Published in final edited form as:

J Neurosci Methods. 1994 August ; 53(2): 129–136.

Determination of extracellular bicarbonate and carbon dioxide concentrations in brain slices using carbonate and pH-selective microelectrodes

M. Chesler^{a,*}, J.C.T. Chen^a, and R.P. Kraig^b

^a Departments of Physiology and Biophysics and of Neurosurgery, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA

^b Departments of Neurology and Pharmacological and of Physiological Sciences, University of Chicago, Chicago, IL 60637, USA

Abstract

The extracellular pH of the brain is subject to shifts during neural activity. To understand these pH changes, it is necessary to measure $[H^+]$, $[HCO_3^-]$, $[CO_3^{2-}]$ and $[CO_2]$. In principle, this can be accomplished using CO_3^{2-} and pH-sensitive microelectrodes; however, interference from HCO_3^- and Cl^- , and physiological changes in $[HCO_3^-]$, complicate measurements with CO_3^{2-} electrodes. Calibration requires knowledge of slope response, interference constants and corrections for $[HCO_3^-]$ shifts. We show that when $[HCO_3^-]$ is altered at constant $[CO_2]$ in the absence of Cl^- , the HCO_3^- interference cancels and the Nikolsky equation reduces to the Nernst equation for CO_3^{2-} . Measurement of CO_3^{2-} slope response by this method yielded a value of 28.5 ± 0.72 mV per decade change in $[CO_3^{2-}]$. In Cl^- -containing solutions, interference coefficients for HCO_3^- and Cl^- were determined by altering $[HCO_3^-]$ at constant $[CO_2]$, changing $[CO_2]$ at constant $[HCO_3^-]$, then solving the simultaneous Nikolsky equations for each transition. The mean interference constants corresponded to selectivity ratios of 245:1 and 1150:1 for CO_3^{2-} over HCO_3^- and Cl^- respectively. To correct for possible changes in $[HCO_3^-]$, the equilibrium relation between CO_3^{2-} and HCO_3^- was substituted into the Nikolsky equation to yield an equation in $[CO_3^{2-}]$ and $[H^+]$. By simultaneously measuring shifts in $[H^+]$ with a pH microelectrode, this equation is readily solved for $[CO_3^{2-}]$. These methods were tested by measuring $[HCO_3^-]$ and $[CO_2]$ in experimental solutions, and in the extracellular fluid of rat hippocampal slices.

Keywords

Ion-selective microelectrode; Extracellular space; Extracellular pH; Hippocampal slice; pH Regulation; Carbonic acid

1. Introduction

The extracellular space of the brain is subject to significant shifts in local pH which result from metabolic and electrical activity (Kraig et al., 1983; Mutch and Hansen 1984; Somjen 1984;

*Corresponding author. Tel.: (212) 263-6318; FAX: (212) 689-0334.

Davis et al., 1987; Chesler and Chan, 1988; Rice and Nicholson, 1988; Sykova and Svoboda, 1990), for reviews see (Chesler, 1990; Chesler and Kaila, 1992). The advent of liquid membrane, H^+ selective microelectrodes has permitted highly resolved measurements of these phenomena (Ammann et al., 1981). To fully understand the origin of physiological pH changes, however, the concomitant behavior of HCO_3^- and CO_2 must be determined.

The reported development of a liquid membrane, HCO_3^- selective electrode based around tri-*n*-octylpropylammonium chloride (Wise, 1973) held promise for microelectrode studies (Khuri et al., 1974). Its composition, however, resembled that of a CO_3^{2-} -selective mixture (Herman and Rechnitz, 1975), and its selectivity for HCO_3^- was later questioned (Kraig and Cooper, 1987). By demonstrating appropriate voltage shifts in response to changes in CO_2 tension, Wietasch and Kraig (1991) revealed that microelectrodes fabricated with this cocktail were selective for CO_3^{2-} over HCO_3^- .

In principle, CO_3^{2-} and pH-selective microelectrode recordings can be combined to provide a measure of $[HCO_3^-]$ and $[CO_2]$, by using appropriate equilibrium relations. Attention to sources of interference is an important caveat in such studies, particularly when the concentration of an interfering ion and the ion of interest are both changing. In the case of the CO_3^{2-} measurements, interference from HCO_3^- warrants particular consideration. Although the CO_3^{2-} microelectrodes are preferentially selective for CO_3^{2-} over HCO_3^- (Wietasch and Kraig, 1991), the baseline concentrations of these ions differ by approximately 600-fold in favor of HCO_3^- . Moreover, since these ions are in equilibrium, they tend to shift simultaneously.

In this report, we demonstrate significant HCO_3^- and Cl^- interference on CO_3^{2-} -selective microelectrodes near the physiological range of concentration. A means of accurately determining the slope and interference of these microelectrodes is presented, and a method of calibration is developed which permits accurate determination of $[CO_3^{2-}]$, despite significant HCO_3^- interference, and changes in $[HCO_3^-]$. The combined use of a CO_3^{2-} and pH-sensitive microelectrode for the determination of $[HCO_3^-]$ and $[CO_2]$ is demonstrated both in solutions, and in the extracellular fluid of brain slices.

