for use in constructing differential pCO_2 sensors.[19,20]

Polyurethanes, a family of block copolymers consisting of alternating hard- and soft-segment units, have also been attracted particular interest, especially in biomedical applications.[37,38] Some polyurethanes such as Tecoflex (an aliphatic polyether-based thermoplastic polyurethane resin), designated as medical-grade biomaterials, have been exploited not only for manufacturing implantable medical devices but also for formulating all-solid-state miniaturized in vivo or in vitro ion sensors and biosensors.[30,39−43] They yield adhesive, physically durable, chemically stable, and biocompatible sensing membranes with electrochemical properties comparable to those of PVC-based membranes. Although several reports have demonstrated the usefulness of polyurethane-based membranes for ion sensing and biosensing devices, there has been little study related to gas sensor applications. Thus, our initial effort to develop a pH-sensitive gas-permeable membrane was devoted to optimizing the silicone rubber membrane composition and to investigating the feasibility of using polyurethane as a gas-permeable membrane for pCO_2 electrodes. In addition, the effect of incorporating a silanizing reagent (silicon tetrachloride) in the polyurethane matrix to achieve even stronger adhesion on the potentiometric response of a pH electrode was also examined.[39]

Figure 3 shows the pH response of 3140 RTV− and TPU−matrix TDDA-doped electrodes employing different membrane compositions, as tested in conventional electrode bodies. As can be seen, the potentiometric behavior of 3140 RTV-based pH sensors is non-Nernstian, exhibiting an inflection point at pH ∼7

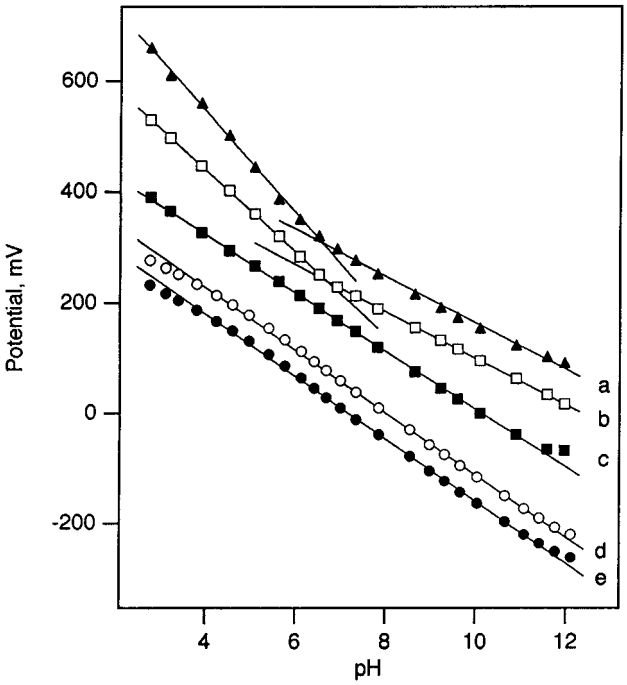

Figure 3. pH response of 3140 RTV− and TPU−matrix TDDA-doped electrodes with different membrane compositions: (a) 3140 RTV, (b) 3140 RTV/KT*p*ClPB, (c) 3140 RTV/KT*p*ClPB/DOS, (d) TPU/KT*p*ClPB/NPOE, and (e) TPU/KT*p*ClPB/NPOE/$SiCl_4$. The membranes were tested in conventional electrode bodies.

