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# An Improved Potentiometric $p\text{CO}_2$ Microelectrode

Pingsan Zhao and Wei-Jun Cai\*

Department of Marine Sciences, The University of Georgia, Athens, Georgia 30602

**The classic Severinghaus  $p\text{CO}_2$  microelectrodes have been greatly improved. The drift is reduced from  $\sim 10$  to  $0.4$  mV/h, and the lifetime has been extended from hours or days to one month. The detection limit has been lowered to  $200 \mu\text{atm}$ , and the  $t_{98}$  for low  $p\text{CO}_2$  response is 2 min. Therefore, the microelectrodes can be extended to low  $p\text{CO}_2$  measurements. In addition, the microelectrodes can be fabricated more easily. The major problems with the classic  $p\text{CO}_2$  microelectrodes are caused by Pyrex and the reference electrode used for constructing the microelectrodes. Pyrex can release chemicals into the internal bicarbonate solution and cause serious drift and a short lifetime. Pyrex has been replaced by aluminosilicate glass, and appropriate Ag/AgCl reference electrodes have been used. The enzyme carbonic anhydrase has been added to the internal bicarbonate solution to accelerate the hydration reaction, which greatly enhances the response rate and improves the detection limit. The internal pH glass microelectrodes have been replaced by pH liquid membrane microelectrodes, which makes fabrication easier and further improves response rate. The theory of the electrode's response characteristics at low  $p\text{CO}_2$  is presented as well as a discussion on the effects of glass materials, the enzyme and pH electrodes.**

The first potentiometric  $p\text{CO}_2$  electrode was introduced by Stow et al. in 1957,<sup>1</sup> and considerable improvements were made to it by Severinghaus and Bradley in 1958.<sup>2</sup> The Severinghaus-type  $p\text{CO}_2$  electrode was miniaturized by Cafilisch et al. in 1974.<sup>3</sup> Since then, much effort has been made to improve the performance of  $p\text{CO}_2$  microelectrodes.<sup>4–13</sup> Although some progress has been made, the  $p\text{CO}_2$  microelectrode still needs improvement in several important areas including stability, construction, response time, and detection limit.

The  $p\text{CO}_2$  microelectrodes are notoriously unstable and short-lived. The best performance results for  $p\text{CO}_2$  macroelectrodes

were obtained by Siesjö,<sup>14</sup> who reported a  $0.2$  mV/h drift and a very long lifetime. All published microelectrode works did not perform at this level. The drift from a few to  $20$  mV/h and a lifetime from several hours to as long as one week were reported for  $p\text{CO}_2$  microelectrodes.<sup>3–13</sup> Precise measurements cannot be made with such microelectrodes. It was not reported why  $p\text{CO}_2$  microelectrodes had a severe drift and a short lifetime.

pH glass electrodes were used as internal pH-sensitive electrodes in most potentiometric  $p\text{CO}_2$  electrodes<sup>1–8</sup> while metal oxide pH electrodes were used in the others.<sup>9–10</sup> pH glass microelectrodes have excellent selectivity and cover a wide dynamic range, but they are difficult to fabricate and have very high resistance and short lifetimes.<sup>15</sup> The advantage of metal oxide pH electrodes is their very low inner resistance, but they may be subject to severe interference by redox pairs.<sup>6,15</sup> Ion-selective liquid membrane microelectrodes (LMM) based on neutral carriers are now the most advanced microelectrode technology.<sup>16</sup> The neutral carrier-based pH LMM has similar dynamic response characteristics to pH glass electrodes, but unlike a pH glass microelectrode, it can be constructed easily and its inner resistance is not very high. In fact, pH glass microelectrodes have been replaced to a large extent by pH LMMs in physiological studies<sup>16–18</sup> and they have been used as the internal pH microelectrodes in  $p\text{CO}_2$  microelectrodes.<sup>11</sup>

It is well-known that the  $\text{CO}_2$  hydration reaction in the living body is accelerated by the enzyme carbonic anhydrase (CA).<sup>19</sup> Some studies have shown that adding CA to the bicarbonate solution in  $p\text{CO}_2$  electrodes could substantially reduce response times and hysteresis (the response curve for an increase in  $p\text{CO}_2$  differs significantly from that of a decrease in  $p\text{CO}_2$ ). Severinghaus initially found that the enzyme had no effect on response rate<sup>2</sup> but later reported that adding  $1 \text{ mg/mL}$  CA might accelerate the response.<sup>20</sup> Donaldson confirmed that CA could improve the response and hysteresis of Severinghaus'  $p\text{CO}_2$  electrodes.<sup>21,22</sup> CA was also used in the  $p\text{CO}_2$  microelectrodes based on PVC membrane<sup>12</sup> and in the optical fiber  $p\text{CO}_2$  sensor.<sup>23</sup> Botrè even used a  $p\text{CO}_2$  electrode to measure the concentration of the carbonic anhydrase.<sup>24</sup>

\* Corresponding author: (phone) 706-542-1285; (e-mail) wcai@uga.cc.uga.edu.

- (1) Stow, R. W.; Bear, R. F.; Randell, B. F. *Arch. Phys. Med. Rehab.* **1957**, *38*, 646–650.
- (2) Severinghaus, J. W.; Bradley, A. F. *J. Appl. Physiol.* **1958**, *13*, 515–520.
- (3) Cafilisch, C. R.; Carter, N. W. *Anal. Biochem.* **1974**, *60*, 252–257.
- (4) Cai, W.-J.; Reimers, C. E. *Limnol. Oceanogr.* **1993**, *38*, 1762–1773.
- (5) Collany, H. T.; Schumacher, T. E.; Rue, R. R.; Liu, S. Y. *Microchem. J.* **1993**, *48*, 42–49.
- (6) Pucacco, L. R.; Carter, N. W. *Anal. Biochem.* **1978**, *90*, 427–434.
- (7) Oliveier, C. N.; Berkenbosch, A.; Quanjer, P. H. *Pflügers Arch.* **1978**, *373*, 269–272.
- (8) Lai, N. C.; Liu, C. C.; Brown, E. G.; Neuman, M. R.; Ko, W. H. *Med. Biol. Eng.* **1975**, *876*–881.
- (9) Sohtell, M.; Karlmark, B. *Pflügers Arch.* **1976**, *363*, 179–180.
- (10) Coon, R. L.; Lai, N. C. J.; Kampine, J. P. *J. Appl. Physiol.* **1976**, *640*, 625–629.
- (11) Ma, Y. *Anal. Biochem.* **1990**, *186*, 74–77.
- (12) Voipio, J.; Kaila, K. *Pflügers Arch.* **1993**, *423*, 193–201.
- (13) Bomsztyk, K.; Calalb, M. K. *Am. J. Physiol.* **1986**, *251*, F933–F937.

