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Multiple glass electrode and signal averaging approach to making long-term calibration-free potentiometric seawater pH measurements

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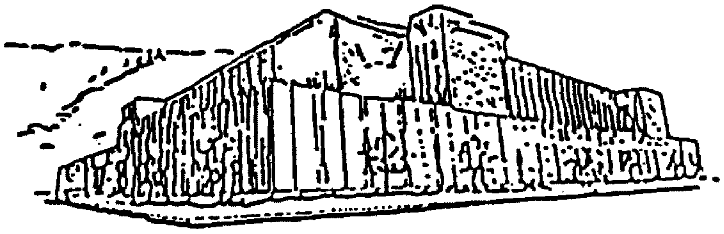

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A MULTIPLE GLASS ELECTRODE AND SIGNAL AVERAGING APPROACH TO MAKING LONG-TERM CALIBRATION-FREE POTENTIOMETRIC SEAWATER pH MEASUREMENTS

By

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A Multiple Glass Electrode and Signal Averaging Approach to Making Long-Term Calibration-Free Potentiometric Seawater pH Measurements

Director: Michael D. DeGrandpre

The carbonate system regulates the pH of seawater and controls the distribution of carbon dioxide (CO_2) between the atmosphere and the oceans. The measurement of any two of the four carbonate parameters [pH, partial pressure of CO_2 (pCO_2), total alkalinity (TA) and total CO_2 (TCO_2)] enables calculation of the remaining two through thermodynamic relations. The processes controlling oceanic CO_2 variability are best described through TCO_2 measurements. Shipboard TCO_2 measurements, however, are infrequent, short in duration and, consequently, fail to give the continuous data that is needed to fully characterize oceanic CO_2 variability. Ideally, continuous long-term TCO_2 measurements should be made on moorings with autonomous instruments.

Currently, it appears that the most technically-feasible means to determining TCO_2 is through *in situ* pH and salinity-derived TA measurements. Although autonomous *in situ* salinity measurements are routinely made, autonomous *in situ* pH measurements have not been possible. Electrode drift has been the major obstacle to making potentiometric seawater pH measurements with relatively good accuracy and precision.

The premise of this research was that using multiple glass electrodes with signal averaging would improve the accuracy and precision of potentiometric seawater pH measurements by reducing electrode drift. This improvement would be a major advancement in the development of an autonomous *in situ* potentiometric seawater pH instrument. The pH measurements from this instrument could then be used in conjunction with pCO_2 or salinity-derived TA to calculate TCO_2 and effectively study the processes which control oceanic carbon dioxide variability.

In assessing the use of a multiple electrode / signal averaging approach to reduce electrode drift in potentiometric pH measurements, several long-term drift experiments were conducted in the laboratory using Orion Ross electrodes. Results from a 56-day experiment indicate that long-term calibration-free potentiometric seawater pH measurements can be made with a precision of ±0.004-0.005 pH units and an accuracy of ±0.015 pH units.

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CHAPTER 1

INTRODUCTION

1.1 Overview

The concept of pH was first introduced by Sørensen (1909) and was originally defined as the negative logarithm of the hydrogen ion concentration. The introduction of the activity concept three years later (Lewis, 1912) eventually led to the definition of pH as the negative logarithm of the hydrogen ion activity ($pH = -\log a_{H^+}$). Since this introduction almost 90 years ago, pH has been an important parameter used to characterize chemical equilibria.

pH is useful in characterizing natural waters because it is dependent upon biological and chemical processes which occur in both fresh and saline waters. Biological processes such as photosynthesis and respiration are intimately linked with pH through carbon dioxide (CO_2) uptake and release. These links have been established with correlations observed between pH and biological indicators such as chlorophyll-*a* (Fuhrmann and Zirino, 1988). Other biologically-driven reactions which affect the hydrogen ion concentration of natural waters include oxygenation, denitrification and sulfate reduction (Westcott, 1978).

pH also has an influence on a wide variety of chemical processes in natural waters. Trace-element concentrations, for example, can vary with pH due to the adsorption and desorption of elements onto mineral surfaces (Fuller and Davis, 1989). Other examples of chemical processes influenced by pH include metal complexation and calcium carbonate ($CaCO_3$) dissolution.

Measuring oceanic pH is important because it reveals the thermodynamic state of all the acid-base systems present in seawater. The pH of the oceans is primarily regulated by the carbonate system. The carbonate system consists of four parameters: pH, total CO_2 (TCO_2), partial pressure of CO_2 (pCO_2) and total alkalinity (TA). TCO_2 is defined as the sum of CO_2 (aq), HCO_3^- and CO_3^{2-} while TA is equal to the concentration of all the bases that can accept a proton. The measurement of any two of these four parameters enables calculation of the others through thermodynamic relations (Millero, 1995).

The processes controlling oceanic pH have become increasingly studied over the past five years because of the interest in understanding the role of the oceans in carbon dioxide cycling. Since the Industrial Revolution began in the late 1800's, atmospheric CO_2 concentrations have been increasing due to the burning of fossil fuels and deforestation. It has been estimated that 30-40 % of all anthropogenic CO_2 added to the atmosphere is taken up by the oceans (Sarmiento, 1993). This influx adds approximately 1 μM of TCO_2 to the oceans each year (Millero et al., 1993a).

This uptake of anthropogenic CO_2 is controlled by the pCO_2 gradient between the atmosphere and the ocean. An atmospheric pCO_2 which is higher than the oceanic pCO_2 results in an influx of carbon dioxide into surface seawaters. The oceanic CO_2 is thermodynamically controlled by the following equilibria (Millero, 1996):

$$\begin{gathered} CO_2(g) \Leftrightarrow CO_2(aq) \\ CO_2(aq) + H_2O \Leftrightarrow H_2CO_3 \\ H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \\ HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \\ Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3(s) \end{gathered} \tag{1-5}$$

Using the well-known equilibrium constants (Goyet and Poisson, 1989) with these equations, the hydrogen ion concentration can be used with another carbonate parameter (TA, TCO_2 or pCO_2) to calculate the remaining two; thus, making pH an effective tool for characterizing the carbonate system and understanding the role the oceans have in CO_2 cycling.

All four parameters of the carbonate system (TA, pH, TCO_2 and pCO_2) are commonly measured on board ships, but due to the expense of ship-based research these measurements are infrequent, short in duration and fail to offer the long term data needed to fully characterize and understand CO_2 variability at the sea surface. Ideally, autonomous *in situ* TCO_2 measurements should be made on ocean moorings over long periods of time (i.e., 3-6 months) because any accumulation or loss of CO_2 will be reflected in the TCO_2 concentration. Unfortunately, current methods for determining TCO_2 are not readily adaptable to autonomous *in situ* analysis. Therefore, the only practical means (at present) to monitoring long term TCO_2 fluctuations is through any two *in situ* measurements of pCO_2, TA (deduced from salinity) or pH.

One possible approach to long-term mooring-based TCO_2 measurements is through *in situ* pCO_2 and pH measurements. Autonomous *in situ* pCO_2 measurements are well established (DeGrandpre et al., 1995; DeGrandpre et al., 1997) and could easily be combined with *in situ* pH measurements to calculate TCO_2. The major shortcoming of this approach, however, is that the accuracy of the TCO_2 calculation from the pCO_2/pH pair is less than ideal. An accuracy of ±2 μatm in pCO_2 and ±0.002 in pH yields an accuracy of only ±18 μmol kg^{-1} for the TCO_2 calculation (Millero et al., 1993b). An improvement in the accuracy of the pCO_2 and pH measurements would reduce the error in the TCO_2 calculation.

A second and perhaps more practical approach to measuring TCO_2 is through *in situ* pH and salinity measurements. The estimated accuracy of calculating TCO_2 using pH and TA is ± 3.8 μmol kg^{-1} (Millero et al., 1993b). Although TA measurements are not readily adaptable to an autonomous instrument, TA concentrations can be derived from *in situ* salinity measurements because of the tight correlation between the two in surface waters (Millero, 1996). This correlation arises because the TA variability at the sea surface is mostly due to dilution and evaporation, resulting in a ratio of HCO_3^- to salinity which is essentially constant. In deeper waters, though, the dissolution of $CaCO_3$ results in a higher TA concentration at a constant salinity. Thus, the relationship between TA and salinity breaks down below the sea surface.

Although autonomous *in situ* salinity measurements are routinely made, no instrument capable of making long-term *in situ* pH measurements has been developed. The objective of this research, then, was to improve the accuracy and precision of potentiometric seawater pH measurements by reducing electrode drift (discussed in the following section). This approach would further the development of an autonomous instrument capable of making long-term *in situ* pH measurements. These measurements can then be combined with salinity-derived TA or pCO_2 data to calculate TCO_2 and effectively study the processes which control oceanic CO_2 concentrations.

1.2 Seawater pH Measurements

An autonomous pH instrument could be developed based upon either a spectrophotometric or potentiometric approach, which are the two common methods used for shipboard pH measurements. This section will give a brief overview of these two methods and will also examine the major problems associated with potentiometric seawater pH measurements. A historical overview of seawater pH measurements will be given in Chapter 2.

Spectrophotometric seawater pH measurements are based on the absorbance characteristics of sulfonephthalein indicator dyes (Byrne, 1987; Byrne and Breland, 1989; Clayton and Byrne, 1993). These indicators (represented as I in the unprotonated form) exist in three distinct forms (H_2I, HI^- and I^{2-}), with each having a unique visible absorbance spectrum in solution. Once an indicator is added to a sample, it will dissociate according to the pH, temperature, ionic strength and pressure of that solution. Therefore, if an indicator is used under standard temperature and pressure, the pH of a given sample can be determined from the absorbance of both the acid (HI^-) and base (I^{2-}) forms of the indicator dye. The ratio (I^{2-}/HI^-) of these forms is then used in conjunction with the molar absorptivities of each form to calculate the pH. H_2I is not considered because it does not exist at pH's typical of natural waters.

Choosing a suitable indicator for spectrophotometric pH measurements should be based on the expected pH of the sample being measured. As a general rule, the log of the formation constant of the sulfonephthalein indicator ($-\log K_n$) should fall within ±1 pH unit of the anticipated pH of the sample; i.e. [$\log K_n$ (*indicator*) – 1] < pH (*sample*) ≤ [$\log K_n$ (*indicator*) + 1] (Clayton and Byrne, 1993). A variety of indicator dyes have been used in spectrophotometric seawater pH measurements. Cresol red (Byrne and Breland, 1989), for example, has been used for surface waters while *m*-cresol purple (Clayton and Byrne, 1993) has been used for surface-to-deep pH profiles in the open ocean.

The potentiometric determination of seawater pH involves measurement of the electromotive force (e.m.f. or E) of an appropriate galvanic cell (Bates, 1973). This cell consists of two reversible electrodes and an external circuit (the pH meter). The two electrodes of this cell, the indicating electrode (typically glass-type) and the reference electrode, have distinct roles in e.m.f. measurements (Westcott, 1978). When the indicating electrode is placed in a sample, the alkali metal ions in the glass bulb equilibrate with the hydrogen ions in solution and create a potential which is dependent upon the hydrogen ion activity (a_{H^+}). Any change in the a_{H^+} of the sample will shift this equilibrium and alter the measured potential. The indicating electrode alone, though, cannot measure this potential. The reference electrode completes the circuit by supplying a constant potential to which the potential from the indicating electrode can be compared. The difference between these potentials is detected and displayed by the pH meter. A schematic of a typical combination electrode is given by Figure 1-1.

A conventional reference electrode consists of an internal wire element (usually Hg/Hg_2Cl_2 or Ag/AgCl) surrounded by an aqeous solution saturated with AgCl and KCl. The reference electrode filling solution allows electrical contact between the wire element and the sample through the liquid junction. Two potentials exist in the reference electrode. In a Ag/AgCl reference electrode, for example, the two potentials arise from (1) the reduction of AgCl to Ag(s) + Cl^- and (2) the liquid junction. These two potentials must remain constant in order for the reference electrode to supply a constant potential to which the potential from the indicating electrode can be compared.

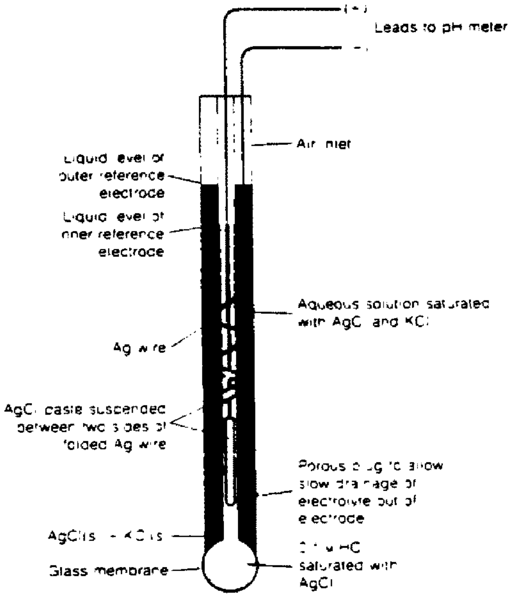

Figure 1-1: Schematic of a Ag/AgCl reference electrode (Harris, 1995)

While the potential arising from the reduction of AgCl remains constant, the potential of the liquid junction can vary. If the liquid junction fails or becomes partially clogged the inner filling solution will not be allowed to continually flow into the sample, resulting in a non-constant liquid junction potential. A non-constant liquid junction potential leads to pH measurments which are slowly drifting and in error. The problems associated with drifting electrodes have been well documented (Bellerby et al., 1993; Dickson, 1993a).