2. Methods

2.1. Electrode fabrication

Carbonate and pH-sensitive microelectrodes were fabricated from 1.5 mm, thin-walled borosilicate glass (AM Systems 6170). For carbonate electrodes, micropipettes were formed on a vertical puller, then broken to a tip diameter of 3–5 μm . The microelectrodes, were silanized by exposure to vapors of *N,N*-dimethyltrimethylsilylamine (Fluka) for 30 min, followed by baking at 250°C for 60 min. Positive pressure was used to backfill the silanized pipettes with 10 mM $NaHCO_3$ in 150 mM $NaCl$. The tip was then filled with carbonate-selective cocktail by gentle suction. Double-barreled pH-sensitive microelectrodes were formed by heating a bound pair of 1.5 mm thin-walled capillaries on the puller, twisting the pair 180°, and then pulling in a conventional manner. The ion and reference barrels were filled with phosphate-buffered saline (pH 7.4, 0.15 M $NaCl$) and 0.15 M $NaCl$ respectively, and were then broken to a tip diameter of 3–5 μm . The ion-sensitive barrel was rendered hydrophobic by repeated suction and ejection of 4% trimethyl chlorosilane in xylene. Gentle suction was used to fill the ion barrel with H^+ selective cocktail (Fluka 82500) which was based on the ionophore tridodecylamine (Ammann et al., 1981).

The CO_3^{2-} -selective cocktail was identical to that employed previously (Wise, 1973; Wietasch and Kraig, 1991). It consisted of a 3:1:6 (volume) mixture of tri-*n*-octylpropylammonium chloride (Kodak cat. No. 135 2194), 1-octanol (Fluka cat. no. 74850) and trifluoroacetyl-*p*-butylbenzene (Specialty Organics, Irwindale, CA). Three batches of this mixture were used. One was made approximately 2 years prior to this study, and a second prepared during the course of the experiments. A third batch of CO_3^{2-} selective cocktail was obtained from World Precision Instruments (IE-310). No differences in the performance of these 3 mixtures was noted.

Electrodes were studied in a conventional brain slice chamber, positioned at the solution inlet. This permitted relatively rapid changes in solution near the tips of the electrodes. In later experiments, a device was constructed in which solutions continuously ran from the outlet of 3 adjacent 20-ga stainless steel tubes, mounted on a micromanipulator. With 1 mm lateral displacements of the tubes, the solution around the electrode tip could be switched in less than 1 s. For studies in slice chambers, CO_3^{2-} and pH microelectrodes were mounted on a dual micromanipulator (Narashige MD-4) and lowered in tandem with a tip separation of approximately 20 μm . A pipette filled with 3 M KCl in 3% agar fitted with an AgCl-coated silver wire served as the bath indifferent electrode. Microelectrodes were connected to high impedance, unity gain electrometers (input impedance: $> 10^{13} \Omega$). Potentials on the reference barrel of the pH microelectrode were subtracted on-line from the CO_3^{2-} and pH signals. Data were measured directly from the strip chart records. The raw voltage measurements were entered into a computer spreadsheet in which interference values and concentrations were calculated, as described below. Calibration experiments were conducted at room temperature (22°–25°C).

2.2. Experimental preparation

Hippocampal brain slices were prepared using standard procedures which have been previously described (Chen and Chesler, 1992a). Slices were incubated in a submersion-style chamber and superfused with saline at a rate of approximately 5 ml/min. The saline consisted of 115 mM NaCl, 3 mM KCl, 35 mM NaHCO_3 , 1.0 mM NaH_2PO_4 , 2.5 mM CaCl_2 , 1.5 mM MgCl_2 , and 10 mM glucose, equilibrated with 95% O_2 and 5% CO_2 (pH 7.54). A bicarbonate concentration of 35 mM was employed to combat the extracellular acidosis of the submerged brain slices (Chen and Chesler, 1992a). The superfused physiological saline was heated to 32°C.

2.3. Determination of CO_3^{2-} -selective microelectrode slope

If interference is due exclusively to HCO_3^- and Cl^- , the potential in millivolts (V) of a carbonate-selective microelectrode is described by the Nikolsky equation (Eisenman, 1961; Wietasch and Kraig, 1991)

$$V = \emptyset + m \log \left\{ a[\text{CO}_3^{2-}] + i(b[\text{HCO}_3^-])^2 + j(c[\text{Cl}^-])^2 \right\} \quad (1)$$

where $\emptyset$ is an offset potential, m is the electrode slope in mV, i and j are interference constants for HCO_3^- and Cl^- , respectively, and a , b and c are activity constants for CO_3^{2-} , HCO_3^- and Cl^- . For a and b we used values of 0.42 and 0.79, respectively (Wietasch and Kraig, 1991). A value of 0.74 was used for c (Weast, 1972). For a shift between two solutions at constant $[\text{Cl}^-]$, the voltage change on a CO_3^{2-} electrode is given by