- (14) Siesjö, B. K. *Acta Physiol. Scand.* **1961**, *51*, 297–313.
- (15) Opdycke, W. N.; Parks, S. J.; Meyerhoff, M. E. *Anal. Chim. Acta* **1983**, *155*, 11–20.
- (16) Ammann, D. *Ion-Selective Microelectrodes Principles, Design and Application*; Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, 1986.
- (17) Zhao, P.; Ammann, D.; Oesch, U.; Simon, W.; Lang, F. *Pflügers Arch.* **1988**, *411*, 216–219.
- (18) Ammann, D.; Lanter, F.; Steiner, R. A.; Schulthess, P.; Shijo, Y.; Simon, W. *Anal. Chem.* **1981**, *53*, 2267–2269.
- (19) Bauer, C.; Gros, G.; Bartels, H. *Biophysics and Physiology of Carbon Dioxide*; Springer-Verlag: Berlin, Heidelberg, New York, 1980; pp 3–63, 133–150.
- (20) Severinghaus, J. W. *Ann. N. Y. Acad. Sci.* **1968**, *148*, 115–132.
- (21) Donaldson, T. L.; Palmer, H. J. *AIChE J.* **1979**, *25*, 143–151.
- (22) Donaldson, T. L.; Ho, S. P. *Chem. Eng. Commun.* **1985**, *37*, 223–231.
- (23) Marazuela, M. D.; Bondi, M. C. M.; Orellana, G. *Sens. Actuators B* **1995**, *29*, 126–131.

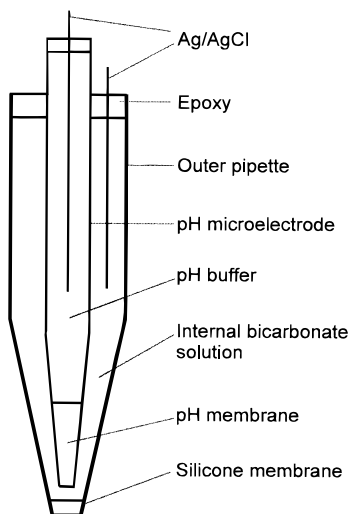


Figure 1. Schematic representation of a  $p\text{CO}_2$  microelectrode. It is not drawn proportionately.

Nearly all  $p\text{CO}_2$  electrodes were developed for physiological applications.<sup>1–3,5–13</sup> Occasionally, however, they have been used in aquatic applications.<sup>4,25</sup> The major difference between aquatic and physiological environments is that the  $p\text{CO}_2$  is much higher in the latter (e.g., ~5% in blood) than in the former (e.g., ~350  $\mu\text{atm}$  in the waters equilibrated with the air and 0.1% to a few percent in sediment interstitial waters). Until now, the  $p\text{CO}_2$  electrode's response characteristics at low  $p\text{CO}_2$  have not yet been characterized completely nor has the possibility for low  $\text{CO}_2$  measurements been fully explored.

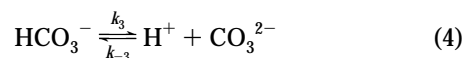
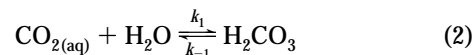
This paper details important modifications made to the  $p\text{CO}_2$  microelectrode which have improved its performance greatly, particularly at low  $p\text{CO}_2$ . The theory of response characteristics at low  $p\text{CO}_2$  is presented, and the effects of glass materials, enzyme, and Ag/AgCl reference electrodes are discussed. An application of the electrodes to natural environments is given.

## THEORETICAL SECTION

**Response Characteristic at low  $p\text{CO}_2$ .** The response behaviors in the steady state<sup>26,27</sup> and in the transient state<sup>21,22</sup> have been studied and are reasonably well understood at high  $p\text{CO}_2$ . But the response behavior under low  $p\text{CO}_2$ , especially the detection limit of the  $p\text{CO}_2$  microelectrodes, has not been reported. The potentiometric  $p\text{CO}_2$  microelectrode is shown schematically in Figure 1. The response mechanism is as follows: First,  $\text{CO}_2$  in the gas state ( $\text{CO}_{2(g)}$ ) diffuses through the membrane and reaches a thin film of bicarbonate solution where part of it dissolves in water and forms the dissolved  $\text{CO}_2$  ( $\text{CO}_{2(aq)}$ ):



Second, the  $\text{CO}_{2(aq)}$  undergoes a hydration reaction and forms  $\text{H}_2\text{CO}_3$ . The  $\text{H}_2\text{CO}_3$  then undergoes two steps of dissociation reactions:



where  $k$  is the respective kinetic rate constant. In the meantime, all the components including  $\text{CO}_{2(g)}$  diffuse through the thin film and result in a pH change in the film. If we neglect the dissociation of  $\text{HCO}_3^-$  and water, the equilibrium pH in the film is as follows:

$$[\text{H}^+] = \frac{K_1[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]} = \frac{K_1 K_H p\text{CO}_2}{[\text{NaHCO}_3]} \quad (5)$$

Therefore, pH will change with  $\log p\text{CO}_2$  linearly. This is the basis by which  $p\text{CO}_2$  electrodes are used to measure  $p\text{CO}_2$ . The true equilibrium pH in the film can be obtained by considering all reactions above, the water dissociation reaction, and the charge balance equation. The pH is governed by the following equation:<sup>26</sup>

$$[\text{H}^+]^3 + [\text{NaHCO}_3][\text{H}^+]^2 - (K_1 K_H p\text{CO}_2 + K_w)[\text{H}^+] - 2K_1 K_2 K_H p\text{CO}_2 = 0 \quad (6)$$