A non-constant liquid junction potential is also a source of error in pH calibrations. This error is best illustrated by examining the operational definition (specific to glass electrodes) of pH. The pH of any aqueous sample is operationally defined by the equation

$$pH(x) = pH(s) + \frac{(E_s - E_x)F}{2.303RT} \qquad (6)$$

where pH(x) = the pH of a sample, pH(s) = the pH of a standard buffer, E_s = the measured potential of the buffer, E_x= measured potential of the sample, R = gas constant, T = measured temperature in K, F = Faraday constant and 2.303 RT/F = Nernst slope (0.05916 mV/pH at 25 °C for an ideal electrode).

Equation 6 assumes that the liquid junction potential is the same when both E_s and E_x are measured. This assumption can result in significant error if the ionic strength (I) of the buffer and sample are not equal (Bellerby et al., 1993). This error is especially large when using low ionic strength NIST (National Institute of Standards and Technology) buffers (I = 0.1 M) to measure the pH of seawater (I = 0.7 M). In this case, the difference in mobilities of the ions in seawater (compared to the buffer) plus the higher concentrations of these ions will cause a larger potential at the reference electrode, resulting in a systematic error of up to 0.5 pH units when working between the two media. Therefore, a buffer with an ionic medium similar to that of seawater must be used in order to maintain a constant liquid junction potential.

1.3 Rationale

Although both approaches to measuring seawater pH have have been made on board ships with relatively good accuracy and precision (Fuhrmann and Zirino, 1988;Clayton and Byrne, 1993), an autonomous instrument using glass electrodes would be simpler and more cost effective than an instrument utilizing the spectrophotometric approach. Electrode drift, however, has limited the usefulness of electrodes for long-term autonomous pH measurements. Therefore, the purpose of this research was to test a hypothesis that the effects of electrode drift could be reduced by using multiple glass electrodes and signal averaging. A thorough literature search indicated that this relatively straightforward approach has never been attempted. Reducing the effects of electrode drift would be a major advancement in the development of an autonomous *in situ* instrument capable of making long-term potentiometric seawater pH measurements. These measurements could then be used in conjunction with $p\mathrm{CO_2}$ or salinity-derived TA data to calculate $\mathrm{TCO_2}$ and effectively study the processes which control oceanic $\mathrm{CO_2}$ variability.

A multiple electrode and signal averaging approach would offer several advantages to making autonomous potentiometric seawater pH measurements. One advantage is that it would greatly simplify an autonomous pH instrument by circumventing the need for periodic calibrations. This approach would eliminate the need for the pumps, flow cells and buffers normally used in shipboard potentiometric pH measurements. Once the electrodes are initially calibrated, subsequent calibrations would not theoretically be needed because a multiple electrode approach would enable the detection and possible removal of any electrode that is drifting or noisy. The remaining non-drifting signals would then be averaged for the resultant pH. Also, this approach assumes that the electrode drift is random. Thus, the average of several signals would be more accurate than any single signal.

Using multiple electrodes and signal averaging would also, in principle, improve the signal-to-noise ratio (i.e., precision) of the data (Skoog and Leary, 1992). To illustrate this, consider four electrodes measuring the pH of the same sample. As the four signals are summed, the noise from each signal is added as well. Since these noise signals are random they will accumulate as the square root of the number of measurements. Therefore as the number of signals n increases, the noise only increases by the square root of n; whereas the signal increases by n. In the case of four measurements, then, the result would be 2 times the single measurement signal-to-noise ratio (S/N). The S/N for the average is

$$\frac{S}{N} = \frac{nS_x}{\sqrt{n}N_x} = \sqrt{n}\frac{S_x}{N_x} \tag{7}$$

where n = number of signals, S_x = value of a single electrode measurement and N_x = noise of a single electrode measurement.

In addition to using a multiple electrode/signal averaging approach, Orion Ross combination electrodes were selected to help reduce the possibility of drift. The double junction design of these electrodes allows the inner reference solution to be isolated from the sample, reducing the chance of precipitates forming and clogging the liquid junction. Junction clogging is also minimized by using a saturated potassium chloride outer filling solution (as opposed to some electrodes that use KCl saturated with AgCl). Since K^+ and Cl^- have similar mobilities ($K^+ = 7.62\times10^{-8}$ $m^2/(s{\cdot}V)$; $Cl^- = 7.91\times10^{-8}$ $m^2/(s{\cdot}V)$), ion movement will not be hindered, providing a virtually constant liquid junction potential.

The proprietary internal system of the Ross electrodes has been reported to offer additional advantages over conventional Ag/AgCl and calomel reference electrodes (Orion Research Inc., 1991). One advantage is a better temperature response. The silver-silver chloride and calomel references normally used in electrodes are based on a chemical equilibrium which is affected by temperature. Any sudden change in the temperature of the pH measurement will cause this equilibrium to shift. This equilibrium shift takes place so slowly, however, that the electrode response begins to drift. This error has been virtually eliminated in Ross electrodes since the proprietary inner filling solution is essentially unaffected by temperature fluctuations. The internal system of Ross electrodes has also been reported to offer better stability, faster response and more accurate and reproducible measurements than either Ag/AgCl or calomel reference electrodes.

In order to test the hypothesis that a multiple electrode/signal averaging approach would improve potentiometric seawater pH measurements, several experiments were performed in which multiple (4-6) electrodes were placed in a suitable buffer, calibrated and then used to record the pH of a buffer over an extended period of time (i.e. 6-8 weeks) without any further standardizations. Electrode drift was monitored by observing the standard deviation of the signals and by performing periodic accuracy checks. The remaining sections of this chapter will discuss the preparations required for performing these experiments.

1.4 pH Scales

Over the past 20 years researchers have measured seawater pH using three different scales which are based on the hydrogen ion concentration rather than the activity of the proton (Dickson, 1984). The three pH scales that have been used are the free hydrogen ion scale, the total hydrogen ion scale and the seawater scale. The difference between these scales is based simply on the ions which are incorporated into each. The free hydrogen ion scale (Bates and Culberson, 1977) accounts for only the hydrogen ion, and is defined as:

$$pH_F = -\log m(H^+) \tag{8}$$

where m = molal concentration (mol kg^{-1}). The total hydrogen ion scale (Hansson, 1973) includes sulfate and is defined as

$$pH_T = -\log m^*(H^+) \tag{9}$$

where $m^*(H^+) = m\,(H^+) + m\,(HSO_4^-)$. The seawater scale (Dickson and Riley, 1979) accounts for fluoride as well as sulfate ions and is given by

$$pH_{SWS} = -\log m(H^+)_{SWS} \tag{10}$$

where $m\,(H^+)_{SWS} = m\,(H^+) + m\,(HSO_4^-) + m\,(HF)$.

Although these three scales are all practical and have been widely used, there is one major disadvantage to using more than one pH scale in oceanographic research; it prevents the comparison of pH measurements between various researchers. Since comparing seawater pH measurements from different times and locations is a necessity for understanding long-term oceanic pH variability, there should be one universal pH scale.

One obvious question for this research, then, was the following: on which pH scale should the seawater pH measurements and calibration buffers be based? Work by

Dickson (1993b) clarified the confusion regarding which pH scale to use in seawater pH measurements. It was concluded that a scale which incorporates sulfate, but not fluoride, would be the most logical. His conclusion is based on the fact that fluoride is a minor acid-base species and should not be measured in order to make the pH definition clearer. Thus, all seawater pH measurements and calibration buffers should be based on the total hydrogen ion concentration scale (Equation 9). In the experiments of this research, calibration buffers based on the total hydrogen ion concentration scale were used (Dickson, 1993b).

CHAPTER 2

BACKGROUND

2.1 Historical Overview of Seawater pH Measurements

Prior to the mid-1980's, most shipboard pH measurements were made using potentiometric techniques. These measurements, however, varied greatly between researchers in regards to the method of sample collection, sample preservation and calibration. Indeed, as late as 1981 there was no standard method for even reporting pH data (Culberson, 1981). Seawater samples were collected in either glass or plastic bottles or glass syringes. Although samples were analyzed as quickly as possible, errors still arose from CO_2 outgassing as the samples warmed to room temperature. Contamination was also a problem. Park (1968), for example, found an error of ±0.04 pH units from imperfections in the lining of PTFE-coated Nansen bottles.

As discussed briefly in Chapter 1, calibration buffers have been a source of error in measuring seawater pH. Most trouble arose from using dilute NIST buffers. Once the electrodes were calibrated in the low ionic strength buffers, they had to be soaked in seawater for approximately 15 minutes to allow the liquid junction to equilibrate with the seawater before making the pH measurement (Zirino, 1975). Even with this procedure, it took several minutes for the electrodes to stabilize in the seawater sample and errors were still prevalent. Almgren et al. (1975) was one of the first groups to use artificial seawater buffers for pH calibrations. When using seawater buffers, it was observed that the electrodes reached a steady state much more rapidly when working between the buffer and sample.

The obvious improvement in seawater pH measurements from using high ionic strength buffers led to an increased interest in evaluating a variety of seawater buffers for pH calibrations. Bates and co-workers published several papers from the late 1970's through the mid-1980's which gave e.m.f. measurements for a series of amine-based synthetic seawater buffers. The buffers evaluated were: 2-amino-2-hydroxymethyl-1,3-propanediol ("tris"-Ramette et al., 1977); 2-amino-2-methyl-1,3-propanediol ("bis"-Bates and Calais, 1981); tetrahydro-1,4-isoxazine ("morpholine"-Czerminski et al., 1982) and 2-aminopyridine (Bates and Erickson, 1986). These measurements, however, were based on the "free" hydrogen ion concentration scale rather than the "total" hydrogen ion concentration scale (Equations 8 and 9). Dickson (1993b) re-evaluated the original e.m.f. measurements made by Bates and calculated the pH of the four buffers based on the total hydrogen ion concentration.

Another historical problem with calibration buffers has been maintaining them at a constant pH. Biological activity or contamination by CO_2 will alter the pH of the buffers quite significantly (Bates, 1973). In the late 1980's, Byrne (1987) proposed a method by which buffers could be standardized using visible spectrometry. This method, based on the dissociation of sulfonephthalein indicator dyes (as described in Section 1.2), has become the primary basis on which spectrophotometric seawater pH measurements are currently made.

A major advancement in potentiometric seawater pH measurements came from the work of Fuhrmann and Zirino (1988). Their method eliminated discrete seawater samples and the problems associated with them (e.g. contamination) by placing the electrodes in a constant-flowing stream of seawater. Buffers were periodically introduced for calibrations. The pH was calculated at a later time using empirically-determined temperature characteristics of the electrode and buffer system. The pH measurements were made with Corning combination glass electrodes (Hg/Hg_2Cl_2 reference), with the analog signals being fed through an analog to digital converter (ADC) and collected on a portable computer. Calibrations were made by diverting a constant stream of buffer past the electrodes and recording the potential of each electrode. The two buffers used were Tris and Tris-HCl which have a pH of 8.875 and 7.458, respectively. Results indicated that potentiometric seawater pH measurements could be made with an accuracy of better than $\pm$ 0.01 pH units and a precision of $\pm$ 0.003 pH units.

Although the work by Fuhrmann and Zirino greatly improved potentiometric seawater pH measurements, researchers began to adopt the spectrophotometric method developed by Byrne for seawater pH measurements. The departure from potentiometric seawater pH measurements was due mostly to the better accuracy and precision that could be achieved with spectrophotometric methods. Byrne and Breland (1989), for example, made a detailed study on pH anomalies associated with hydrothermal venting using a cresol red indicator. The spectrophotometric seawater pH measurements had an accuracy of ± 0.001 and a precision of ± 0.0005 pH units. Clayton and Byrne (1993) obtained similar results using an *m*-cresol purple indicator. Although the accuracy of the spectrophotometric pH measurements made on this cruise were somewhat uncertain, the at-sea precision was reported to be ± 0.0004 pH units. Bellerby et al. (1995) developed an automated flow injection system which eliminated the need for discrete samples in spectrophotometric seawater pH measurements. This method gave a shipboard accuracy of ±0.005 pH units and a precision of ±0.005 pH units.