due to the drastic change in its response slope from 93 (for pH <7) to 42 (for pH >7) mV/pH (see curve a in Figure 3). It is shown herein that the incorporation of a lipophilic additive (i.e., KT*p*ClPB) into the membrane (curve b) does not improve the electrode performance significantly. The calibration curve becomes linear gradually as the content of a plasticizer (i.e., DOS) is increased, eventually achieving a Nernstian response shape in the pH 3−12 range when it becomes 35 wt % of the total composition (see curve c). However, for such a highly plasticized silicone membrane, the slope somewhat decreases to 52.4 mV/pH, and the exudation of DOS is observed, considerably deteriorating its adhesive property. As a result, the solid-state pCO_2 gas sensor using the highly plasticized pH sensing RTV film as a gas-permeable membrane yielded unsatisfactory performance, in terms of potentiometric response, stability, and lifetime. On the other hand, TPU-based pH-sensitive membranes, even with the addition of $SiCl_4$ (curves d and e in Figure 3), exhibited the typical potentiometric response toward pH changes (slope: 57.7 and 57.8 mV/pH, respectively), as shown in Figure 3. Furthermore, it has been our experience in solid-state ion sensor and biosensor experiments that the $SiCl_4$-doped polyurethane membranes usually exhibit much better adhesion, stability, and lifetime. Thus, we selected the polyurethane−matrix TDDA-doped membrane system incorporating $SiCl_4$ as a pH-sensitive gas-permeable membrane for use in the present planar differential pCO_2 sensing device.

To investigate the effect of the enzyme CA, which catalyzes the hydration and dehydration reactions of CO_2 and accelerates the reaction rate ∼5000-fold, on several important parameters (i.e., preconditioning and response times, stability, and slope), pCO_2 electrodes using varying amounts of CA (i.e., 0, 0.1, 0.3, 1.0, and

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Table 1. Effect of the Enzyme on the Potentiometric Response Characteristics of the Miniaturized Differential pCO_2 Gas Sensor[a,b]

	preconditioning time[c] t_{pre}, min	response time[d] $t_{90\%}$, s				recovery time[e] t_{rec}, min	drift, mV/h	slope,[f] mV/dec
		B[g] → 5 mM	5 → 15 mM	15 → 30 mM	30 → 50 mM	50 mM → B[g]		
without enzyme	5.2 ± 0.5	456 ± 4	196 ± 4	172 ± 4	72 ± 4	>10	0.4 ± 0.05	53.8 ± 0.5
with enzyme[h]	1.7 ± 0.5	38 ± 4	24 ± 4	24 ± 4	20 ± 4	1.5 ± 0.5	0.1 ± 0.05	55.1 ± 0.5

[a] Hydrogel layer composition: 4 wt % PVA, 5 mM $NaHCO_3$, and 0.5 mM NaCl in water. pH-sensitive gas-permeable membrane compostion: 44.5 wt % TPU/PVC (75 wt % TPU and 25 wt % PVC), 4.0 wt % TDDA, 1.0 wt % KT*p*ClPB, and 50.5 wt % NPOE. [b] Number of samples, $n = 5$. [c] Time required by virgin electrodes to reach 0.1 mV/min drift, which is a limiting stability recommended by IUPAC for clinical applications. [d] Time required to reach 90% of the equilibrium potential. [e] Time required to reach 90% of the background potential. [f] Range, 5−50 mM. [g] Background solution: 0.2 M Tris-H_2SO_4, pH 7.4. [h] Incorporation of 0.3 mg/mL carbonic anhydrase into the hydrogel layer.

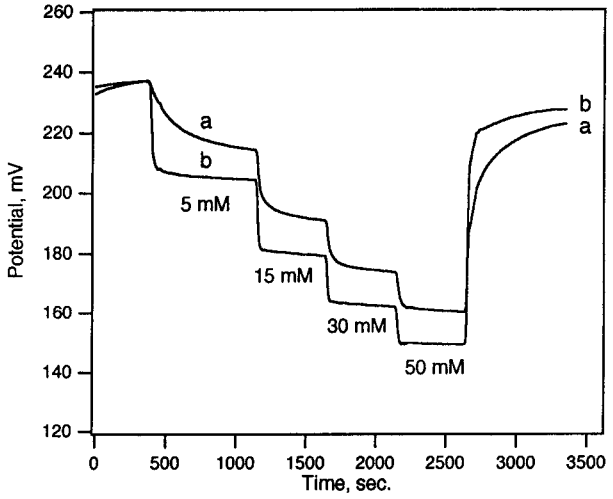

Figure 4. Effect of the enzyme CA on the performance of differential pCO_2 gas sensors: (a) no enzyme and (b) with enzyme.