In eqs 5 and 6,  $K_H$  is Henry's law constant,  $K_1$  and  $K_2$  are the first and second dissociation constants of  $\text{H}_2\text{CO}_3$ , and  $K_w$  is the water dissociation constant. We define

$$\Delta\text{pH} = (\text{pH})_t - \text{pH} \quad (7)$$

where  $(\text{pH})_t$  is governed by eq 6 and pH by eq 5. Apparently,  $\Delta\text{pH}$  is the function of sample  $p\text{CO}_2$  and the internal  $[\text{NaHCO}_3]$ . Figure 2 and Table 1 show the dependence of  $\Delta\text{pH}$  (or  $\Delta E = 59\Delta\text{pH}$  in mV) on  $p\text{CO}_2$  under different  $[\text{NaHCO}_3]$ . It can be seen from Figure 2 and Table 1 that considerable deviation from linearity can occur at low  $[\text{NaHCO}_3]$  and high  $p\text{CO}_2$  or at high  $[\text{NaHCO}_3]$  and low  $p\text{CO}_2$ . For example,  $\Delta E$  is 38.8 mV when  $p\text{CO}_2 = 10\%$  and  $[\text{NaHCO}_3] = 0.01$  mM and  $-26.7$  mV when  $p\text{CO}_2 = 0.01\%$  and  $[\text{NaHCO}_3] = 0.1$  M (see Table 1). Siesjö mentioned that there was a small deviation from linearity with 0.1 mM  $\text{NaHCO}_3$  at ~10%  $p\text{CO}_2$ .<sup>25</sup> Table 1 indicates a 3.4 mV error under these conditions. Therefore, the high  $[\text{NaHCO}_3]$  should be used for high  $p\text{CO}_2$  measurement (e.g., in blood) and low  $[\text{NaHCO}_3]$  for low  $p\text{CO}_2$  measurements. Table 1 also shows the lowest  $p\text{CO}_2$  the electrodes can detect when the deviation  $\Delta E$  is set to 1 mV. It is apparent that  $p\text{CO}_2$  electrodes may be applied to very low  $p\text{CO}_2$  measurements.

There are two compartments of the internal bicarbonate solution in the  $p\text{CO}_2$  microelectrodes: the thin film and the reservoir of bicarbonate.<sup>4</sup> Between these two compartments there is exchange of  $\text{CO}_2$  and other carbon species if there is a concentration difference in the two compartments.<sup>26</sup> Usually we neglect the  $\text{CO}_2$  exchange between the two compartments. The bicarbonate solution itself can produce a certain amount of  $\text{CO}_2$ , depending on its initial concentration.<sup>4,26</sup> The  $p\text{CO}_2$  is ~700  $\mu\text{atm}$

(24) Botrè, C.; Botrè, F. *Anal. Biochem.* **1990**, *185*, 254–264.

(25) Midgley, D. *Analyst* **1975**, *100*, 386–389.

(26) Jenson, M. A.; Rechnitz, G. A. *Anal. Chem.* **1979**, *51*, 1972–1977.

(27) Ross, J. W.; Riseman, J. H.; Krueger, J. A. *Pure Appl. Chem.* **1973**, *36*, 473–487.

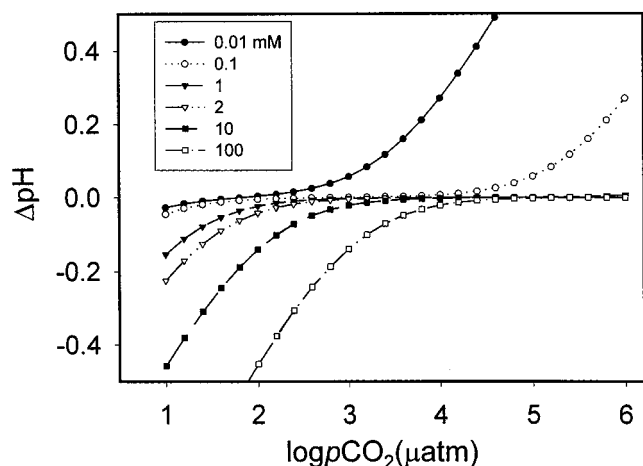


Figure 2. Theoretical dependence of the deviation from linearity ( $\Delta pH$ ) of a  $pCO_2$  electrode on sample  $pCO_2$  and the initial concentration of the internal bicarbonate solution.

Table 1. Theoretical Deviation from Linearity ( $\Delta E$ ) of  $pCO_2$  Microelectrodes on Sample  $pCO_2$  and the Concentration of the Internal Bicarbonate Solution [ $NaHCO_3$ ]

[ $NaHCO_3$ ] (mM)	0.01	0.1	1	2	10	100
[ $CO_2$ ] ( $\mu M$ ) <sup>a</sup>	0.47	1.81	11.31	21.60	103.8	1028
$pCO_2$ ( $\mu atm$ ) <sup>b</sup>	14	54	334	637	3062	30336
$\Delta E$ (mV)						
0.001% <sup>c</sup>	-1.61	-2.64	-9.09	-13.29	-27.01	-52.48
0.01%	0.24	-0.28	-1.38	-2.45	-8.28	-26.71
0.04%	1.47	-0.06	-0.37	-0.68	-2.86	-14.30
0.1%	3.38	0.01	-0.15	-0.28	-1.25	-8.20
1%	15.99	0.40	-0.01	-0.03	-0.13	-1.24
10%	38.84	3.40	0.04	0.01	-0.01	-0.13
100%	66.17	16.00	0.41	0.10	0.00	-0.01
( $pCO_2$ ) <sub>L</sub> ( $\mu atm$ ) <sup>d</sup>	16	25	150	251	1200	12000