$$\Delta V = m \log \frac{a[\text{CO}_3^{2-}]_2 + i(b[\text{HCO}_3^-]_2)^2 + j(c[\text{Cl}^-])^2}{a[\text{CO}_3^{2-}]_1 + i(b[\text{HCO}_3^-]_1)^2 + j(c[\text{Cl}^-])^2} \quad (2)$$

where the subscripts 1 and 2 denote concentrations in starting and final solutions respectively. Use of this relation requires knowledge of the slope response, m , and the interference constants, i and j .

A means of determining m is evident upon consideration of the response to a change in $[\text{HCO}_3^-]$ in Cl^- -free media. At constant $[\text{CO}_2]$, the response is

$$\Delta V = m \log \frac{a[\text{CO}_3^{2-}]_2 + i(b[\text{HCO}_3^-]_2)^2}{a[\text{CO}_3^{2-}]_1 + i(b[\text{HCO}_3^-]_1)^2} \quad (3)$$

The equilibrium relation between $[\text{CO}_3^{2-}]$ and $[\text{HCO}_3^-]$ is

$$[\text{HCO}_3^-] = \frac{[\text{CO}_3^{2-}][\text{H}^+]}{K_b} \quad (4)$$

where K_b is the bicarbonate dissociation constant of 6×10^{-11} M (MacInnes and Belcher, 1935). Substitution of Eq. 4 into Eq. 3 and rearranging yields

$$\Delta V = m \log \frac{[\text{CO}_3^{2-}]_2 \left\{ a + \frac{ib^2[\text{CO}_3^{2-}]_2[\text{H}^+]_2^2}{K_b^2} \right\}}{[\text{CO}_3^{2-}]_1 \left\{ a + \frac{ib^2[\text{CO}_3^{2-}]_1[\text{H}^+]_1^2}{K_b^2} \right\}} \quad (5)$$

Given an apparent dissociation constant for carbonic acid (K_a), constant $[\text{CO}_2]$, and substitution of Eq. 4 for $[\text{HCO}_3^-]$,

$$\begin{aligned} [\text{HCO}_3^-][\text{H}^+] &= K_a[\text{CO}_2] = \frac{[\text{CO}_3^{2-}]_2[\text{H}^+]_2^2}{K_b} \\ &= \frac{[\text{CO}_3^{2-}]_1[\text{H}^+]_1^2}{K_b} = g \end{aligned} \quad (6)$$

where g is a constant. Substitution of Eq. 6 into the numerator and denominator of Eq. 5 and rearranging yields

$$\Delta V = m \log \frac{[\text{CO}_3^{2-}]_2(a + gib^2/K_b)}{[\text{CO}_3^{2-}]_1(a + gib^2/K_b)} = m \log \frac{[\text{CO}_3^{2-}]_2}{[\text{CO}_3^{2-}]_1} \quad (7)$$

Thus, for a change of $[\text{HCO}_3^-]$ at constant $[\text{CO}_2]$, a CO_3^{2-} -selective microelectrode will behave according to the Nernst equation, as if there were no HCO_3^- interference. This atypical relationship between a measured ion and an interfering species arises because the concentrations are linked through their equilibrium relation. The value of m can therefore be determined by measuring the voltage change in response to a shift in $[\text{HCO}_3^-]$ (in the absence of Cl^-). As shown in the Results, the value of m determined in this fashion was always very close to the theoretical value of 29.5. This value was therefore chosen for all subsequent calculations. Fixing m can be justified because small deviations in its value would be largely compensated by adjustments in the determination of i and j (detailed below). From an operational standpoint, small variations in the value of m are therefore irrelevant to the final determination of $[\text{CO}_3^{2-}]$, $[\text{HCO}_3^-]$ and $[\text{CO}_2]$.

2.4. Determination of interference values and pH electrode slope

To determine i and j it was necessary to alter $[\text{CO}_3^{2-}]$ by two different means, thereby allowing the solution of two independent equations. This entailed the use of two solution changes. First, $[\text{HCO}_3^-]$ was switched from 35 mM (subscript 1) to 15 mM (subscript 2) at constant $[\text{Cl}^-]$ (150 mM) and constant CO_2 (5%). The slope of the pH microelectrode was determined from its voltage change in response to this transition (ΔV_a), which is given by

$$\Delta V_a = m \log \frac{a[\text{CO}_3^{2-}]_2 + i(b[\text{HCO}_3^-]_2)^2 + j(c[\text{Cl}^-])^2}{a[\text{CO}_3^{2-}]_1 + i(b[\text{HCO}_3^-]_1)^2 + j(c[\text{Cl}^-])^2}. \quad (8)$$