Even though the current trend in seawater pH measurements has been to use a spectrophotometric approach, potentiometric measurements are still being made. Several research groups (Copin-Montegut and Avril, 1995; Rogachev et al., 1996) have recently used potentiometry with good results.

2.2 Ross Electrodes

As stated in Chapter 1, Ross electrodes were selected for this research because of the reported advantages over conventional reference electrodes. In order to determine if Ross electrodes have performed better in terms of accuracy and precision than either Ag/AgCl or calomel reference electrodes, a thorough literature search was made for any seawater pH measurements made with Ross electrodes. Work by Mackey et al. (1989) is the first reported use of Ross electrodes in measuring seawater pH. This shipboard study utilized the pumping design of Fuhrmann and Zirino (1988) to measure the pH of a constant-flowing stream of seawater. Over the course of a two week cruise, the researchers reported a precision of ± 0.002 pH units and an accuracy of ± 0.008 pH units.

Two groups have used Ross electrodes to make continuous shipboard pH measurements and then used the data in conjunction with salinity-derived TA to calculate *p*CO2. Butler and Mackey (1992) made pH measurements off the southern coast of New Zealand using a setup comprised of a peristalic pump, digital pH meter, flow-cell, pH electrode and temperature probe. The Ross electrodes used in this 2-week cruise were calibrated using tris seawater buffers with a resulting accuracy of ± 0.008 pH units. Salinity was measured by pumping seawater past the conductivity sensor of a thermosalinograph system (Ocean Data TSG-103). The pCO_2 was then calculated from the pH and TA using the dissociation constants of carbonic acid and boric acid given by Edmond and Gieskes (1970). The accuracy of the pCO_2 calculation was checked using two methods. First, the pH system used in the shipboard study was used in a laboratory experiment where the pH of a seawater sample (with a known alkalinity and salinity) was measured. Once the sample equilibrated with air of a known pCO_2, the observed pH was used with the TA to calculate the atmospheric pCO_2. The calculated pCO_2 of 346 μatm was within experimental error of the measured concentration of 349 μatm. The accuracy was also checked periodically during the cruise. Here, a small headspace was allowed to equilibrate with a seawater sample of known pH. The pCO_2 concentration of the headspace was then measured using an infra-red gas analyzer and compared to the calculated concentration. Although the accuracy of the measured pCO_2 was not fully verified, the calculated pCO_2 was at most only 20 μatm lower than the measured concentration.

Copin-Montegut and Avril (1995) also used measured pH and salinity-deduced TA data to calculate pCO_2 during a 1992 cruise near the Canary Islands. The Ross electrodes used in this study had a precision of $\pm$ 0.001 and an accuracy of $\pm$0.010 pH units. Salinity was measured using a shipboard thermosalinometer. The pCO_2 was calculated from pH and TA using the dissociation constants of Goyet and Poisson (1989) for carbonic acid and Dickson (1990) for boric acid. Routine pCO_2 measurements were made using a Maihak Infra-red Analyser and then compared to the calculated pCO_2 for accuracy. The standard deviation between the measured and calculated values was 1.2 μatm for 2283 samples.

Although only a few research groups have used Ross electrodes in seawater pH measurements, the results suggest that Ross electrodes perform better than conventional reference electrodes (e.g. compare to the results obtained by Fuhrmann and Zirino, 1988). The results from these groups also demonstrate the ability of using *in situ* pH and salinity-derived TA measurements to calculate a third carbonate parameter (i.e., pCO_2 or TCO_2).

2.3 *In Situ* Autonomous pH Instruments

Although several commercial instruments have been designed for *in situ* pH measurements, their relatively poor pH accuracy prevents these instruments from being useful for long-term pH studies. The following is a brief overview of these instruments. A multi-parameter instrument has recently been developed by Yellow Springs Instruments (YSI). The YSI 600 is a low-cost sonde which requires an external power supply and data logger and is capable of measuring parameters such as pH, dissolved oxygen, conductivity and temperature. The field-replaceable pH electrodes used in this instrument have a reported precision of ± 0.01 and an accuracy of only ± 0.2.

Sea-Bird Electronics (SBE) has developed a pH sensor (SBE 18) intended as a "bolt-on" auxiliary sensor for the SBE 9 Conductivity-Temperature-Depth (CTD) Underwater Unit. The SBE 18 pH sensor uses a pressure-balanced Ag/AgCl reference pH electrode to measure the pH-dependent voltages. The reported accuracy of the pH measurement is ± 0.1 pH units.

An *in situ* instrument using a spectrophotometric approach to measuring seawater pH has also been developed (Waterbury et al., 1996). This pH sensor consits of two separate flow cells. The first flow cell performs optical measurements on seawater alone (blank), while the second performs measurements on the seawater/indicator (thymol blue) mixture. The upper sealed section of this instrument houses the optics and various electronics while the lower or "wet" section contains the flow cells, dye pump motor and dye bladder. Laboratory experiments used to quantify the precision of the sensor were less than ideal for several reasons. First, it was difficult to maintain a constant pH since the sensor was discharging thymol blue into the test tank. Since tap water was used, CO_2 exchange between the air and water also had an effect on the pH in the thousandths and ten-thousandths of a pH unit range. Nonetheless, the precision was estimated to be within the range of ± 0.0002 to ± 0.0008, which was within the design goal of ± 0.001 pH units. The instrument was field tested in 1996 during a cruise in the South Pacific. The sensor was deployed once a day for a total of 60 casts and the results were compared to shipboard spectrophotometric pH measurements. An accuracy on the order of ± 0.010 was observed for the *in situ* measurements. Although this instrument performed well over short periods of time, the complexity and power requirements may limit its usefulness for long-term pH measurements.

2.4 Chapter Summary

Although the research reviewed in this chapter indicates that spectrophotometric seawater pH measurements may offer better precision and accuracy (Byrne and Breland, 1989; Clayton and Byrne, 1993), we concluded that a potentiometric approach should be reconsidered for several reasons. First, if using multiple glass electrodes and signal averaging would reduce electrode drift, one of the major limitations of pH electrodes would be overcome. Also, work by Dickson (1993b) has improved the accuracy of seawater pH buffers and, in turn, the accuracy of potentiometric seawater pH measurements. These reasons, combined with the fact that several research groups continue to make potentiometric seawater pH measurements with good results, convinced us that an autonomous *in situ* potentiometric seawater pH instrument could be successfully developed.

CHAPTER 3

EXPERIMENTAL

3.1 Chapter Overview

Since the ultimate goal of this research was to make long-term autonomous potentiometric seawater pH measurements, laboratory experiments had to be performed to show that multiple Ross electrodes could provide stable, non-drifting signals over long periods of time. A large amount of preliminary work had to be performed in order to carry out these long-term experiments. The preliminary work included devising a computer interfacing scheme, software development, preparing seawater buffers, measuring electrode slopes and measuring the temperature response of the electrodes. Once this work was completed the long-term experiments were performed. A description of the preliminary work as well as the experimental details of each long-term experiment follow in this chapter.

3.2 Experimental Setup and Electrode Characterization

3.2a Computer Interfacing

The pH measurements were made using a multi-pH meter computer interface (Figure 3-1). The pH meters were controlled by a program written in QuickBasic (Microsoft Corp.). The program code is given in Appendix I. The QuickBasic program was run on an IBM AT personal computer. This computer was interfaced with up to four pH meters and an analog to digital converter (ADC).

Two types of pH meters were used in the multi-meter configuration. One pH meter was a microprocessor based meter (Fisher Scientific, Accumet Model 25) which has dual input channels and is capable of bi-directional communication to a computer via RS-232 ports. This model has two available modes. Single channel mode displays the pH or mV of the selected electrode, while dual channel mode displays the pH or mV of both electrodes simultaneously. The other pH meter was a digital display meter (Fisher Scientific, Accumet Model 10), but it allows only a single electrode input and has no RS-232 communication. Data was collected from the model 10 meters through the analog recorder output and ADC.

All temperature measurements were made with a Yellow Springs Instrument (YSI) model 44031 precision thermistor (resistance 10,000 ohms at 25 °C). The voltages from a resistor bridge were converted to a resistance within the QuickBasic program, and then converted to a temperature using the polynomial regression of a plot of resistance versus temperature (obtained from YSI). An accuracy of ± 0.05 °C was observed for this thermistor.

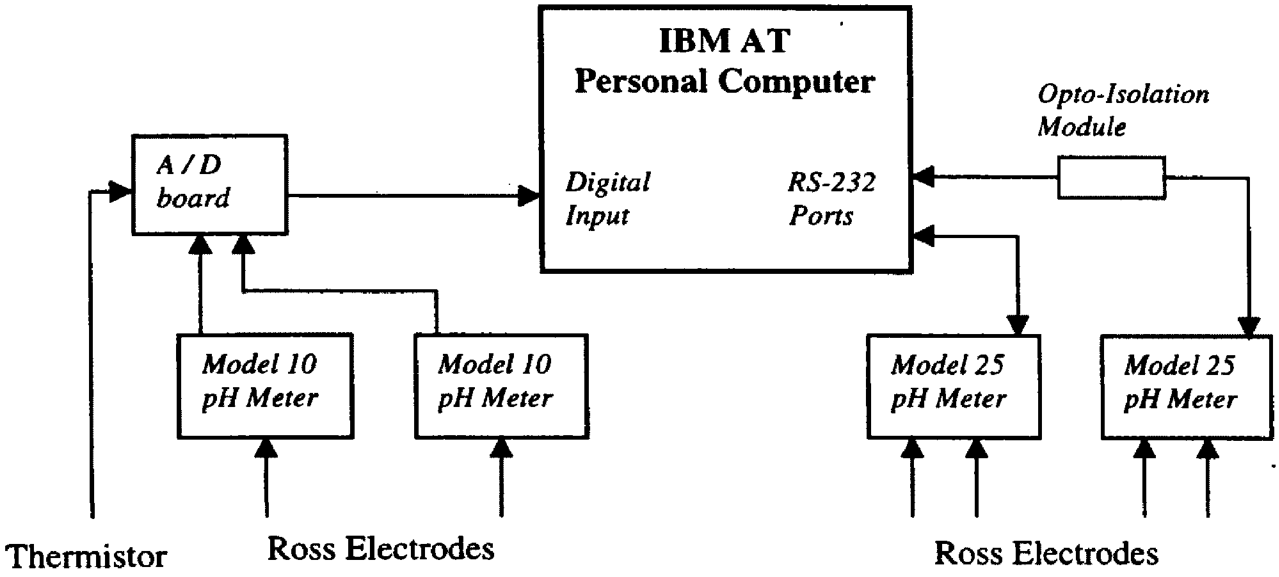

Figure 3-1: Interfacing Scheme

The analog signals from the model 10 pH meters and thermistor were digitized and sent to the computer by an ADC (Keithley model ADC-16). The eight channel ADC board has an input resolution of 16 bits (15 plus a sign bit), a range of ±3.2768 V and a sampling rate of 16 samples/second. The absolute accuracy (at gain=1) of the ADC is ±0.01% of the range and the resolution is 0.1 mV. The resulting digital signal is sent to the computer through a 37-pin D-type connector.

A major obstacle that had to be overcome with this extensive electronic interfacing was ground loops. The ground loops arose from having several electrodes in a single sample, with each electrode attached to the same floating ground. Being on the same ground caused the electrodes to influence one another, leading to noisy and inaccurate potentiometric measurements. Two specific ground loops were identified in the instrument configuration. One ground loop (hereafter referred to as a "local" ground loop) was discovered between two electrodes interfaced to the same model 25 pH meter (see Figure 3-1). In this case, an obvious interference was observed while the meter was in dual channel mode and the two electrodes were in the same solution. If electrode A, for example, was placed in the solution and allowed to stabilize, and then electrode B was introduced into the solution the potential of electrode A would "jump" erratically to ±10-50 mV of the original potential. This altered potential would never stabilize to the original starting potential. The second type of ground loop, hereafter referred to as a "cross" ground loop, occurred when two electrodes in the same solution were connected to separate model 25 pH meters. The characteristics of this ground loop were identical to the "local" ground loop.

Ground loops were eliminated by two techniques. The "cross" ground loop was eliminated by use of an RS-232 optical isolator (Omega Model 268), as shown in Figure 3-1. This opto isolation module provides for a complete full duplex (including control sign) electrical isolation channel between two RS-232 devices. It consists of two DB-25 pin connectors for RS-232 connections and has a data rate of 0 to 19,200 bits per second. This module was placed in-line between one of the model 25 pH meters and the computer, effectively isolating the ground for each of the two pH meters.