<sup>a</sup> [ $CO_2$ ] here is produced by the  $NaHCO_3$  solution itself. [ $CO_2$ ] is calculated by the equation  $[NaHCO_3]/K_1 ((K_2[NaHCO_3] + K_w)/(1 + [NaHCO_3]/K_1))^{1/2}$ .  $K_1$ ,  $K_2$ , and  $K_w$  in the infinitely diluted solution at 25 °C are used. <sup>b</sup>  $pCO_2 = [CO_2]/K_H$  and 0.034 is used for  $K_H$ . <sup>c</sup> Sample  $pCO_2$  in atm. <sup>d</sup> The lowest sample  $pCO_2$  that the electrodes can detect when  $\Delta E = 1$  mV. The numbers in italic show that the deviation is less than 1% within the  $pCO_2$  range of interest when 2 mM  $NaHCO_3$  solution is used as the internal solution.

for 2 mM bicarbonate solution (see Table 1). If this solution is used as the internal solution and if the  $pCO_2$  of the sample is higher than 700  $\mu atm$ , the  $CO_2$  in the reservoir does not diffuse to the film and the effect from the  $CO_2$  in the reservoir is negligible. When the  $pCO_2$  of the sample is less than 700  $\mu atm$ , however, the  $CO_2$  in the reservoir will diffuse to the film and will cause an increase in response time and detection limit. Usually the  $pCO_2$  of bicarbonate solution decreases as its concentration decreases (see Table 1). Therefore, for low  $CO_2$  measurements, low concentrations of internal bicarbonate solution should be used. However, diluted solution can be more easily contaminated. Thus, a compromise should be reached. We recommend the use of 2 mM  $NaHCO_3$  solution. As can be seen from the italic values in Table 1, within the  $pCO_2$  range of interest ( $0.04\% < pCO_2 < 10\%$ ), deviation ( $\Delta E$ ) is less than 1 mV and the lowest  $pCO_2$  the electrodes can detect is less than 250  $\mu atm$  for 2 mM internal bicarbonate solution. Cai and Reimer<sup>4</sup> have recommended the use of 1–3 mM  $NaHCO_3$  solution.

## EXPERIMENTAL SECTION

**Reagents.** The reagents used for the liquid membrane solution were tridodecylamine (TDDA), 2-nitrophenyl octyl ether

(2-NPOE), potassium tetrakis(4-chlorophenyl) borate ( $KT_4CIPB$ ), and poly(vinyl chloride) (PVC). The silanization reagent was *N,N*-dimethyl(trimethylsilyl)amine. They were all acquired from Fluka. The gas-permeable membrane was made from a Silastic medical adhesive (Silicone Type A, Dow Corning). The glass material for inner pH microelectrodes is Pyrex (1.5 mm o.d., Fisher). Glass materials for the outer pipet of the  $pCO_2$  microelectrodes were aluminosilicate (Al) glass (1720), Pyrex (7740), and lead glass (0120) from Houde Glass, Inc. (4 mm o.d., 3.2 mm i.d.).  $CO_2$  gas standards, 0.099, 0.48, and 2.6%  $CO_2$  and 100% nitrogen gas ( $N_2$ ) are from BOC Group, Inc. Tetrahydrofuran (THF) was from Fluka, and carbonic anhydrase II was from bovine erythrocytes (activity, 3000 W-A units/mg of protein, Sigma). All other reagents used are analytical grade.

**pH Microelectrodes.** The preparation and properties of pH liquid membrane microelectrodes based on neutral carriers for this study will be reported elsewhere in detail.<sup>28</sup> A brief description is given here. The liquid membrane solution for the pH electrode consists of 67% membrane solution (10% TDDA, 1%  $KT_4CIPB$ , and 89% 2-NPOE)<sup>17</sup> and 33% PVC that was dissolved in the excess THF. When a homogeneous membrane solution formed, THF was then allowed to evaporate until the membrane solution had a viscosity suitable for filling the microelectrode. The micropipets were prepared from Pyrex or Al glass by a microelectrode puller (Sutter Instrument Co.). The pipet tips were broken at 5–50  $\mu m$  diameter, and the pipets were silanized to make the glass wall hydrophobic before they were filled.<sup>17,18,29</sup> At first, the silanized micropipets were filled with a pH 7 phosphate buffer and then with a PVC membrane solution. Finally, a Ag/AgCl wire was inserted into the shank of the micropipet as a reference electrode and sealed with Parafilm. The electrodes were conditioned overnight before use.

**$pCO_2$  Microelectrode.** A  $pCO_2$  microelectrode based on the above pH microelectrode is shown in Figure 1. It mainly consists of an outer pipet with a gas-permeable membrane in the very tip and an inner pH microelectrode as described above. The outer pipet was pulled by a micropipet puller in two steps, first to  $\sim 2$  mm and then to  $\sim 1 \mu m$ . The temperature selected for the pulling depended on the hardness of the glass used. Temperatures were highest for alumina glass and lowest for lead glass. The tip of the outer pipet was cut at 50–300  $\mu m$ . The silicone membrane was formed in the tip by dipping the tip into the silicon liquid while the process was observed under a microscope. The original silicone material for gas-permeable membrane was too viscous for dipping and had to be diluted by THF to a suitable viscosity in order to prepare a very thin membrane. The silicone membrane was cured in air for at least 24 h. The micropipet, with silicone membrane, was then filled with internal solution (2 mM  $NaHCO_3$ , 0.5 M NaCl). An air gap was formed between the silicone membrane and the bicarbonate solution and it was dispelled by several methods. If the volume of the air gap was very small, pressure was applied to the other end to compress the air in the tip and expel it through the membrane. The air in the tip could also be expelled by supersonic wave or in a vacuum oven.

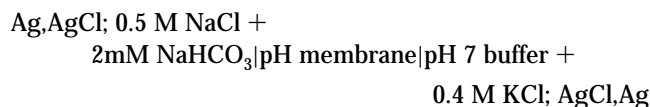
The next step involved matching the inner pH microelectrode and the outer pipet while observing them under a microscope. The relative positions of the outer pipet and the pH microelectrode were adjusted carefully in order to set the pH electrode in the

(28) Zhao, P.; Cai, W.-J., in preparation.

(29) Ammann, D.; Zhao, P.; Simon, W. *Neurosci. Lett.* **1987**, 74, 221–226.

center of the outer pipet at a distance  $\sim 5\text{--}30\ \mu\text{m}$  from the silicone membrane. Then UV-curable epoxy was used to fix the inner and outer pipets. An Ag/AgCl reference electrode was inserted into the internal solution in the outer pipet. The  $p\text{CO}_2$  microelectrode was then ready for use.