The solution was then changed from 35 mM bicarbonate at 5% CO_2 , to 35 mM bicarbonate and elevated (10–15%) CO_2 (subscript 3). The response of the carbonate microelectrode for the second solution change (ΔV_b) is given by

$$\Delta V_b = m \log \frac{a[\text{CO}_3^{2-}]_3 + i(b[\text{HCO}_3^-]_3)^2 + j(c[\text{Cl}^-])^2}{a[\text{CO}_3^{2-}]_1 + i(b[\text{HCO}_3^-]_1)^2 + j(c[\text{Cl}^-])^2}. \quad (9)$$

Values for ΔV_a and ΔV_b were determined for every CO_3^{2-} -selective microelectrode. Assuming 29.5 as a value for m , these data were then used to determine electrode interference constants i and j by solving the simultaneous Eqs. 8 and 9.

2.5. Use of carbonate microelectrodes in physiological experiments

Once the values for m , i and j are determined, a CO_3^{2-} -selective microelectrode can be used for physiological measurements, providing $[\text{Cl}^-]$ is constant. In normal brain, activity-induced changes in extracellular $[\text{Cl}^-]$ ($[\text{Cl}^-]_o$, etc.) amount to a few millimolar against a background of approximately 120 mM (Dietzel et al., 1982). This represents an insignificant change from the standpoint of Cl^- interference on the CO_3^{2-} microelectrode. Given a constant $[\text{Cl}^-]_o$, the carbonate electrode response is described by Eq. 2, where ΔV is the voltage change between a known reference solution (subscript 1) and an unknown solution (subscript 2). In a brain slice experiment the reference solution is the superfused physiological saline, and the extracellular fluid is the unknown solution.

The remaining unknown variables in Eq. 2 are $[\text{CO}_3^{2-}]_2$ and $[\text{HCO}_3^-]_2$. By substitution of Eq. 4 into Eq. 2, the unknown $[\text{HCO}_3^-]_2$ is replaced by $[\text{H}^+]_2$, which can be readily measured with a pH-sensitive microelectrode. This yields the following quadratic in $[\text{CO}_3^{2-}]_2$:

$$\left\{ \frac{ib^2[\text{H}^+]_2^2}{K_b^2} \right\} [\text{CO}_3^{2-}]_2^2 + a[\text{CO}_3^{2-}]_2 + y = 0 \quad (10)$$

where y is a constant given by

$$y = \left\{ jc^2[\text{Cl}^-]^2 - (a[\text{CO}_3^{2-}]_1 + ib^2[\text{HCO}_3^-]_1^2 + jc^2[\text{Cl}^-]^2)10^{\Delta V/m} \right\}. \quad (11)$$

Note that $[\text{CO}_3^{2-}]_2$ can be determined despite simultaneous changes in the concentration of interfering HCO_3^- ions. The changes in $[\text{HCO}_3^-]_2$ are compensated for, because $[\text{H}^+]_2$ is measured.

To obtain $[\text{CO}_3^{2-}]_2$, Eq. 10 was solved using the quadratic formula. The value of $[\text{HCO}_3^-]_2$ was then calculated through Eq. 4. The partial pressure of $(\text{CO}_2)_2$ (in torr) was obtained using $[\text{HCO}_3^-]_2$, $[\text{H}^+]_2$ and the Henderson Hasselbach equation

$$(\text{pCO}_2)_2 = [\text{HCO}_3^-]_2 \times 10^{\text{p}K_c - \text{pH}_2} \quad (12)$$

where K_c is a constant combining the apparent dissociation constant for carbonic acid and the solubility constant for CO_2 . The values for $\text{p}K_c$ were empirically determined by measuring the pH of a solution of 35 mM NaHCO_3 in 150 mM NaCl , equilibrated with a certified 5% CO_2 , 95% O_2 , clinical blood-gas standard. The resulting molar values at 22°C and 32°C were 10.52 and 10.58, respectively.

2.6. Data analysis

For electrode calibrations, and for Figs. 1 and 2, voltages were measured by hand from strip chart records. To obtain continuous, real-time records of CO_2 , HCO_3^- and CO_3^{2-} (Fig. 3) the microelectrode voltages were archived onto video tape (Neurodata DR-484) and analyzed off-line. The replayed analogue signals were sampled at 10 Hz and converted to tab delimited text files using an Instrutech ITC 16 data acquisition board, Axodata 1.1 software (Axon Instruments) and a Macintosh IICx computer. The text files were then opened in the wave analysis program Igor (Wavemetrics) and converted to continuous records of $[\text{HCO}_3^-]$ and PCO_2 for individual time points, using a macro incorporating the CO_2 , HCO_3^- and CO_3^{2-} equilibrium relations given by Eqs. 4 and 12. The records were imported into Adobe Illustrator for final editing and presentation. Statistics were calculated as means $\pm$ SE.