Elimination of the "local" ground loops required investigation into the operations of the model 25 pH meters. These meters had initially been used in dual channel mode so that the potential of each electrode could be collected as quickly as possible by the computer (the meter will send the potentials of both electrodes with a single command in dual channel mode, as opposed to several commands in single channel mode). However, through communication with the manufacturer of these meters (Denver Instruments) it was discovered that in dual channel mode the electrodes are on the same floating point ground, thus leading to the observed ground loop. In contrast, the two electrodes are electronically isolated from one another in single channel mode. The electrodes were then tested in single channel mode, and no ground loop effect was observed. The QuickBasic program was modified accordingly to read the electrode potentials with the meters in single channel mode.

3.2b Buffer Preparation

The calibration buffers used in this research were adopted from the recipes given by Dickson (1993b). These recipes use synthetic seawater of salinity 35.0 ppt buffered with one of four possible buffering agents. These four are 2-aminopyridine, 2-amino-2-hydroxymethyl-1,3-propanediol (tris), tetrahydro-1,4-isoxazine (morpholine) and 2-amino-2-methyl-1,3-propanediol (bis). The pH (at 25 °C) of each of these buffering agents in the synthetic seawater is 6.770, 8.073, 8.573 and 8.810, respectively.

Tris and bis buffers were used in this research. Artificial seawater buffered with tris was used as the sample for long-term experiments because the pH of this buffer (8.073 at 25°C) falls well within the expected pH range of most surface seawaters (7.8-8.2). Tris and bis were used to determine the slopes of the electrodes.

Dickson's buffers were used for several reasons. First, these buffers are based on the total hydrogen ion concentration scale. Since Dickson had previously (1993b) set this scale as the universal scale on which all seawater pH measurements should be based, using these buffers seemed to be the most logical choice. Also, Dickson (1993b) has reported the pH of these buffers as a function of temperature and salinity. Thus, the accuracy of the measurements can be checked at any temperature using a sample of carefully prepared buffer which has been sealed from the atmosphere.

Table 1 lists the constituents used in the synthetic seawater buffers. In preparing the buffers, 1000 mg of deionized (DI) water was weighed into a 1 L borosilicate glass bottle. This water was then degassed using N_2 and capped securely to prevent any CO_2 transfer into the solution. The salts were then dried at approximately 130 °C for two hours and allowed to cool in a dessicator. Each salt was weighed out and dissolved in the 1 L of DI water. The appropriate buffer (tris, bis or $NaHCO_3$) was then weighed out and added to the salt solution. Finally, 40.40 mL of 0.990 N HCl were added to the tris and bis to adjust the buffers to the proper pH.

***Table 1:* Synthetic seawater buffer recipe based on 1 kg H_2O(Dickson, 1993b)**

Constituent	Moles	Weight (g)
NaCl	0.4246	22.6457
KCl	0.0106	0.7884
$MgCl_2$	0.0547	5.2123
$CaCl_2$	0.0108	1.5805
Na_2SO_4	0.0293	4.1563
Buffer (tris or bis)	0.0800	-

3.2c Spectrophotometric Accuracy Checks

In addition to using a preserved buffer, spectrophotometric measurements were used to check the accuracy of the electrodes in the long-term pH evaluations. The spectrophotometric pH measurements were made with a UV\VIS spectrophotometer (Perkin Elmer Lambda 11) and a 10 cm quartz cylindrical cell (Starna Cells, Inc.). Constant temperature was maintained (± 0.02 °C) with a microprocessor-based water bath (Neslab RTE-111). A 2.0 mM *m*-cresol purple solution (introduced by Clayton and Byrne, 1993) was used as an indicator. The pH values measured by this method were based on the total hydrogen ion concentration scale, which was consistent with the tris-bis buffer scale.

The spectrophotometric accuracy checks were made in the following manner: (1) the 10 cm cell was filled with approximately 30 mL of sample (no headspace) and placed in the constant temperature water bath; (2) after temperature equilibration, the cell was removed from the water bath, quickly dried and placed in the spectrophotometer; (3) a blank scan was made over a range of 430 to 735 nm; (4) the cell was removed from the spectrometer and a small volume (~1 mL) was poured out of the cell; (5) 80 μL of the indicator was added to the cell; (6) the cell was then "topped off" with the sample solution (leaving no headspace), capped, and mixed; (7) the cell was placed back in the water bath; (8) after allowing the temperature to equilibrate again, the cell was removed from the bath, quickly dried and placed in the spectrophotometer; (9) absorbances at 434, 578 and 730 nm were then recorded. The pH was calculated using the absorbance ratio of the acid and base forms of the indicator in combination with the equations of Clayton and Byrne (1993).

3.2d Electrode Slope

The response of each electrode, the change in electrode potential per unit pH change, was determined experimentally using a 2 point calibration. The calibration buffers, tris and bis, have a pH of 8.071 and 8.808 at 25 °C, respectively (Dickson, 1993b). The procedure for this 2 point calibration was quite straightforward. The buffers were kept at 25 °C ±0.02 °C and well stirred in an external water bath. Each electrode was then placed in the buffers and the potential of each electrode was measured. A plot of electrode potential vs. pH was constructed for each electrode, with the slope of this line being the pH response of the electrode (Figure 3-2). Table A2-1 in Appendix II lists the slope for each electrode used in this work.

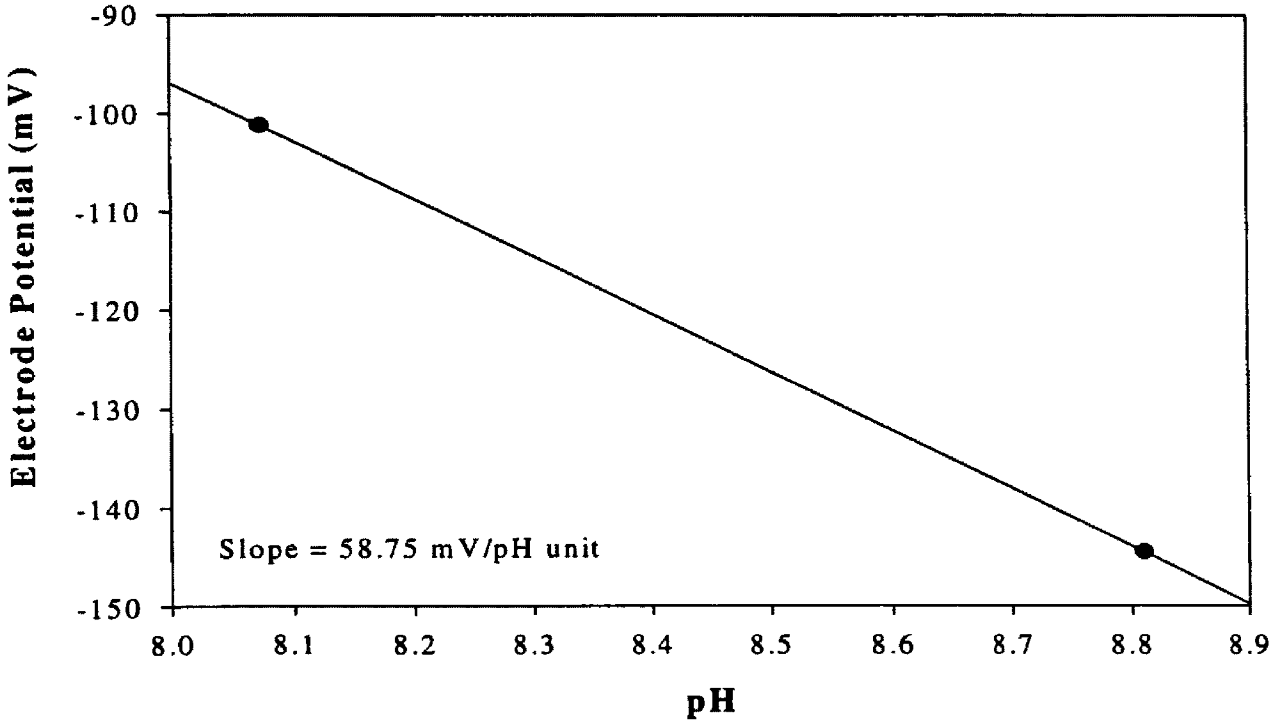

Figure 3-2: Example of slope determination for a Ross electrode

The reproducibility of the slope determinations was tested using a single electrode. In this experiment, an aliquot of fresh tris and bis was placed in an external water bath which was maintained at a constant temperature of 25 °C. The electrode was then placed in each buffer and the corresponding potential was recorded. This procedure was repeated six times over the course of an eight hour period, with a fresh aliquot of the buffers being used each time. Figure 3-3 shows the results for this experiment. From this plot, it is obvious that the slope determinations are quite reproducible. All but the second measurement had an identical slope of 58.62 mV/pH unit.

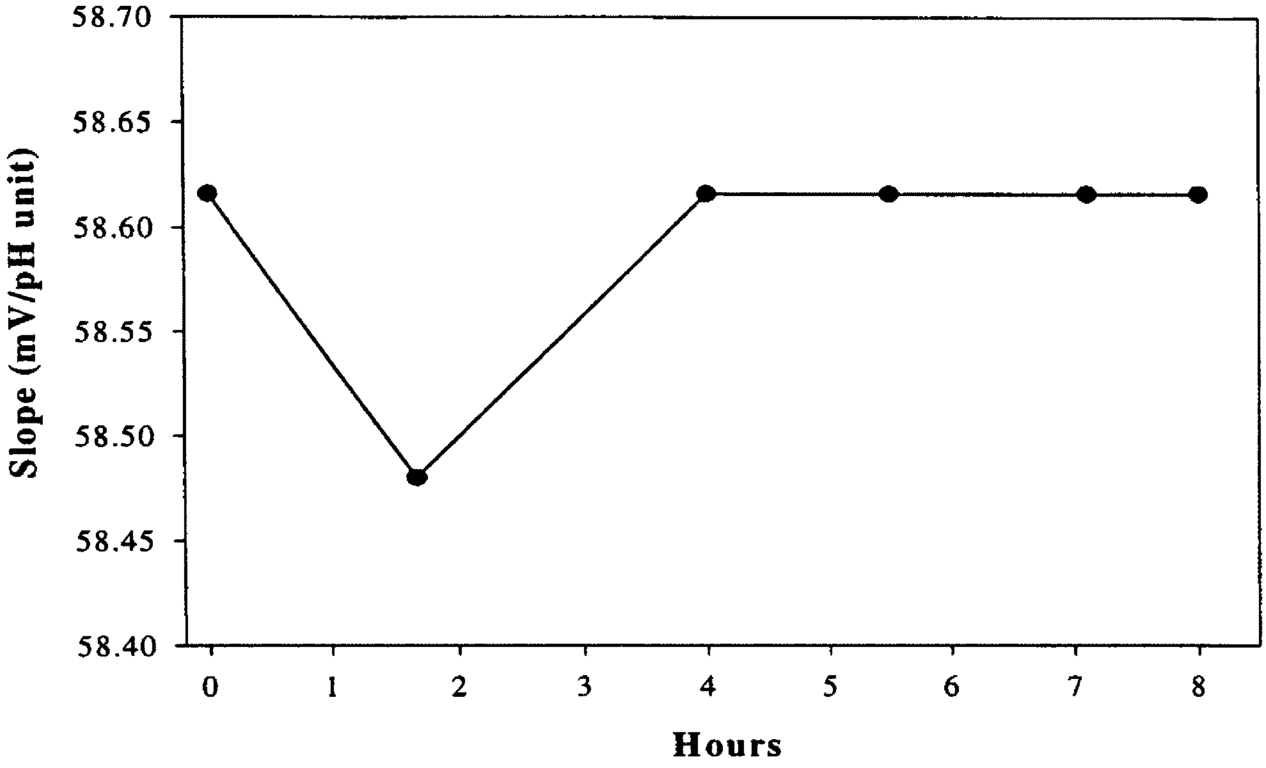

Figure 3-3: Reproducibility of slope determinations using electrode E5

The long-term change in the slope of electrode E5 was also monitored. As a new electrode, the slope was found to be 59.29 mV/pH unit. Ninety-seven days later the slope had dropped to 58.75 mV/pH unit. After 112 days, the slope had dropped to 58.62 mV/pH unit. These results correspond to a drop in the slope of electrode E5 by approximately 0.006 mV/pH unit per day. In order to determine if a change of 0.006 mV/pH unit per day in the slope of an electrode could cause an error in pH, the role of electrode slope in Equation 6 (Section 1.2) must be examined. In this example, the most extreme slopes of electrode E5 (59.29 and 58.62 mV/pH unit) will be used for comparison. Assuming that the temperature is the same for both the calibration and the hypothetical pH measurement, T/T_c would be equal to one, leaving the electrode slope (K) and the potential difference (E_s-E_x) as the only variables in Equation 6. It must be determined from this equation at what potential difference the drift in electrode slope begins to become important.