**Measurement and Calibration Methods.** The following cell was employed for the EMF measurements:



An Accumet pH meter (inner impedance,  $>10^{12}\ \Omega$ ) was used to measure EMF, and a Compaq computer was connected to the pH meter for data acquisition. The response time was defined as the time when 98% response was reached ( $t_{98}$ ). The calibration apparatus consisted of a 140 mL bottle with a rubber cap in which a  $p\text{CO}_2$  microelectrode and two glass pipes, one for introducing  $\text{CO}_2$  gas or for adding bicarbonate solution and the other for balancing inner and outer gas pressure, were mounted. Two methods were employed for the calibration. In the first one, the bottle was empty and different gas standards (saturated by water vapor) were introduced from the inlet pipe. Usually, three  $\text{CO}_2$  gas standards (0.1, 0.5, and 2.5% atm) as well as laboratory air ( $\sim 500\ \mu\text{atm}$ ) and  $\text{N}_2$  were used for calibration. Other  $\text{CO}_2$  standards were prepared by mixing the 0.5%  $\text{CO}_2$  gas with  $\text{N}_2$ . The  $\text{CO}_2$  concentration was measured by a Li-Cor  $\text{CO}_2$  Analyzer (Model LI-6252). In the second method, the bottle was filled with 120 mL of 0.1 M HCl and the  $p\text{CO}_2$  microelectrode was immersed into the solution. A known amount of  $\text{CO}_2$  gas was produced by adding bicarbonate solution using a micrometer burette (Gilmont) with a resolution of 0.0001 mL. In this way, we could also check as to whether the gas-permeable membrane leaked.

**Sediment Measurements.** Sediment samples were taken from the Savannah River estuary on the Georgia coast. The sediments were incubated in the laboratory at room temperature ( $22\ ^\circ\text{C}$ ) for one month to allow the sediment to develop vertical  $\text{CO}_2$  concentration gradients similar to those of real sediments. The  $p\text{CO}_2$  microelectrodes were mounted onto a manipulator. Before profiling, the  $p\text{CO}_2$  microelectrodes were kept in the overlying water for more than 10 min. Before and after measurements, the  $p\text{CO}_2$  microelectrodes were calibrated with  $\text{CO}_2$  gas standards.

## RESULTS AND DISCUSSION

Typical response and calibration curves for the new  $p\text{CO}_2$  microelectrodes are shown in Figure 3. The microelectrodes were prepared from Al glass and contained the enzyme CA in the internal bicarbonate solution. Compared with previous  $p\text{CO}_2$  microelectrodes, several significant improvements have been made with this new one: (a) The new microelectrode is very stable. The drift is only 0.4 mV/h compared to 6–10 mV/h for previous models.<sup>5,6</sup> The new microelectrode has a lifetime of more than one month while the older models lasted only hours or days.<sup>4–6,13</sup> (b) It can be seen from Figure 3 that the new electrodes have a Nernstian slope from  $\sim 500\ \mu\text{atm}$  to 5%  $p\text{CO}_2$  and that the detection limit of the new  $p\text{CO}_2$  microelectrodes is  $\sim 200\ \mu\text{atm}$   $p\text{CO}_2$ . Therefore, the new electrodes can be extended to low  $p\text{CO}_2$  measurements. Most previous electrodes are only used for very

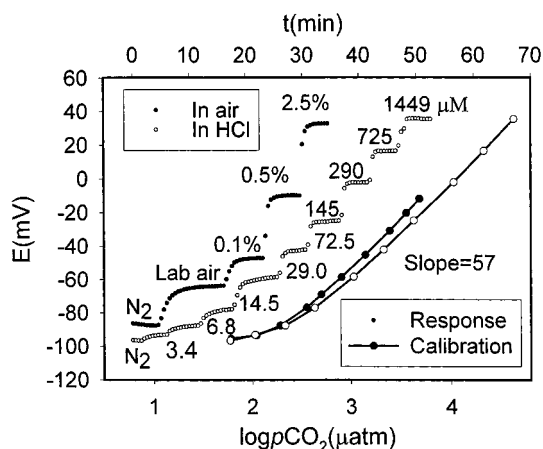


Figure 3. The response ( $E-t$ ) and calibration ( $E-\log p\text{CO}_2$ ) curves of a typical  $p\text{CO}_2$  microelectrode. The internal solution contains 2 mM  $\text{NaHCO}_3$  and 0.75 mg/mL CA. The outer pipet was made from alumina glass with a tip diameter of  $130\ \mu\text{m}$ . The inner pH microelectrode was made from Pyrex and the thickness of the gas-permeable silicone membrane was  $35\ \mu\text{m}$ . The numbers by the curve indicate partial pressure (%) for curve (●) and concentration ( $\mu\text{M}$ ) for curve (○) of  $\text{CO}_2$ . Laboratory air contains  $\sim 400\ \mu\text{atm}$   $\text{CO}_2$ .

high  $p\text{CO}_2$  measurements in physiological studies.<sup>5–13</sup> Midgley tested the possibility of using commercial macroelectrodes for the routine analysis of power station water and found that the detection limits of the commercial electrodes were too high to do so.<sup>25</sup> Cai and Reimers'  $p\text{CO}_2$  microelectrode was able to detect as low as 0.1%  $p\text{CO}_2$  for the study of a marine  $\text{CO}_2$  system.<sup>4</sup> (c) The response of the new electrode is relatively fast. It can be seen from Figure 3 that  $t_{98}$  is  $\sim 2$  min, even at low  $p\text{CO}_2$ . (d) The construction of the new electrodes is easier and the success rate of fabricating the new electrodes is very high because of the use of PVC liquid membrane microelectrodes based on neutral carriers. In addition, the resistance of the  $p\text{CO}_2$  microelectrode is  $\sim 10^9\ \Omega$ . More details about the performance of  $p\text{CO}_2$  microelectrodes are given in Table 2.