3. Results

3.1. Slope response of CO_3^{2-} electrodes

Calibration records of a CO_3^{2-} -selective microelectrode are shown in Fig. 1. In the absence of Cl^- , the $[\text{CO}_3^{2-}]$ was raised from 12 μM to 64 μM by lowering the pCO_2 from 201 to 38 torr at a constant $[\text{HCO}_3^-]$ of 35 mM. The empirical slope response (m_e) to such a transition is given by

$$m_e = \frac{\Delta V}{\log \frac{[\text{CO}_3^{2-}]_2}{[\text{CO}_3^{2-}]_1}}. \quad (13)$$

In this instance the slope response was only 17.8 mV per decade change in $[\text{CO}_3^{2-}]$. Next, the $[\text{CO}_3^{2-}]$ was lowered back to 12 μM by decreasing the $[\text{HCO}_3^-]$ from 35 to 15 mM at constant pCO_2 of 38 torr. This produced a response of 27.7 mV per decade change in $[\text{CO}_3^{2-}]$. The difference in the empirical slopes can be attributed to the HCO_3^- interference, i , which in this instance was 1.01×10^{-2} . As predicted from Eq. 7, this interference cannot be discerned from changes in $[\text{HCO}_3^-]$ alone. Thus, for nine CO_3^{2-} -selective microelectrodes in which $[\text{HCO}_3^-]$ was changed between 15 and 35 mM, the mean value for m_e was 28.5 ± 0.72 (range: 25.8–31.5). This value is very close to 29.5, the theoretical value of m based on the Nernst equation at 25°C.

In the presence of Cl^- , however, the slope response for the same change in $[\text{HCO}_3^-]$ was much less, as shown in Fig. 1B. In this case, transition from 15 to 35 mM $[\text{HCO}_3^-]$ produced a voltage change of only 14.9 mV, corresponding to an m_e of 20.2 mV per decade change in $[\text{CO}_3^{2-}]$. Since HCO_3^- interference should not be evident for a pure change in $[\text{HCO}_3^-]$ (as demonstrated in Methods — see paragraph following Eq. 7), the low empirical slope in this instance can be attributed to the addition of Cl^- interference. When the pCO_2 was subsequently altered at fixed $[\text{HCO}_3^-]$, the slope response of the CO_3^{2-} microelectrodes was reduced further. In the second response of Fig. 1B, the pCO_2 was elevated from 38 to 114 torr (calculated from the pH and $[\text{HCO}_3^-]$), causing a fall in $[\text{CO}_3^{2-}]$ from 64 to 21 μM . The empirical slope response in this instance was only 17.2 mV per decade change in $[\text{CO}_3^{2-}]$. This further reduction in m_e was attributed to the interference from HCO_3^- ions.

The data of Fig. 1B can be used to determine the bicarbonate interference (i) and chloride interference (j) from simultaneous solution of Eqs. 8 and 9. For this electrode, the values were of i and j were 7.74×10^{-3} and 6.75×10^{-4} respectively. For 22 carbonate electrodes, i averaged $1.67 \pm 0.24 \times 10^{-2}$, corresponding to a selectivity ratio of 245: 1 for CO_3^{2-} over HCO_3^- . The mean value for j was $7.52 \pm 0.63 \times 10^{-4}$, corresponding to a selectivity ratio of 1150: 1 for CO_3^{2-} over Cl^- .

3.2. Determination of unknown $[\text{HCO}_3^-]$ and $[\text{CO}_2]$

The accuracy of the calibration technique was verified by performing measurements of coded solutions, the composition of which were unknown to the experimenter. A known solution with a pCO_2 of 38 torr (35 mM HCO_3^- in 150 mM NaCl, pH 7.48), was changed to an ‘unknown’

in which the $p\text{CO}_2$ was elevated to 124 torr (35 mM HCO_3^- in 150 mM NaCl, pH 6.97). The calculated $p\text{CO}_2$ was 120 ± 1.6 torr ($n = 4$) representing an error of 3%.

In a separate experiment, the $[\text{CO}_2]$ was ramped upward, by slowly bubbling 100% CO_2 in a flowing mixture of 150 mM NaCl and 35 mM HCO_3^- . At various time points, the $[\text{HCO}_3^-]$ was calculated from the equilibrium between CO_3^{2-} and H^+ . This value, and the solution pH, were then used to calculate the $[\text{CO}_2]$ in the chamber from the Henderson-Hasselbach equation. The mean $[\text{HCO}_3^-]$ calculated from 14 different $[\text{CO}_3^{2-}]$ and pH values was 36.7 ± 0.70 mM, representing an error of 4.9%. The values of $[\text{CO}_2]$ calculated from each determination of $[\text{HCO}_3^-]$ are displayed in Fig. 2 as partial pressure. The experimental determinations, based on the calculated $[\text{HCO}_3^-]$, agree well with the theoretical values, based on the measured pH and the actual $[\text{HCO}_3^-]$ (dashed line).