2.0 mg/mL) in the *unbuffered* hydrogel layer were prepared. In this experiment, we found that 0.3 mg/mL CA is the optimal amount for the proposed pCO_2 sensor system: a lower amount (i.e., 0.1 mg/mL) of CA is not sufficient for improving the response time of the electrode, whereas larger amounts (i.e., 1.0 and 2.0 mg/mL) of CA give somewhat reduced slopes for CO_2 concentrations. This is believed to be due to a slight buffering effect of the enzyme protein.[44] In Table 1, the effects of the enzyme CA on the potentiometric response characteristics of the resulting pCO_2 sensors are summarized, in terms of the preconditioning period (t_{pre}, the hydration time required by virgin electrodes to reach 0.1 mV/min drift, which is a limiting stability recommended by IUPAC for clinical applications), response time ($t_{90\%}$, the time required to reach 90% of the equilibrium potential), recovery time (t_{rec}, the time required to reach 90% of the background potential), stability, and response slope in the range of clinical interest.[45] As can be seen, the potentiometric performance is improved greatly after adding the enzyme: the sensor employing CA exhibited much faster response properties, enhanced stability, and greater CO_2 response slope than without the enzyme (53.8 ± 0.5 mV/decade without CA and 55.1 ± 0.5 mV/decade with CA) (see Table 1 and Figure 4). It is believed that the incorporation of the prepared adhesion promoter (i.e., $SiCl_4$) into the TPU-based gas-permeable membrane contributes greatly in improving the signal drift and response slope of the pCO_2 microelectrode.

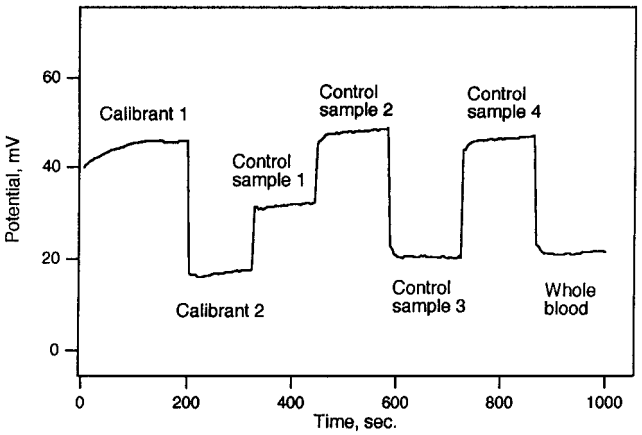

Figure 5. Continuous monitoring of various sample measurements with the differential pCO_2 microelectrode assembled in a flow cell cartridge.

Table 2. Determination of pCO_2 Levels in Various Control and Blood Samples with the Differential pCO_2 Gas Sensor and a Commercial Clinical Analyzer[a,b]

sample type	manufacturer's specification	pCO_2 value determined	
		this work	Nova[c]
control sample 1[d]	43 ± 6	38 ± 3	47 ± 3
control sample 2[d]	19 ± 5	16 ± 2	22 ± 2
control sample 3[e]	63.8 ± 5.0	61.7 ± 2.5	69.7 ± 2.0
control sample 4[e]	21.7 ± 2.5	19.9 ± 1.5	23.7 ± 1.0
whole blood[f]		58.5 ± 2.5	52.9 ± 2.0

[a] In mmHg. [b] Number of samples, $n = 5$. [c] The Severinghaus-type electrode installed in the Nova Stat Profile Ultra M clinical analyzer. [d] Blood gas/electrolyte control samples (model A701-001) from Alko Diagnostic Corp.. [e] Blood gas/electrolyte control samples (model Ultra Control) from Nova Biomedical. [f] Samples obtained from a local blood bank.