This improved performance is the result of several design modifications. The Pyrex glass used in Severinghaus  $p\text{CO}_2$  microelectrodes for several decades was replaced by Al glass, and glass (or metal oxide) pH microelectrodes have been replaced by pH LMM microelectrodes based on a neutral carrier. CA and specifically prepared reference electrodes have been used in the new microelectrodes. A discussion of the major factors that affect the performance of  $p\text{CO}_2$  microelectrodes follows.

**Effect of Glass Materials.** The glass materials used for constructing the  $p\text{CO}_2$  microelectrodes have a great effect on the performance of the microelectrodes. Previously, almost all  $p\text{CO}_2$  microelectrodes were constructed using Pyrex glass. However, we discovered that the drift and short lifetime of  $p\text{CO}_2$  microelectrodes were mainly caused by Pyrex. Satisfactory results were obtained after Al glass was substituted for Pyrex. The reason is that Pyrex can release a significant amount of chemicals into the internal bicarbonate solution, which changes the pH value of the solution. The chemical durability of Pyrex is not sufficient for constructing the  $p\text{CO}_2$  microelectrodes. The tests for chemical durability showed that the weight loss in 5% NaOH at  $100\ ^\circ\text{C}$  for 6 h was  $1.4\ \text{mg}/\text{cm}^2$  for Pyrex and only  $0.35\ \text{mg}/\text{cm}^2$  for Al glass.<sup>30</sup>

(30) Bansal, N. P.; Doremus, R. H. *Handbook of Glass Properties*; Academic Press: Orlando, FL, 1986; pp 31–45, 647–656.

Table 2. Effects of the Enzyme and the Thickness of Gas-Permeable Membrane and Tip Diameter on the Performance of  $p\text{CO}_2$  Microelectrodes

	response time ( $t_{98}$ ) (min)				response amplitude (mV)				hysteresis (mV) <sup>a</sup>		
	0.5→2.5%	2.5→0.5%	air→0.1%	0.1%→air	$\text{N}_2$ →air	air→0.1%	0.1→0.5% <sup>b</sup>	0.5→2.5% <sup>b</sup>	0.5%	0.1%	air
no enzyme											
Tip 170–260 $\mu\text{m}^d$	3.6 ± 0.6 <sup>c</sup>	8.0 ± 1.9	4.7 ± 0.6		10.1 ± 1.3	8.5 ± 3.3	55.4 ± 3.8	56.4 ± 3.1	3.2 ± 0.7	7.8 ± 2.4	
Mem 20–50 $\mu\text{m}^d$	( $n = 7$ )	( $n = 6$ )	( $n = 3$ )		( $n = 2$ )	( $n = 6$ )	( $n = 6$ )	( $n = 6$ )	( $n = 6$ )	( $n = 6$ )	
enzyme											
Tip 50–220 $\mu\text{m}$	2.0 ± 0.0 <sup>c</sup>	3.6 ± 0.8	3.3 ± 0.4	2.7 ± 0.4	30.5 ± 6.0	18.1 ± 1.8	56.2 ± 2.0	57.8 ± 0.8	0.3 ± 0.4	1.8 ± 0.4	2.0 ± 0.8
Mem 15–35 $\mu\text{m}$	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )	( $n = 4$ )
Mem 85 $\mu\text{m}$	3.0	6.0	3.0	4.0	18.1	17.2	54.1	56.3	1.3	3.4	5.4
Mem 360 $\mu\text{m}$	5.0	>10	>10	>10	7.2	17	55.7 <sup>e</sup>	40.8	6.4	24.7	25.1
Tip 310 $\mu\text{m}$	2.0	5.0	3.0	3.0	13.8	15.9	49.0	55.0	1.2	2.7	4.3
Tip 50 $\mu\text{m}$	2.0	3.0	3.0	2.0	39.0	20.0	55.1	58.0	0.	2.0	2.0

<sup>a</sup> Hysteresis here means potential difference in equilibrium caused by opposite direction in concentration change. <sup>b</sup> The data for the column has been converted to the slope (mV/log  $p\text{CO}_2$ ). <sup>c</sup> The data form is mean value ± standard deviation.  $n$  is the number of measurements. <sup>d</sup> Tip and Mem signify the tip diameter and membrane thickness, respectively. <sup>e</sup> The  $\Delta E$  value must be larger than it should be because the reading at 0.1%  $p\text{CO}_2$  was not at equilibrium yet (lower than it should be) due to slow response.

Table 3. Kinetic Parameters of a  $\text{CO}_2$  System and their Damköhler Numbers ( $N_k$ )

	$k_1$	$k_{-1}$	$k_{\text{cat}}$	$k_2$	$k_{-2}$
$k$ ( $\text{s}^{-1}$ ) <sup>a</sup>	0.04	28	110	$1.5 \times 10^7$	$4.7 \times 10^{10}$
$N_k(\text{S})^b$	$8.6 \times 10^{-3}$	6.1	23.8	$3.2 \times 10^6$	$1.0 \times 10^{10}$
$N_k(\text{M})^c$	$6.7 \times 10^{-3}$	4.7	18.3	$2.5 \times 10^6$	$7.8 \times 10^{10}$

<sup>a</sup> The data are from ref 19. <sup>b</sup>  $N_k(\text{S})$  and  $N_k(\text{M})$  signify  $N_k$  in solution and  $N_k$  in membrane, respectively.  $D_{\text{CO}_2}$  in solution,  $1.85 \times 10^{-5} \text{ cm}^2/\text{s}$ , and  $L$ , 0.002 cm, are used for calculating  $N_k(\text{S})$ . <sup>c</sup> The product of diffusion and distribution coefficients of  $\text{CO}_2$  in membrane,  $2.4 \times 10^{-5}$  <sup>27</sup> is used for calculating  $N_k(\text{M})$ .

which indicates that the chemical durability of Al glass is much better than that of Pyrex. We also used lead glass which has a weight loss of 3.6 mg/cm<sup>2</sup>; <sup>30</sup> however, it produced the worst results, which further confirms the effect of glass materials on  $p\text{CO}_2$  microelectrodes.