3.3. Brain slice measurement

A record derived from the simultaneous insertion of a pH and CO_3^{2-} microelectrode into a rat hippocampal slice is shown in Fig. 3. Transients preceding entry into the tissue are due to initial rapid movement of the electrode array. The pH gradient between bath and slice occurred through the unstirred layer and the first 100 μm of the tissue. Note that the transition from bath to the extracellular space was accompanied by no change in $[\text{HCO}_3^-]$, whereas the calculated $p\text{CO}_2$ increased from 38 torr in the bath, to approximately 60 torr in the slice. The corresponding fall in pH and fall in $[\text{CO}_3^{2-}]$ were therefore entirely due to the elevated CO_2 tension in the tissue. In 5 slices, the average $p\text{CO}_2$ and $[\text{HCO}_3^-]_o$ were 75.1 ± 6.61 torr and 34.8 ± 1.12 mM, respectively. This observation agrees with previous findings in brain slices in which extracellular acidosis was attributed solely to elevated $p\text{CO}_2$ (Voipio and Kaila, 1993).

4. Discussion

These results indicate that CO_3^{2-} microelectrodes may be used for physiological measurements despite considerable interference from HCO_3^- and Cl^- . In neurophysiological studies, $[\text{HCO}_3^-]_o$ is likely to vary during neuronal activity (Chesler, 1990; Chesler and Kaila, 1992). As noted, the effect of this changing interference can be eliminated, provided extracellular pH is monitored. Changes in $[\text{Cl}^-]_o$ which may accompany neuronal activity would have a negligible effect on the performance of the CO_3^{2-} microelectrode. Under pathological conditions, however, $[\text{Cl}^-]_o$ can fall dramatically (Kraig and Nicholson, 1978; Hansen and Zeuthen, 1981). Under such circumstances, CO_3^{2-} microelectrode studies would be inaccurate without a corresponding measurement of $[\text{Cl}^-]_o$ shifts.

The observed Cl^- interference was similar to that reported previously (Wietasch and Kraig, 1991); however, the interference from HCO_3^- was found to be considerably greater in the present study. It is notable that the bicarbonate interference was determined over a small range of $[\text{CO}_3^{2-}]$ (approx. 20–70 μM). This may explain the difference from the previous study, in which $[\text{CO}_3^{2-}]$ was varied over several orders of magnitude (1–300 μM) and the interference derived by curve fitting (Wietasch and Kraig, 1991). These collective observations may indicate a pH or concentration dependence of the bicarbonate interference. This possibility, while of interest, is irrelevant to the practical use of the CO_3^{2-} microelectrode, provided the HCO_3^- interference is determined within the experimentally relevant range of $[\text{CO}_3^{2-}]$.

Full characterization of a CO_3^{2-} microelectrode requires two chloride-free solutions to determine the slope of the CO_3^{2-} barrel, and three Cl^- -containing solutions to determine the interference constants. This entire procedure is necessary only if extremely accurate measurements of interference values are desired. It should be emphasized that in practice, it is not necessary to determine m for every CO_3^{2-} microelectrode, because deviations in this value are largely compensated by changes in the calculated interference constants. Given the consistency of m , these compensatory shifts in i and j are unlikely to be large. More importantly, such changes in i and j have little impact on the final determination of $[\text{CO}_3^{2-}]$, $[\text{HCO}_3^-]$ and $[\text{CO}_2]$. Thus, from an operational standpoint, only the three Cl^- containing solutions are required for the calibration and use of CO_3^{2-} microelectrodes once the values and variability of m are established.

The choice of constants used in the determination of $[\text{CO}_3^{2-}]$, $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ is not always critical. The values of the activity constants only influence the calculated interference constants. The constant K_b cancels in the calculation of $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ and only affects the value of $[\text{CO}_3^{2-}]$. However, the value of K_c can influence the calculation of $[\text{HCO}_3^-]$ and pCO_2 and should be chosen appropriately. Moreover, if CO_2 is to be expressed as concentration rather than partial pressure, then separate dissociation and solubility constants for CO_2 must be used.

Although the CO_3^{2-} microelectrode exhibits considerable selectivity over HCO_3^- and Cl^- (245:1 and 1150:1 respectively), incorporation of the interference constants into the calibration is essential. This is because the baseline concentrations of HCO_3^- and Cl^- are so much greater than that of CO_3^{2-} (approx. 600:1 and 2000:1, respectively). The greater the change in $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ from baseline, the greater will be the error if interference is not considered. An example of such error is plotted in Fig. 4 for a hypothetical CO_3^{2-} electrode subjected to changes in $[\text{HCO}_3^-]$ or $[\text{CO}_2]$. In this example, $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ were calculated from the empirical slope of the electrode (m_e), without incorporation of interference constants. It is evident that accurate measurements with the CO_3^{2-} microelectrode are not generally feasible unless the HCO_3^- and Cl^- interference constants are utilized. For similar reasons, it is necessary to simultaneously measure pH in order to compensate for changes in $[\text{HCO}_3^-]$. For example, given a selectivity ratio of 245: 1, a 5 mM increase in $[\text{HCO}_3^-]$ would be misread as a 20 μM shift in $[\text{CO}_3^{2-}]$. Since baseline $[\text{CO}_3^{2-}]$ is of this order, this would result in considerable errors in the calculation of $[\text{HCO}_3^-]$ and $[\text{CO}_2]$.