If the potential change was 1 mV, the change in pH using 59.29 mV/pH unit (hereafter referred to as slope 1) would be 0.0168 pH. Using 58.62 mV/pH unit (slope 2), the change in pH would be 0.017 (which is the same as 0.0168 within the accuracy of the pH meter). A 3 mV change would also yield an identical pH change (0.051) when using either slope. A potential change of 6 mV yields a pH change of 0.101 for slope 1, and a change of 0.102 for slope 2. For a 10 mV change, the pH change for slope 1 is 0.169 and 0.171 for slope 2. Thus it can be concluded that at small potential changes (i.e. 1-3 mV), a slowly drifting slope of the magnitude found in this research will not affect the pH calculations. At larger potential changes (i.e. 4-10 mV), the pH calculations will be affected minimally (0.001-0.002 pH units).

3.2e Temperature Compensation

Equation 6 shows that when calculating the pH of a sample, the electrode potentials measured in both the buffer and the sample must be at the same temperature. In the experiments described here, however, the temperature was not the same for the sample and the accuracy checks because the accuracy checks were made at a single, constant temperature while the sample fluctuated with the temperature of the laboratory. Thus, a method for correcting for temperature differences between sample measurements and calibrations had to be utilized. The following equation, obtained from Fuhrmann and Zirino (1988), enables pH calculations when the sample and calibration temperatures are different:

$$pH_x(t) = pH_s(t) + \frac{E_s(t) - E_x(t)}{KT/T_c} \tag{11}$$

where $pH_x(t)$ = pH of the sample at temperature t, $pH_s(t)$ = pH of the buffer, t = sample temperature (in °C), $E_x(t)$ = electrode potential of the sample, $E_s(t)$ = electrode potential of the calibration buffer, T = sample temperature (in Kelvin), T_c = calibration temperature (in Kelvin) and K = slope of the electrode at T_c.

The electrode potential of the buffer at calibration temperature is corrected to the sample temperature using Equation 12:

$$E_s(t) = E_s(t_c) + f_s \times [t - t_c] \tag{12}$$

where $E_s(t_c)$ = electrode potential of the calibration buffer at T_c, f_s = temperature response of the electrode in the calibration buffer and t_c = calibration temperature (in °C).

3.2f Electrode Temperature Response

In order to make the temperature correction of Equation 12, the temperature response (f_s) of each electrode in a given buffer had to be determined experimentally. For this experiment, a beaker containing approximately 100 mL of a given buffer was placed in an external water bath and constantly stirred. After the initial temperature was reached, the potential of each electrode in the buffer was recorded. The electrodes were then removed (in order to minimize the amount of filling solution which flowed into the buffer) and the temperature was increased by 2 °C. The electrodes were then placed back into the buffer, allowed to stabilize, and recorded once again. This process was continued every 2° over the range of 15-29 °C. A plot of potential versus temperature was then constructed for each electrode. Figure 3-4 is an example plot of the results that were obtained. In this case, the temperature response of electrode E5 in the bicarbonate buffer was found to be 0.6523 mV/°C.

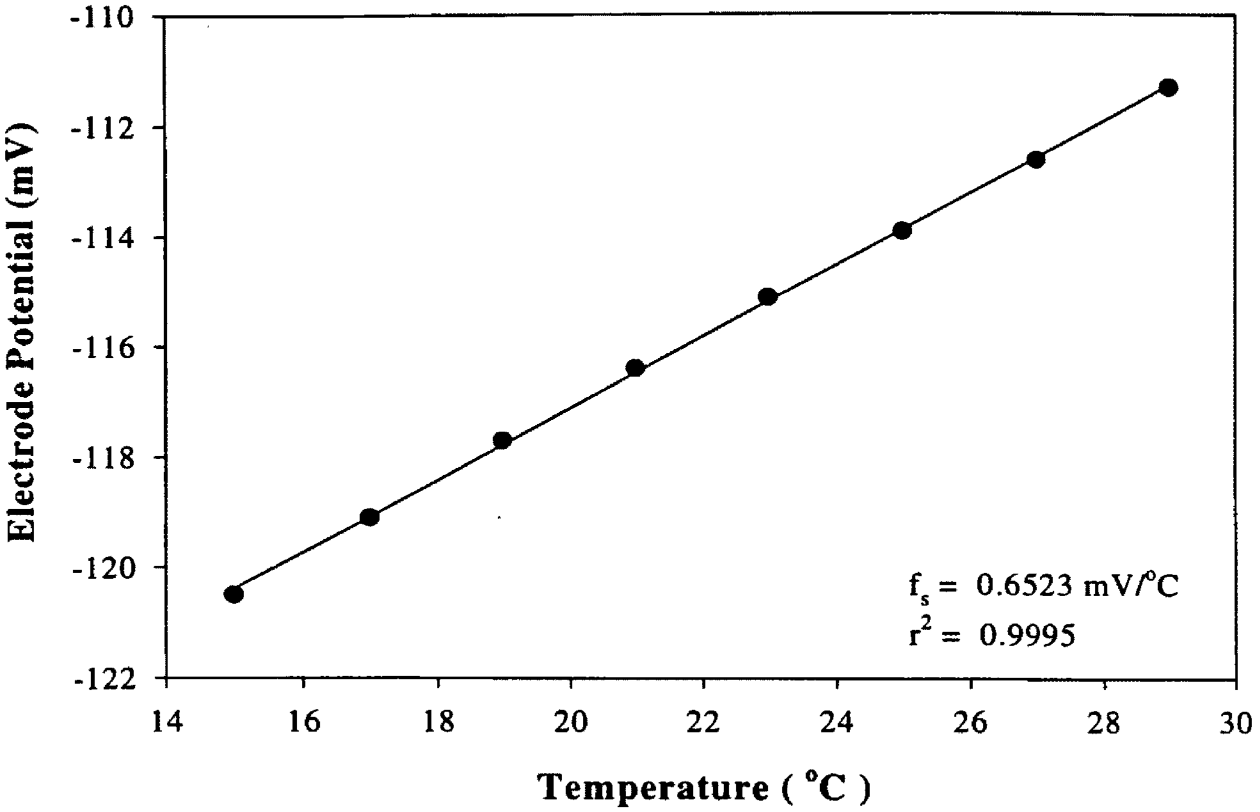

Figure 3-4: Temperature response of electrode E5 in the $NaHCO_3$ buffer

3.3 Overview of the Experiments

The results of this research are comprised of five main data sets. The first three data sets are the sequential results obtained from experiments in which the long-term drift of the electrodes was monitored. The first data set, hereafter referred to as the tris experiment (Section 4-1), consisted of four electrode measurements of artificial seawater buffered with tris-(hydroxymethyl) aminomethane. This experiment was terminated after eleven days because the tris buffer was interfering with the performance of the electrodes. Tris was subsequently eliminated as a buffering agent. The second data set, hereafter referred to as the first bicarbonate experiment (Section 4-2), used the same electrodes as the tris data set, but $NaHCO_3$ was substituted for tris as the buffer. This experiment was much longer in duration than the tris experiment, but the results were less than ideal in terms of accuracy and precision most likely due to damage of the electrodes from the tris buffer. The third long-term drift experiment, the second bicarbonate experiment (Section 4-3), also used $NaHCO_3$ as the buffer, but the old electrodes were replaced with six new electrodes and the results were much improved. The fourth data set (Section 4.4) was an experiment which compared the performance of the Orion Ross electrodes with conventional electrodes. In this experiment, four Ag/AgCl reference electrodes were used to measure the pH of the identical media used in the previous two experiments. The fifth data set (Section 4.5) was an experiment to test the performance of the Ross electrodes in a freshwater (low ionic strength) system. Once these experiments were completed, a prototype electronic circuit board for an autonomous instrument was built and tested. These results are given in Appendix III.

3.3a Tris Experiment

Four electrodes, referred to as E3, E4, E5 and E6, were used in this experiment. E3 and E4 were Orion Ross model 81-15SC epoxy body electrodes. These semi-micro electrodes have a glass fiber junction and are approximately 165 mm in length. E5 and E6 were Orion Ross model 81-62SC glass body electrodes. These electrodes differ from the 81-15SC in that they have a ceramic junction and are shorter (about 120 mm) in length. For the initial calibration, the electrodes were first placed in a beaker containing about 100 mL of freshly prepared tris buffer. After allowing the electrodes to stabilize for approximately one minute, the potential of each was recorded at the calibration temperature (21.0 °C). At 21.0 °C the pH of the tris buffer is 8.199 (Dickson, 1993b), thus the corresponding potential for each electrode was assigned this pH value.

Once the electrodes were calibrated, they were suspended in a beaker containing about 150 mL of continuously-stirred tris seawater. The QuickBasic program and computer recorded the potential of each electrode, as well as the temperature, every 15 minutes over an 11 day period. The accuracy of the electrodes was checked by periodically measuring the potential of a "preserved" aliquot of tris seawater buffer. The preserved aliquot came from a 1 L bottle which was isolated from both the atmosphere and light. It was assumed that the pH of this preserved tris was constant, meaning the potential measured by each electrode at any time should also remain constant. Any significant deviation from this constant potential would indicate that the electrode was drifting. Since the initial calibration was made at 21 °C, subsequent accuracy checks were corrected to 21 °C using Equation 11.

3.3b First Bicarbonate Experiment

Since, as will be discussed in Section 4.1, it was determined that tris might be interfering with the performance of the electrodes, a new buffering agent was used for all subsequent long-term pH experiments. The 0.080 M tris buffer was replaced with 2.0 mM $NaHCO_3$ (which is more representative of seawater). Additional NaCl (~0.6 g) was added to the artificial seawater in order to maintain a salinity of 35. The pH was adjusted to near 8 .0 with a small addition of 0.1 M HCl.

Electrodes E3, E4 and E5 were used again in this drift study, while E6 was replaced with electrode E8-CP. E8-CP is a Cole-Parmer Model 55520-06 epoxy body electrode. This electrode has a dual chamber design which essentially isolates the inner AgCl reference half-cell from the outer KCl solution and, similar to the Ross electrodes, prevents clogging of the ceramic junction by eliminating reactions between ions in the sample and the Ag ions in the electrolyte. This electrode was used in order to compare its performance with that of the Ross electrodes.

Since the pH of the $NaHCO_3$ buffer was not known as a function of temperature, a new approach for checking the accuracy of the electrodes was developed. This approach consisted of sampling the test solution and determining the pH colorimetrically, as described in Section 3.2c. The accuracy was evaluated by comparing the average pH of the electrodes to the pH measured colorimetrically.

In this experiment, the four electrodes were placed in a beaker containing approximately 250 mL of the artificial seawater (S=35) buffered with 2.0 mM $NaHCO_3$. For the initial calibration, an aliquot of seawater was taken from the sample and the pH was determined spectrophotometrically using an *m*-cresol purple indicator solution. After the initial calibration, the QuickBasic program was started and the potential of each electrode, as well as the temperature, was recorded every 20 minutes over a 54 day period. The pH's were calculated at the end of the experiment based on the change in potential from the initial calibration (using Equation 11). Ten colorimetric accuracy checks were made over the duration of this experiment.

3.3c Second Bicarbonate Experiment

Based on the results of the first bicarbonate experiment, which are presented in Section 4.2, the electrodes were malfunctioning and presumably failed to accurately represent the true capabilities (in terms of accuracy and precision) of Ross electrodes. In order to accurately assess whether or not the Ross electrodes are less susceptible to long-term drift than conventional electrodes, a drift study using new Ross electrodes needed to be undertaken. Communication with Orion Research Inc. ultimately resulted in the donation of six Ross model 81-02SC electrodes to this research project. These new electrodes were then used for another long-term drift experiment (the second bicarbonate experiment). Here, the methods and media used were the same as those used in the first bicarbonate experiment, except that the potentials of the six electrodes were measured every 30 minutes over a 56 day period (rather than every 20 minutes over 54 days).

3.3d Conventional Electrode Experiment

In order to determine if the Ross electrodes perform better than other types of glass electrodes, an experiment using conventional combination glass Ag/AgCl electrodes was carried out for comparison with the Ross electrodes. In this experiment four electrodes (Fisher Cat No. 13-620-285) were placed in a beaker containing approximately 250 mL of synthetic seawater buffered with 2.0 mM $NaHCO_3$. The QuickBasic program was started and an initial colorimetric measurement was taken. The change in potential for each electrode was then collected every 15 minutes over a 7 day period and the resultant pH's were calculated using Equation 11.

3.3e Freshwater (Low Ionic Strength) Experiment

The purpose of this experiment was to investigate the performance of Ross electrodes in a low ionic strength system. Here, four electrodes from the second bicarbonate data set were used (#2929, #2876, #2901 and #1823) along with two new electrodes (#4051 and #4034). The six electrodes were placed in a beaker containing approximately 300 mL of water obtained from Placid Lake, Montana, and the potentials of each were recorded every 10 minutes over 7 days.