The tip diameter of the outer pipet is also an important factor regulating the performance of the new  $p\text{CO}_2$  microelectrodes. When Al glass was used as the outer pipet, satisfactory results were obtained if the tip of the microelectrode was larger than 150  $\mu\text{m}$ . If the tip of the outer pipet was less than 150  $\mu\text{m}$ , however, the performance of the microelectrodes depended on what type of glass materials were used to construct the inner pH microelectrode. Our work showed poor results (slope, 30–50 mV, lifetime, <1 week) with Pyrex, but better results (similar to those with large outer tip) with Al glass. When the tip of an outer pipet was less than 50  $\mu\text{m}$ , the stability and lifetime of a  $p\text{CO}_2$  microelectrode decreased to some extent, even if Al glass was used for constructing both the outer pipet and the inner pH microelectrode. For a certain glass material, the ratio of the surface area of glass to the volume of solution (S/V), which is inversely proportional to its tip diameter, determines the extent to which the performance of a  $p\text{CO}_2$  microelectrode is influenced by chemicals released from a glass wall. The smaller the tip diameter, the larger the S/V and the greater the effect exerted by the glass. The literature data showed a similar trend. A lifetime of several hours for 4–10  $\mu\text{m}$  tip, <sup>12</sup> of several days for 2–200  $\mu\text{m}$  tip, <sup>6</sup> and of two weeks for 300  $\mu\text{m}$  tip <sup>4</sup> was reported with Pyrex and soda lime glass, which has a weight loss of 1.1 mg/cm<sup>2</sup>. For  $p\text{CO}_2$  microelectrodes with a tip diameter of less than 50  $\mu\text{m}$ , further improvements in stability and lifetime may be obtained by using a material that is chemically

more durable than Al glass. Unfortunately, the glasses with high chemical durability seem to have a high softening point and are difficult to handle. Therefore, new types of glass with high durability and a low softening point are needed for this purpose.

**Effect of Enzyme.** As described in the Theoretical Section, the response of  $p\text{CO}_2$  electrodes to a  $\text{CO}_2$  sample mainly involves two processes—the diffusion of  $\text{CO}_2$  through the silicone membrane and the film of the internal bicarbonate solution and the simultaneous chemical reactions including the hydration of  $\text{CO}_2$  and sequential dissociation of  $\text{H}_2\text{CO}_3$ . The dissociation of  $\text{H}_2\text{CO}_3$  proved to be very fast, <sup>19</sup> and the response rate of a  $p\text{CO}_2$  microelectrode depends on the slowest step of diffusion or hydration. The Damköhler number ( $N_k$ ), <sup>31</sup> defined as

$$N_k = kL^2/D \quad (8)$$

where  $L$  is the length of the diffusion path and  $D$ , the diffusion coefficient, can be used to compare the relative rates of the two processes. When  $N_k \gg 1$ , diffusion is the rate-limiting step, and when  $N_k \ll 1$ , the chemical reaction is the rate-limiting step. <sup>22</sup> Table 3 gives rate constants and corresponding Damköhler numbers. It can be seen from Table 3 that the hydration and dehydration processes are relatively slow compared to the diffusion through the solution or the silicone membrane. Therefore, the response rate will be governed by hydration and dehydration processes. Therefore, it can be expected that the response rate will increase with the addition of a reagent that can catalyze the hydration reaction. By using the catalytic kinetic rate constant ( $k_{\text{cat}}$ ), a  $N_k$  of 27.4 is obtained (see Table 3). In fact, it has been reported that the hydration rate increased  $10^5$  times after the enzyme was added. <sup>32,33</sup> Therefore, we would expect a faster response after adding the enzyme. Figure 4 shows the response curves of a  $p\text{CO}_2$  microelectrode with and without CA in the internal electrolyte solution. The response is improved greatly after adding the enzyme (also see the second and third rows in Table 2). That is, the response time and hysteresis are reduced and the response slope is enhanced.

It can also be seen from Table 2 that the improvements made by the addition of the enzyme are more pronounced at low  $p\text{CO}_2$

(31) Suchdeo, S. R.; Schultz, J. S. *Biochem. Biophys. Acta* **1974**, 352, 412–440.

(32) Ren, X.; Lindsog, S. *Biochim. Biophys. Acta* **1992**, 1120, 81–86.

(33) Kernohan, J. C. *Biochim. Biophys. Acta* **1965**, 96, 304–317.

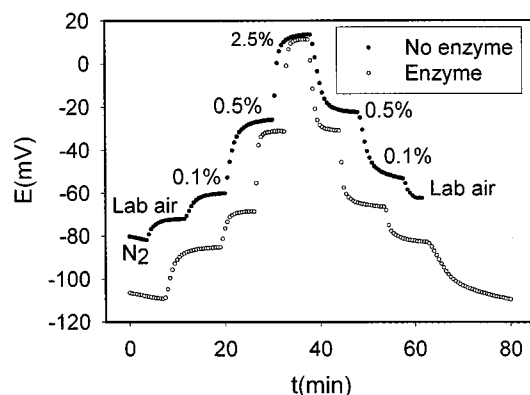


Figure 4. Effect of the enzyme CA on the performance of  $p\text{CO}_2$  microelectrodes. The electrode without the CA has an outer tip of  $170\ \mu\text{m}$ , a gas-permeable membrane with a thickness of  $30\ \mu\text{m}$ , and an inner pH electrode with a tip diameter of  $25\ \mu\text{m}$ . The electrode with the CA is the same as the one in Figure 3. The numbers by the curve indicate partial pressure (%) of  $\text{CO}_2$  gas standards.

than those at high  $p\text{CO}_2$ . In other words, the detection limit is lowered after the addition of the enzyme. For example, the response slopes from 0.1 to 2.5%  $\text{CO}_2$  are similar with and without the enzyme, but the response amplitude from  $\text{N}_2$  to air is 30 mV with the enzyme and only 15 mV without the enzyme. As explained in the Theoretical Section, when the  $p\text{CO}_2$  of a sample is very low, the  $\text{CO}_2$  in the bicarbonate reservoir will diffuse to the film. The influence caused by the  $\text{CO}_2$  in the reservoir depends on the relative flux of the  $\text{CO}_2$  from the silicone membrane and that from the reservoir. The larger the flux from the membrane, the less the influence by  $\text{CO}_2$  from the reservoir and the lower the detection limit. In the presence of CA, the  $\text{CO}_2$  flux from the sample increases, and therefore, lower  $p\text{CO}_2$  can be detected.