The recent development of a CO_2 microelectrode by Voipio and Kaila (1993) provides an alternate means of determining the extracellular pCO_2 and $[\text{HCO}_3^-]$ in brain slices. The availability of an independent method of CO_2 determination presents an opportunity to confirm the accuracy of the brain slice measurements made with CO_3^{2-} and pH microelectrodes. In preliminary experiments on rat hippocampal slices we have observed good agreement using both techniques. Another advantage of combining these methods should arise during the use of carbonic anhydrase inhibitors (Chen and Chesler, 1992a; Kaila et al., 1992). Under such conditions, equilibrium between CO_2 and HCO_3^- would not be expected during the rapid pH transients associated with excitatory synaptic transmission (Chesler 1990; Chen and Chesler, 1992a,b). Consequently, use of the Henderson-Hasselbach equation would be invalid, requiring separate, independent methods to measure changes in $[\text{CO}_2]$ and $[\text{HCO}_3^-]$.

Acknowledgments

The authors thank Professor Charles Nicholson for numerous discussions and suggestions throughout the course of this study. Supported by NINDS Grants NS-32123 and NS-27011. J.C.T. was supported by the Medical Scientist Training Program under NIGMS Grant 5T32 GM-07308. R.P.K. was supported by NINDS grant NS-19108 and an Established Investigator Award from the American Heart Association.

References

- Ammann D, Lanter F, Steiner RA, Schulthess P, Shijo Y, Simon W. Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. *Anal Chem* 1981;53:2267–2269. [PubMed: 7316213]
- Chen JCT, Chesler M. Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J Neurophysiol* 1992;67:29–36. [PubMed: 1348085]
- Chen JCT, Chesler M. pH transients evoked by excitatory synaptic transmission are increased by inhibition of extracellular carbonic anhydrase. *Proc Natl Acad Sci USA* 1992;89:7786–7790. [PubMed: 1380165]
- Chesler M. The regulation and modulation of pH in the nervous system. *Prog Neurobiol* 1990;34:401–427. [PubMed: 2192394]
- Chesler M, Chan CY. Stimulus-induced extracellular pH transients in the in vitro turtle cerebellum. *Neuroscience* 1988;27:941–948. [PubMed: 3252179]
- Chesler M, Kaila K. Modulation of pH by neuronal activity. *Trends Neurosci* 1992;15:396–402. [PubMed: 1279865]
- Davis PK, Carlini WG, Ransom BR, Black JA, Waxman SG. Carbonic anhydrase activity develops postnatally in the rat optic nerve. *Dev Brain Res* 1987;31:291–298.
- Dietzel I, Heinemann U, Hofmeier G, Lux HD. Stimulus-induced changes in extracellular Na⁺ and Cl[−] concentration in relation to changes in the size of the extracellular space. *Exp Brain Res* 1982;46:73–84. [PubMed: 6279427]
- Eisenman G. Cation selective glass electrodes and their mode of operation. *Biophys J* 1961;2:259–323. [PubMed: 13889686]
- Hansen AJ, Zeuthen T. Extracellular ion concentrations during spreading depression and ischemia in the rat brain cortex. *Acta Physiol Scand* 1981;113:437–445. [PubMed: 7348028]
- Herman HB, Rechnitz GA. Preparation and properties of a carbonate ion-selective membrane electrode. *Anal Chim Acta* 1975;76:155–164.
- Kaila K, Paalasmaa P, Taira T, Voipio J. pH transients due to monosynaptic activation of GABA A receptors in rat hippocampal slices. *NeuroReport* 1992;3:105–108. [PubMed: 1377040]
- Khuri RN, Bogharian KK, Agulian SK. Intracellular biocarbonate in single skeletal muscle fibers. *Pflügers Arch* 1974;349:285–294. [PubMed: 4472159]
- Kraig RP, Cooper AJL. Bicarbonate and ammonia changes in brain during spreading depression. *Can J Physiol Pharmacol* 1987;65:1099–1104. [PubMed: 3621035]
- Kraig RP, Ferreira-Filho CR, Nicholson C. Alkaline and acid transients in cerebellar microenvironment. *J Neurophysiol* 1983;49:831–850. [PubMed: 6834101]
- Kraig RP, Nicholson C. Extracellular ionic variations during spreading depression. *Neuroscience* 1978;3:1045–1059. [PubMed: 745780]
- MacInnes DA, Belcher D. The thermodynamic ionization constants of carbonic acid at 38° from electromotive force measurements. *J Am Chem Soc* 1935;57:1683–1685.
- Mutch WAC, Hansen AJ. Extracellular pH changes during spreading depression and cerebral ischemia: mechanisms of brain pH regulation. *J Cereb Blood Flow Metab* 1984;4:17–27. [PubMed: 6693512]
- Rice ME, Nicholson C. Behavior of extracellular K⁺ and pH in skate (*Raja erinacea*) cerebellum. *Brain Res* 1988;461:328–334. [PubMed: 3179721]
- Somjen GG. Acidification of interstitial fluid in hippocampal formation caused by seizures and by spreading depression. *Brain Res* 1984;311:186–188. [PubMed: 6488041]
- Sykova E, Svoboda J. Extracellular alkaline-acid-alkaline transients in the rat spinal cord evoked by peripheral stimulation. *Brain Res* 1990;512:181–189. [PubMed: 2354355]