CHAPTER 4

RESULTS

4.1 Tris Experiment

Figure 4-1 is a plot of the four pH signals and the temperature measured over the 11 day period of this experiment (experimental details are given in Section 3.3a). The large oscillations in both the pH and temperature are due to diurnal heating and cooling.

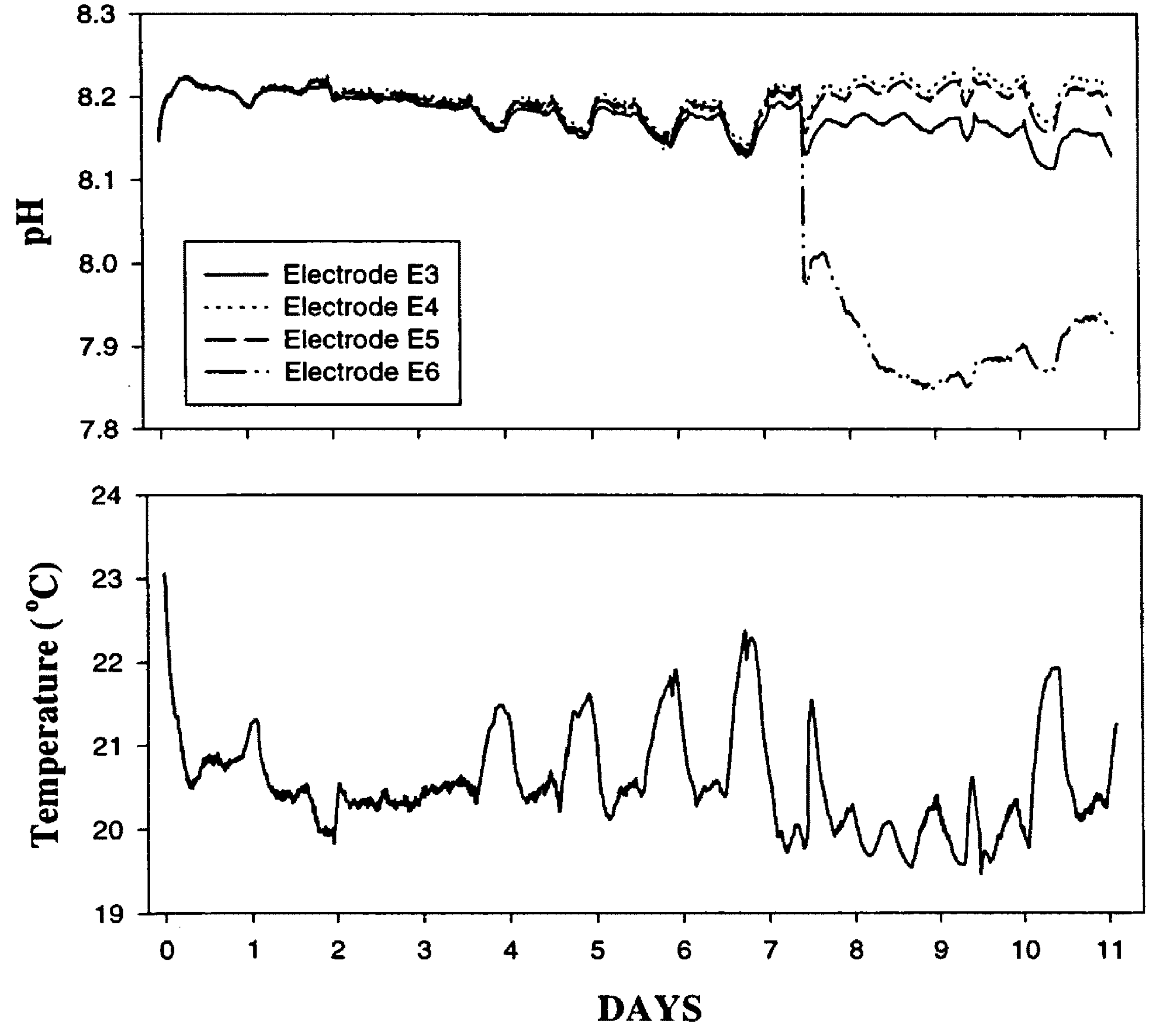

Figure 4-1: Four pH signals (top) and temperature (bottom) measured for the tris experiment

After day 7 of this experiment it was noticed that electrodes E3 and E6 were beginning to drift (according to the accuracy checks) and the filling solution was no longer flowing out of these electrodes. Thus, an attempt was made to unclog the liquid junction of these electrodes in hopes of improving their performance. As recommended by Orion, these two electrodes were placed in a hot (70 °C) 0.1 M KCl solution and allowed to soak for approximately 20 minutes. Once they were placed back in the artificial seawater, however, the electrodes did not return to their original potential (the potential before they were soaked in the KCl), resulting in a large offset in the pH measurement (Fig. 4-1, day 7.5). In addition, this attempt at unclogging the liquid junction of E3 and E6 did little to improve the performance of these electrodes. No apparent improvement was observed in either the electrode drift or flow of the filling solution.

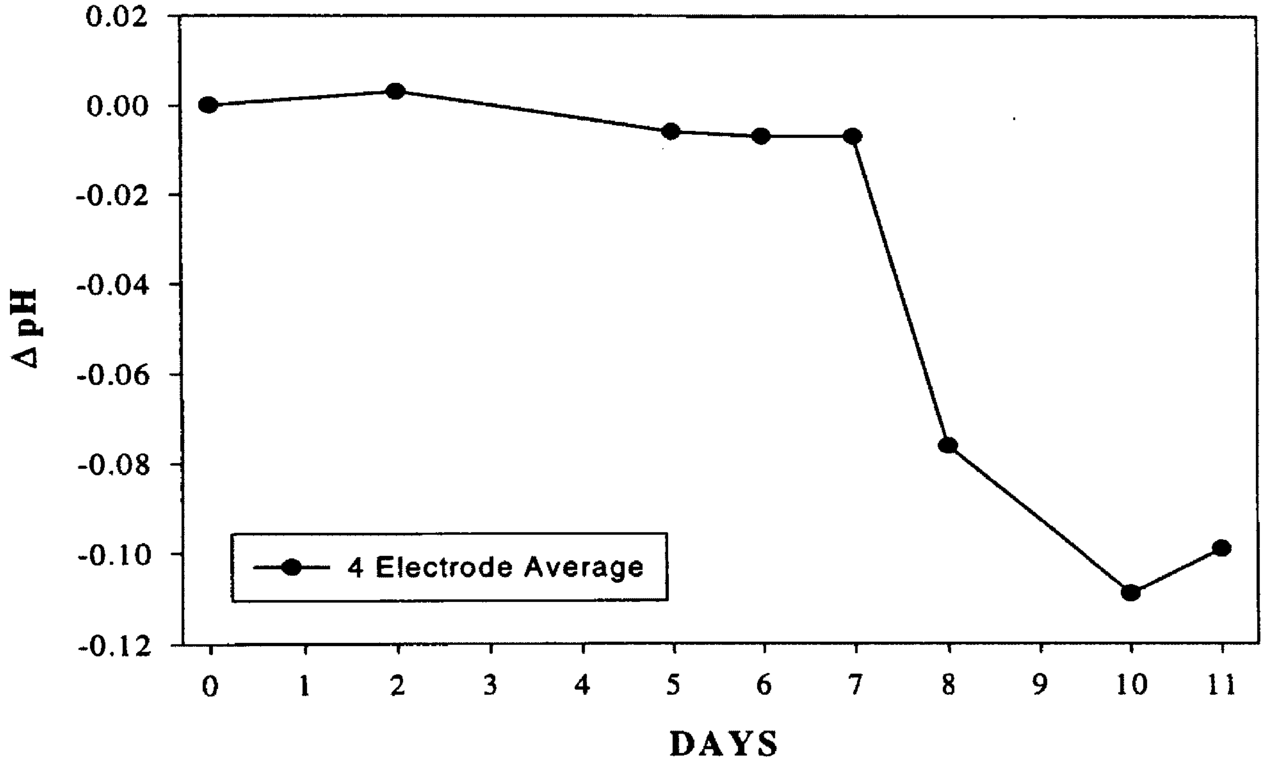

Figure 4-2: Deviation of the average pH from the "preserved" tris seawater buffer (tris data set)

Around day 10 it was noticed that a large amount of fibrous material was floating at the top of the seawater sample. A close inspection of the electrodes revealed that the glass fiber junctions on electrodes E3 and E4 were deteriorating or being "eaten" away. Electrodes E5 and E6, which have ceramic junctions, showed no sign of deterioration.

Since no other reasonable explanation could be made regarding the cause of this deterioration, it was hypothesized that tris was the source. Although electrodes E3 and E4 were performing well up through day 10, the experiment was nonetheless stopped because degradation of the liquid junction would prevent an accurate assessment of the performance of these electrodes.

Figure 4-2 is a plot of the accuracy checks made using aliquots of the "preserved" tris buffer. By day 7 the electrodes had drifted –0.007 pH units away from the initial calibration, due mostly to the drift of electrodes E3 and E6. The large pH offset at days 8-11 is due to the attempt at unclogging the junctions of electrodes E3 and E6. By day 11, the average pH of the four electrodes had deviated by –0.10 pH units from the initial calibration.

Figure 4-3 is a plot of the standard deviation (SD) of the four electrode signals. This plot shows the precision of the average (4 electrodes) pH measurement. Up to day 7, the precision was ± 0.010 pH units. After the attempt at unclogging electrodes E3 and E6, however, the standard deviation rose to a maximum of ± 0.17 pH units on day 9. By the end of the experiment the SD had dropped slightly to ± 0.13 pH units.

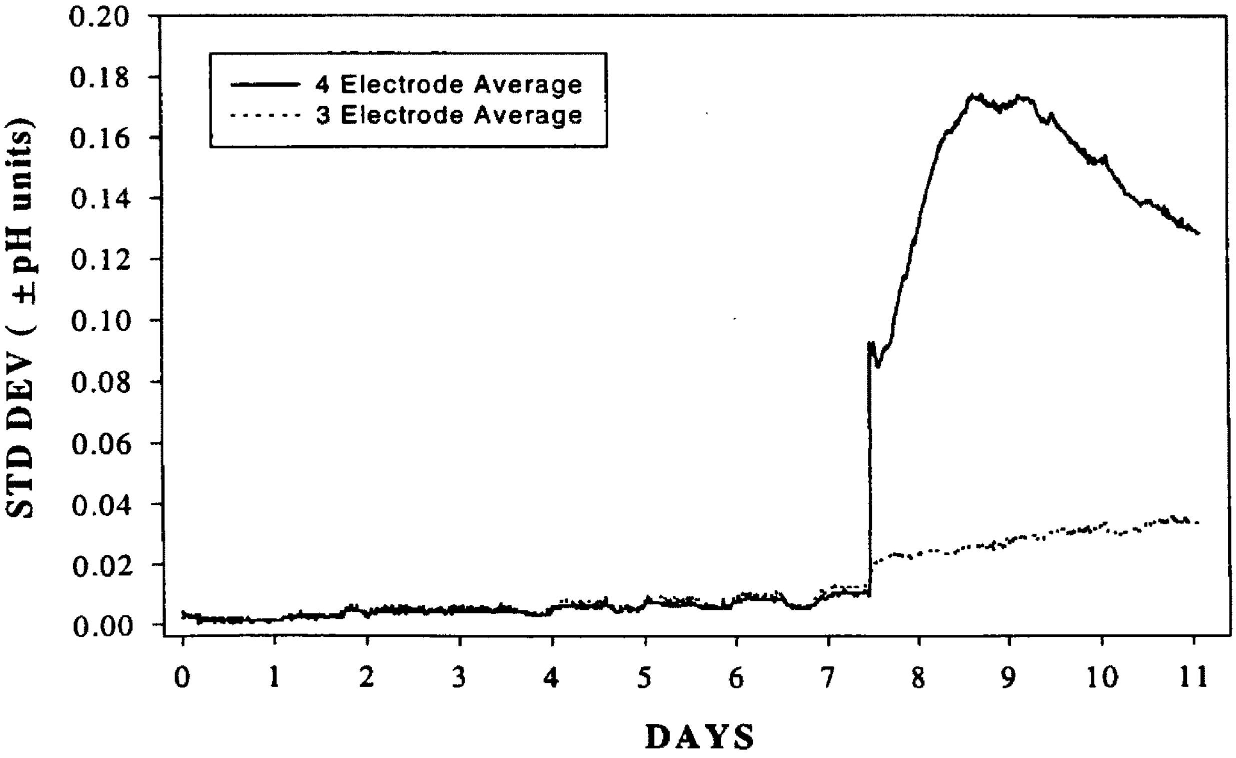

Figure 4-3: Standard deviation of the three and four electrode averages (tris data set)

Although the attempt to revive electrodes E3 and E6 resulted in less than ideal results, the experiment was still beneficial in that it illustrated the advantages of using multiple electrodes and outlier detection. From Figure 4-1 it is obvious that E6 has deviated significantly from the other three electrodes. This electrode was subsequently eliminated from the average in order to improve the accuracy and precision of the measurements. The American Society for Testing Materials (ASTM) T_n test (Mandel, 1979) was used to statistically verify that electrode E6 could be eliminated from the data set. By day 8 of this experiment, electrode E6 was an outlier and could be eliminated from the data set with 99% confidence.