The CA concentration, from 0.5 to 1.0 mg/ml, was tested and showed no difference. CA and its solution should be kept at low temperature, but our tests showed that CA solution left at room temperature for one month still worked.

**Effect of Reference Electrodes.** Unexpectedly, when an Ag/AgCl reference electrode prepared by electroplating was used as the internal reference electrode in the internal bicarbonate solution, the response rate and slope of the  $p\text{CO}_2$  microelectrodes were reduced and the lifetime was only several days, although all other optimized conditions were employed. However, when an Ag/AgCl prepared by dipping an Ag wire into a molten AgCl was used, the lifetime was governed by other factors and could be extended to more than one month. All satisfactory results above were obtained with the latter reference electrode.

Dark AgCl crystals were often observed on the gas-permeable membrane when the electroplated reference electrodes were used. We suspect that the AgCl crystals dropped from reference electrodes may block the silicone membrane, reduce the  $\text{CO}_2$  flux through the membrane, and therefore reduce the response rate and slope of the  $p\text{CO}_2$  microelectrodes. Further improvement may be obtained if the reference electrode only contacts the internal bicarbonate solution indirectly.

**Effect of Membrane Thickness.** According to Ross' theory,<sup>27</sup> if diffusion is the rate-determining step, the response time of a  $p\text{CO}_2$  electrode is proportional to the thickness of the membrane and of the bicarbonate film. When hydration is the rate-limiting

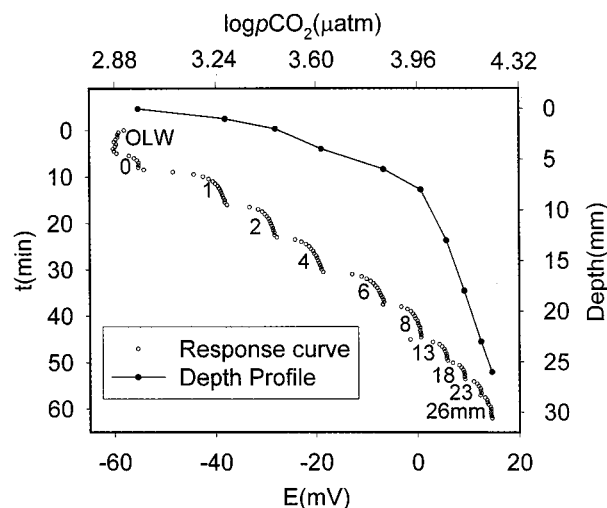


Figure 5. Response curve ( $t$ - $E$  or  $-\log p\text{CO}_2$ ) of a  $p\text{CO}_2$  microelectrode in laboratory-incubated sediment and the corresponding  $p\text{CO}_2$  profile (Depth- $E$  or  $-\log p\text{CO}_2$ ). OLW, overlying water. The numbers by the response curve indicate the measured depth (mm).

step, however, the response time will not be influenced by membrane thickness. In the presence of the enzyme, the response characteristics of  $p\text{CO}_2$  microelectrodes with membrane thickness from 15 to  $35\ \mu\text{m}$  are similar, which indicates that hydration is the rate-limiting step (see third row in Table 2). But when the membrane thickness is  $85\ \mu\text{m}$ , the response rate is reduced and the hysteresis becomes more pronounced. This could provide evidence that the rate-limiting step is being switched from hydration to diffusion (see fourth row in Table 2). When the membrane thickness is  $365\ \mu\text{m}$ , the response is completely governed by the diffusion process (see fifth row in Table 2). Therefore, it is unnecessary to make a silicone membrane thinner than 15– $35\ \mu\text{m}$  under our experimental conditions, and membranes thicker than  $80\ \mu\text{m}$  should be avoided.

**Effect of Tip Diameter.** As can be seen from the last two rows in Table 2, a small tip diameter improves the response at low  $p\text{CO}_2$ . The response amplitude from  $\text{N}_2$  to air is only 14 mV for a  $310\ \mu\text{m}$  tip, but 39 mV for a  $50\ \mu\text{m}$  tip; the hysteresis at 0.5%  $p\text{CO}_2$  is 1.2 mV for the  $310\ \mu\text{m}$  tip, but only 0.2 mV for the  $50\ \mu\text{m}$  tip.

This can be explained by the effect of the reservoir on the film. The internal pH microelectrodes have  $\sim 20\ \mu\text{m}$  of tip diameter. When the tip of the outer pipet is slightly larger than the inner pH microelectrode, the outer pipet and the inner pH microelectrode will be matched tightly. The exchange rate of  $\text{CO}_2$  between the film and the reservoir will be very slow. That is, the influence of the bicarbonate reservoir is not significant and therefore good results can be obtained. However, when the tip of the outer pipet is much larger than the inner pH microelectrode, the match is loose and the influence of the bicarbonate reservoir is significant. Good results cannot be obtained.

Although a small tip is preferred for  $p\text{CO}_2$  microelectrodes from a response point view, it may reduce the stability and the lifetime of  $p\text{CO}_2$  microelectrodes as discussed in the section on the effects of glass materials. We recommend using microelectrodes with a tip diameter of 100– $200\ \mu\text{m}$ .

**Sediment Application.** As an example of the application of the new microelectrodes to in situ low  $p\text{CO}_2$  measurements, we

use the microelectrode to measure the  $p\text{CO}_2$  profile of an incubated estuarine sediment. Figure 5 shows the response curve of our  $p\text{CO}_2$  microelectrode in the sediment and the corresponding curve of  $p\text{CO}_2$  versus depth. It can be seen that the response time ( $t_{98}$ ) is about 2–4 min. The  $p\text{CO}_2$  profile pattern is similar to that obtained by other methods. Since the microelectrode method is an in situ method and has a millimeter resolution, we expect that the  $p\text{CO}_2$  profile obtained with this method will be more accurate and will provide more information on diagenetic reactions in sediments than that with other methods. More studies on the application of this microelectrode to sediments (incubated and field) are under investigation in this laboratory.

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