- Voipio J, Kaila K. Interstitial pCO₂ and pH in rat hippocampal slices measured by means of a novel fast CO₂/H⁺- sensitive microelectrode based on a PVC-gelled membrane. *Eur J Physiol* 1993;423:193–201.
- Weast, RC. Handbook of Chemistry and Physics. The Chemical Rubber Company; Cleveland, OH: 1972.
- Wietasch K, Kraig RP. Carbonic acid buffer species measured in real time with an intracellular microelectrode array. *Am J Physiol* 1991;261:R760. [PubMed: 1653544]
- Wise (Inventor). Bicarbonate Ion-Sensitive Electrode US Patent. 3,723,281. 1973.

Fig. 1.

Calibration of CO_3^{2-} -selective microelectrode. A: determination of slope responses in Cl^- -free solution. Record shows voltage change on CO_3^{2-} electrode (V_c). Empirical slope response (m_e) was 17.8 mV per decade change in $[\text{CO}_3^{2-}]$ when pCO_2 was lowered from 201 to 38 torr. The same change in $[\text{CO}_3^{2-}]$ produced a slope response of 27.7 mV per decade when $[\text{HCO}_3^-]$ was switched from 35 to 15 mM at constant pCO_2 . B: determination of interference constants for HCO_3^- and Cl^- . Changing $[\text{HCO}_3^-]$ from 35 to 15 mM at constant pCO_2 produced an empirical slope response of 20.2 mV per decade change in $[\text{CO}_3^{2-}]$. Increasing pCO_2 at constant $[\text{HCO}_3^-]$ produced an empirical slope response of 17.2 mV per decade. Solution of simultaneous Eqs. 8 and 9 yielded HCO_3^- and Cl^- interference constants of 7.74×10^{-3} and 6.75×10^{-4} respectively.

Fig. 2.

Determination of pCO₂ using CO₃²⁻ and pH sensitive microelectrodes. A solution (35 mM NaHCO₃, 150 mM NaCl) was subjected to increasing pCO₂. At 14 time points, the pCO₂ was calculated from the Henderson-Hasselbach equation, based on the determined [CO₃²⁻], [HCO₃⁻] and pH (black dots). Values corresponded well with theoretical values of pCO₂ (dashed line).

Fig. 3.

Measurement of pCO_2 and $[HCO_3^-]$ in a rat hippocampal slice. The CO_3^{2-} and pH microelectrodes were simultaneously advanced into the tissue. V_c is the raw voltage response of the CO_3^{2-} microelectrode. Noise on traces before entry is an artifact due to movement of the high-resistance microelectrodes. Note that the acidosis was due to the elevation of pCO_2 relative to the bath. There was little difference in the $[HCO_3^-]$ between the bath and the gross extracellular fluid. Calibration bar at bottom represents approximate steps of 100 μm above

and below the surface of the slice. The $[\text{HCO}_3^-]$ and CO_2 were derived from CO_3^{2-} and pH measurements and plotted off-line as described in Data Analysis section in Methods.

Fig. 4.

The impact of interference constants on accuracy. Plots show the per cent error that would arise if the $[\text{CO}_3^{2-}]$ were determined from the empirical slope response of a CO_3^{2-} microelectrode without consideration of interference. A hypothetical electrode was assumed to have HCO_3^- and Cl^- interference constants of 1.67×10^{-2} and 7.52×10^{-4} , respectively. The $[\text{CO}_3^{2-}]_2$ was then calculated as $[\text{CO}_3^{2-}]_2 = [\text{CO}_3^{2-}]_1 \times 10^{\Delta V/m_e}$ where m_e is the empirical slope response of 19.4 mV. A: per cent error calculated at constant pCO₂ (38 torr) for changes in $[\text{HCO}_3^-]$ from a baseline of 26 mM. B: per cent error calculated at constant $[\text{HCO}_3^-]$ (26 mM) for changes in pCO₂ from a baseline of 38 torr. Note that errors become more pronounced as the bicarbonate or pCO₂ changes grow larger, and are greater for negative deviations from baseline.