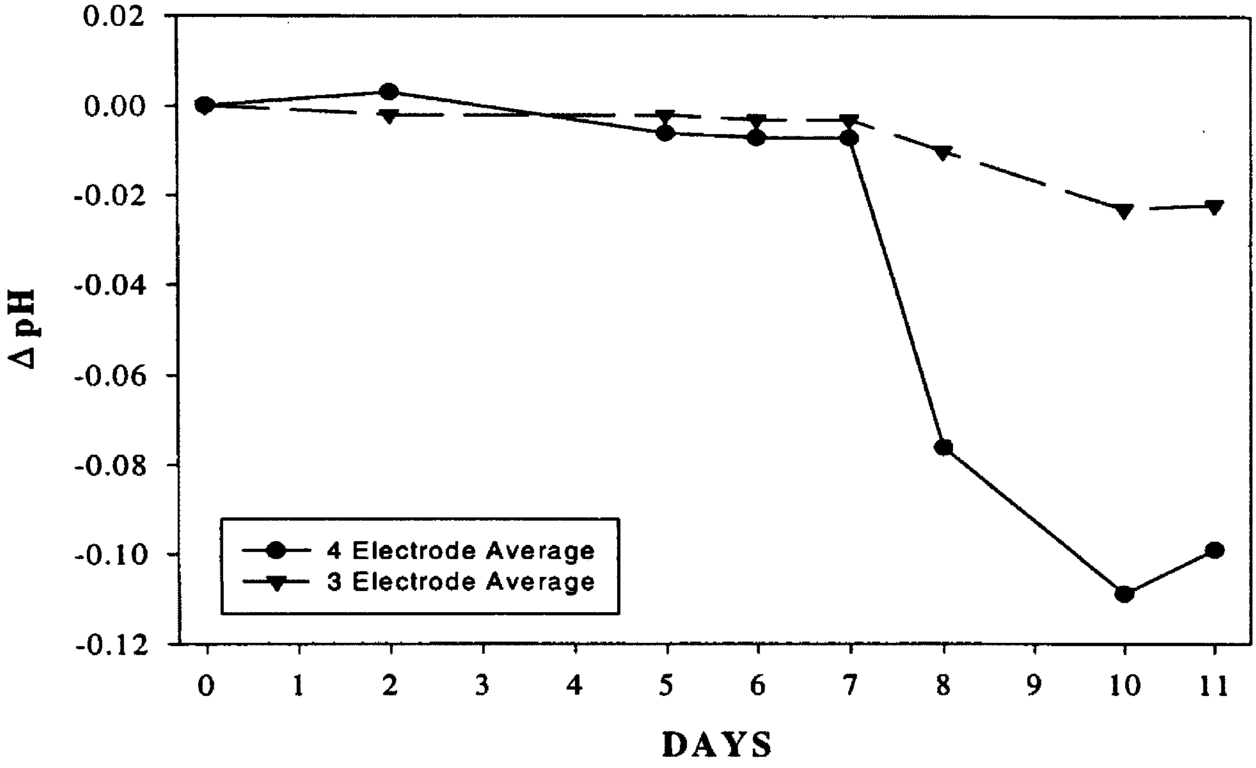

Figure 4-4: Accuracy improvement after eliminating E6 (tris data set)

The removal of electrode E6 greatly improved both the precision and accuracy of the average pH. Figure 4-4 is a plot comparing the deviations from the "preserved" tris seawater buffer of the 3 and 4 electrode averages. At day 8, the deviation of the 3 electrode average was –0.010 pH units, an accuracy improvement of 0.066 pH units over the 4 electrode average. The accuracy check at day 11 showed a 0.077 pH improvement after eliminating E6. The improvement in precision after eliminating E6 is illustrated by Figure 4-3. At day 7.5, the improvement was 0.075 pH units. At day 11 the improvement was 0.095 pH, resulting in a final precision of ± 0.033 pH units for this experiment.

4.2 First Bicarbonate Experiment

Since the tris experiment indicated that tris might deteriorate the glass fiber junctions of the electrodes, a new experiment using a $NaHCO_3$ buffer was performed.

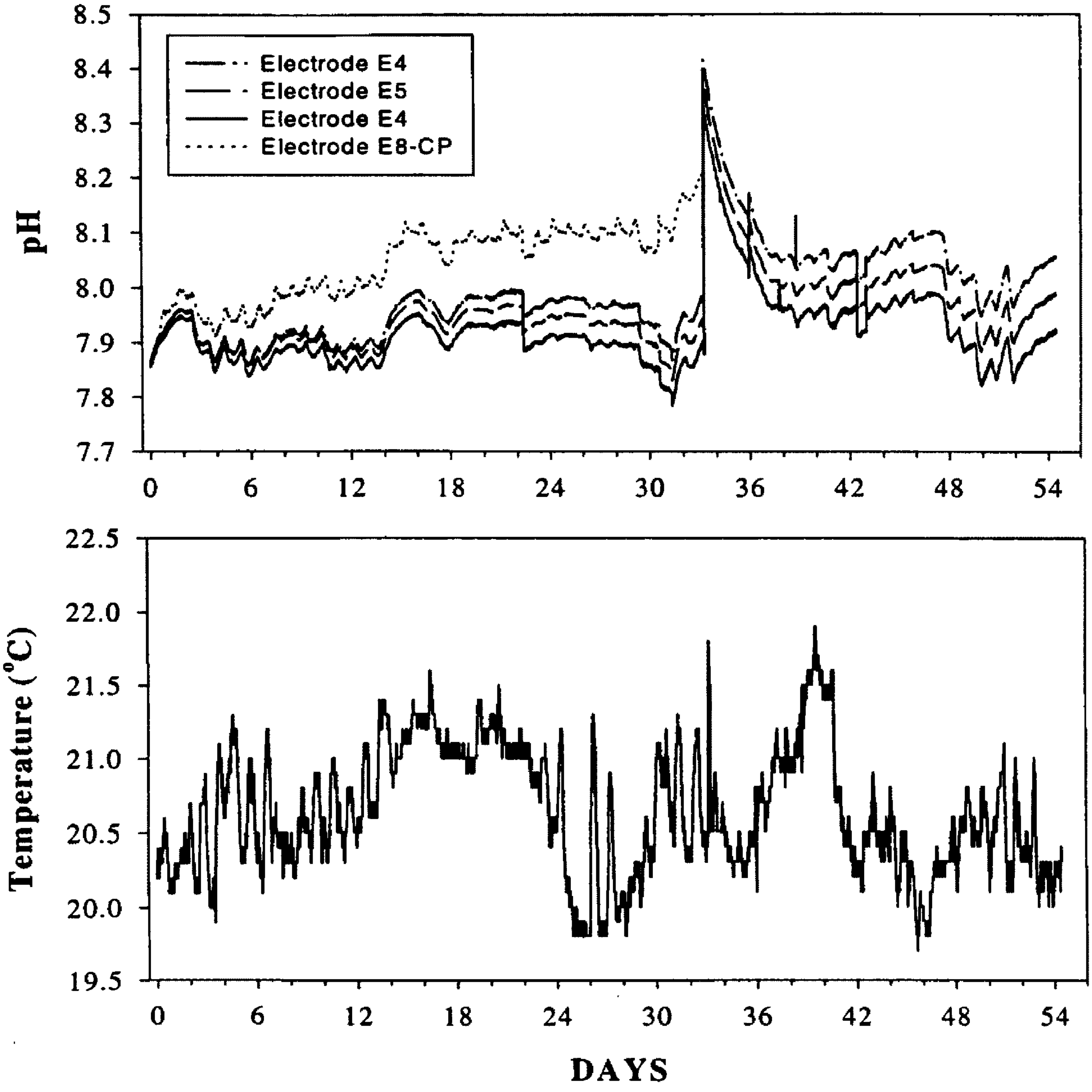

Figure 4-5: Four electrode signals (top) and temperature (bottom) of the first bicarbonate experiment

Experimental details are given in Section 3.3b. Within the first 33 days of this experiment, it was noticed that electrode E8-CP was drifting significantly from the other three electrodes (Figure 4-5). By day 16, E8-CP had drifted 0.120 pH units away from the average of the remaining three electrodes. By day 33 the deviation increased to +0.260 pH units. Periodic visual inspections of electrode E8-CP indicated that the filling solution was flowing much slower compared to the other three electrodes. Since electrode E8-CP was obviously an outlier, it was subsequently removed from the beaker and no longer used for the remainder of the experiment. Although the three remaining electrodes drifted away from one another through the last 21 days of the experiment, no electrode drifted significantly enough from the others to be classified as an outlier.

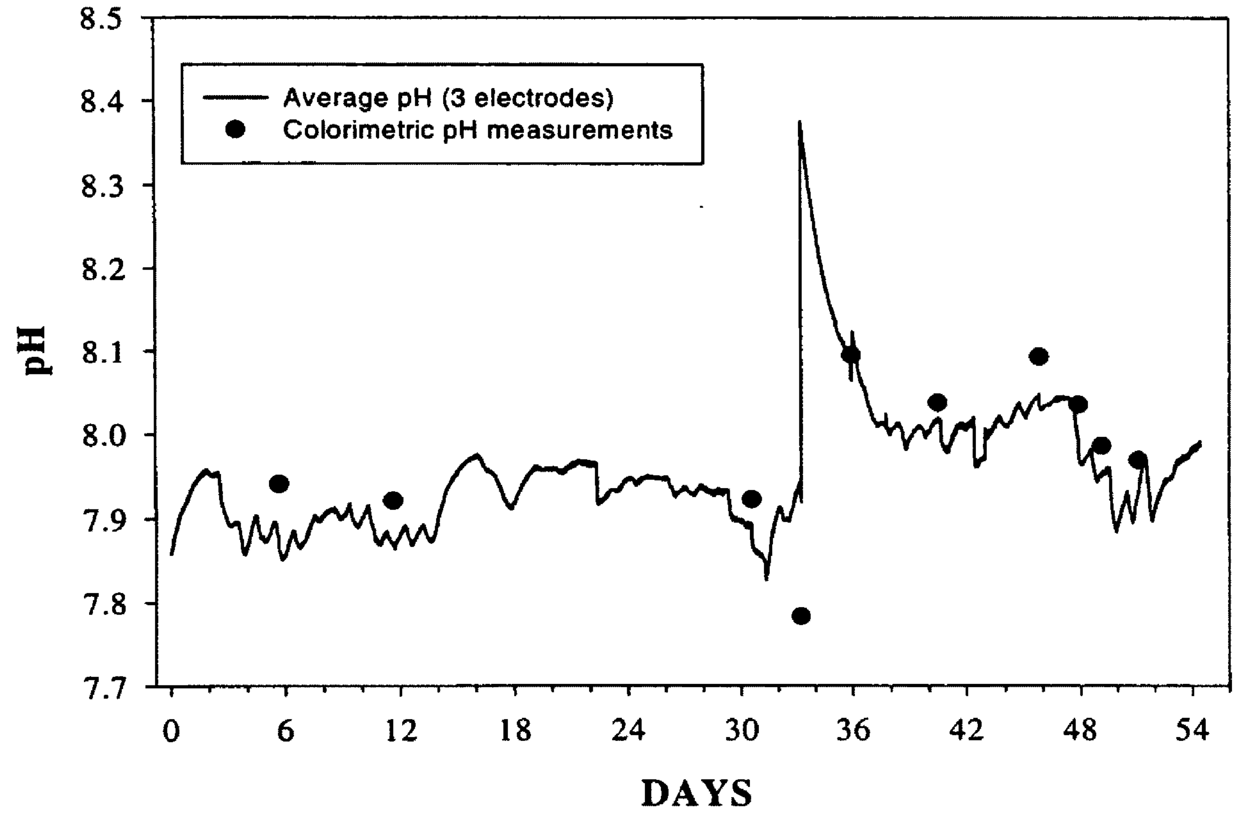

Figure 4-6: Average pH and colorimetric accuracy checks of the first bicarbonate experiment

Figure 4-6 is plot of the average pH measurement (3 electrodes), the colorimetric accuracy checks and the temperature recorded over the entire 54 days of this experiment. From this graph it is apparent that the average pH measurement did not match up well with the colorimetric accuracy checks. At day 6, the first accuracy check yielded a difference of -0.065 pH units between the average pH and colorimetric pH. By day 49, the difference was +0.014 pH units. The differences over the entire experiment are given in Figure 4-7.