With the selected compositions for the internal hydrogel layer (4 wt % PVA, 5 mM $NaHCO_3$, 0.5 mM NaCl, and 0.3 mg/mL CA) and the pH-sensitive gas-permeable membrane (44.5 wt % TPU/PVC, 4.0 wt % TDDA, 1.0 wt % KT*p*ClPB, 50.5 wt % NPOE, and a aliquot amount of $SiCl_4$), the analytical performance of the planar differential pCO_2 sensor device fabricated on a single chip and assembled into a stop-flow cell cartridge as shown in Figure 2B was examined by injecting aliquot (5 μL) samples. The results were compared to those obtained with a commercial blood gas/electrolyte analyzer (Nova Stat Profile Ultra M). Figure 5 illustrates the results of the experiment performed with the proposed differential pCO_2 cartridge employing two commercial calibrants, four different control samples, and a whole blood specimen. As

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(45) IUPAC Recommendations for Nomenclature of Ion-Selective Electrodes. *Pure Appl. Chem.* **1994**, *66*, 2527−2536.

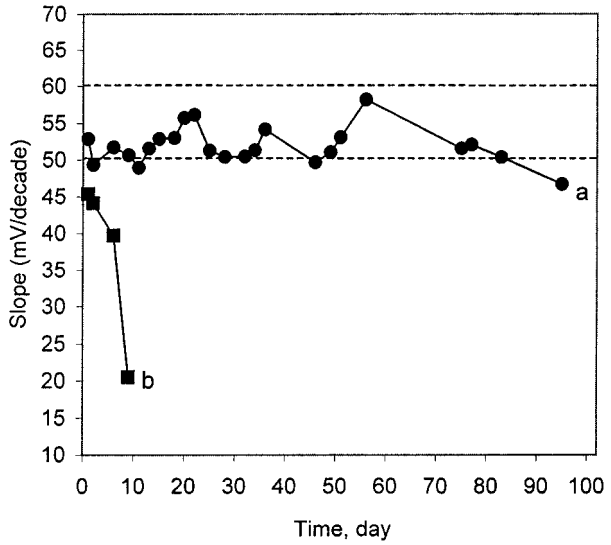

Figure 6. Variation of the response slope over time for planar pCO_2 microsensors using pH-sensitive gas-permeable membranes with (a) and without (b) $SiCl_4$. Between each measurement, gas sensors were stored in a 5 mM $NaHCO_3$ solution at room temperature.

can be seen, the differential pCO_2 microsensor yields very fast preconditioning and response properties, with stable signal outputs. The analytical results determining pCO_2 levels in various samples are in excellent agreement with those obtained with a standard desk-top chemical analyzer (see Table 2).

Since Caflisch and Carter miniaturized the Severinghaus-type pCO_2 electrode in 1974,[46] a great deal of effort has been made to improve the performance of pCO_2 microelectrodes. Despite such effort, only a few analyzers based on miniature pCO_2 sensor systems are currently available on the market. One of the main limitations of such devices is the short lifetime of most pCO_2 microelectrodes: lifetimes from several hours to as long as one month, to our knowledge, have been reported.[27] In this regard, we have investigated the long-term stability of differential electrodes by checking the calibration curve during a rather long period of time. Between each measurement, gas sensors were stored in a 5 mM $NaHCO_3$ solution at room temperature. Figure 6 shows the variation in the response slope measured in the 5−50 mM range for planar pCO_2 microsensors employing pH-sensitive gas-permeable membranes with (a) and without (b) $SiCl_4$. It can be seen that when the silanizing reagent is used, the pCO_2 electrode sensitivity remains stable for at least 80 days. Furthermore, no considerable degradation in the response and recovery times is observed, and the linear part of the dynamic range remains unchanged. The pCO_2 electrode prepared without using $SiCl_4$, however, exhibited an inferior initial response slope and rapidly lost electrode sensitivity after only 2 days of use.

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