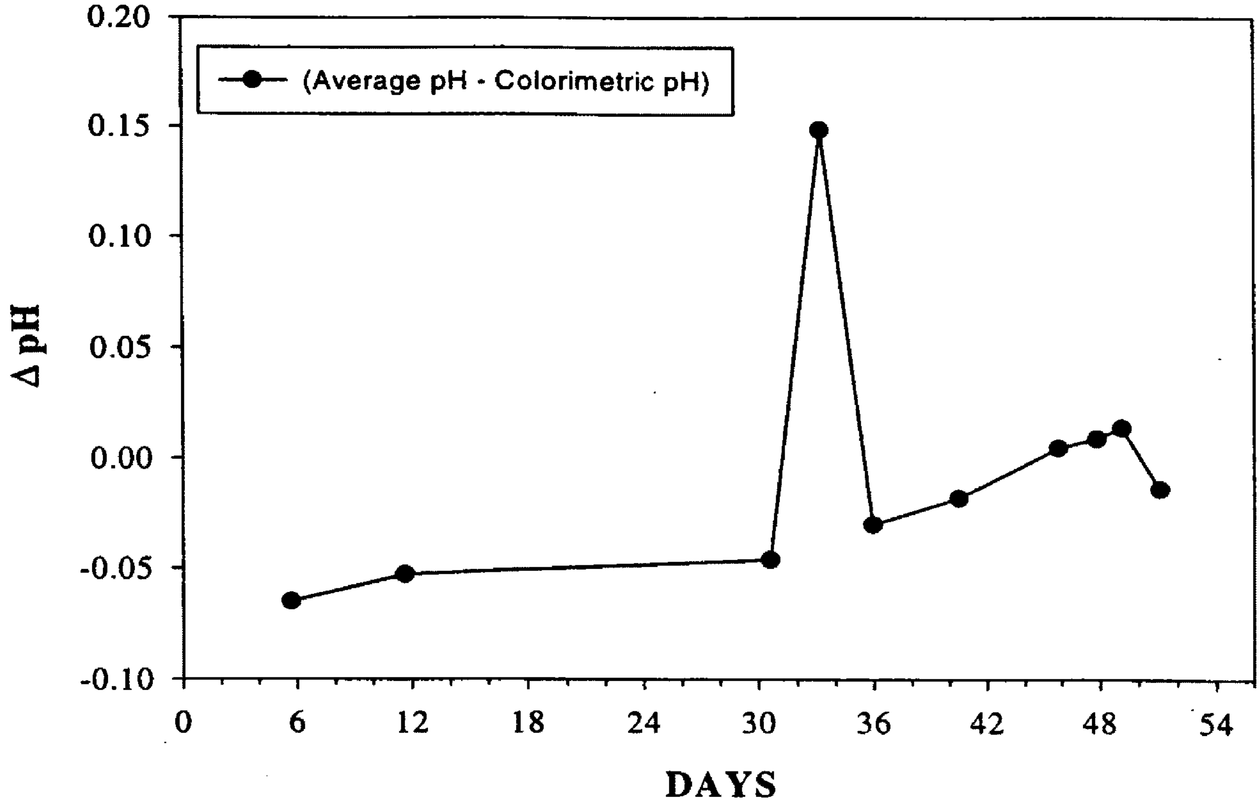

Figure 4-7: Difference between the 3 electrode average pH and the colorimetric accuracy checks for the first bicarbonate experiment

Figure 4-7 shows that the difference between the average pH and colorimetric accuracy checks is decreasing over the course of this experiment. At the outset the average pH is considerably lower than the colorimetric pH, but the difference begins to decrease. The two methods show the best agreement at day 45 when the difference is only +0.005 pH units. However, the difference begins to increase after this point (by day 49 it had increased to +0.014 pH units). The outlier at day 33 is most likely the result of experimental error with the colorimetric pH measurement. Possible sources of this experimental error will be discussed in the following section.

Figure 4-8 is a plot of the standard deviation of the 3 signals for this 54 day experiment. This plot gives a good indication that the electrodes are drifting due to the linear increase in standard deviation over the entire experiment. At day 10 the standard deviation is already 0.015 pH units, and it continues to grow larger with a final value of 0.068 pH units at day 54. Had the electrodes not been drifting, the standard deviation would be expected to have a constant value (since the noise would be constant) rather than continuing to increase.

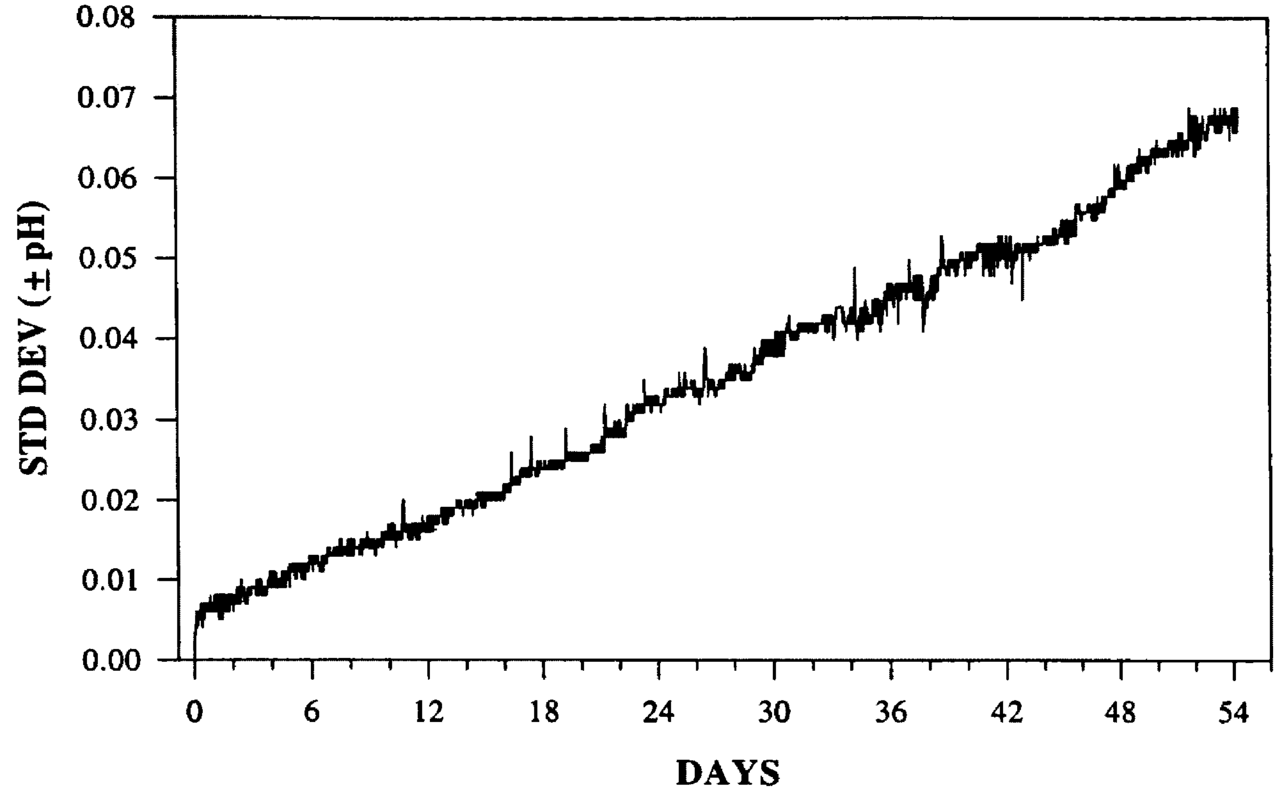

Figure 4-8: Standard deviation of the three electrode average for the first bicarbonate experiment (Electrode E8-CP was removed)

After thoroughly evaluating this data, it was determined that the results most likely did not reflect the true capabilities of the Ross electrodes. There are several possible explanations for the large electrode drift that was observed in this experiment. First, these electrodes were about one year old prior to this experiment and may have been damaged during storage. Electrodes which have been stored over any period of time are quite susceptible to junction clogging and, consequently, electrode drift Secondly, and probably more importantly, these electrodes were used in the previous experiment with the tris buffer. Based on the observations of the tris experiment, the tris most likely permanently damaged the liquid junctions of these electrodes, leading to the observed drift.

4.3 Second Bicarbonate Experiment

After obtaining six new Ross electrodes, a second long-term drift experiment using the $NaHCO_3$ buffer was performed. Details of this experiment are given in Section 3.3c. Figure 4-9 is a plot of the six pH signals and temperature recorded over this 56 day experiment.

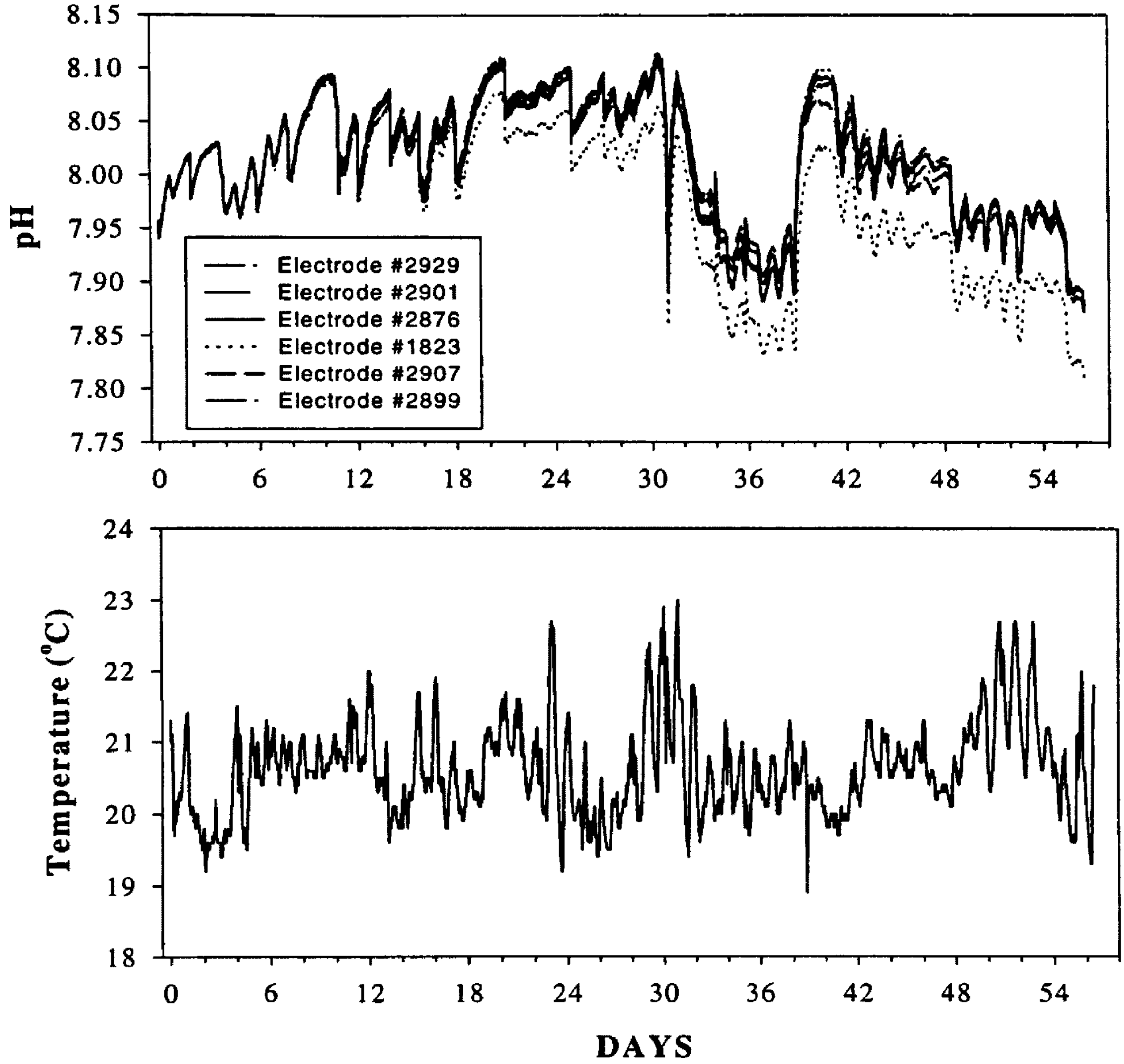

Figure 4-9: Six electrode signals (top) and temperature (bottom) of the second bicarbonate experiment

Through the first 10 days all six electrodes were tracking identically with essentially no drift between the six signals. By day 20, however, it is obvious that electrode #1823 is beginning to drift away from the other five electrodes. Over the remaining 34 days of the experiment, this drift continued to increase. The cause of this drift was most likely a malfunctioning liquid junction although no visible evidence, such as a slow filling solution flow rate, was observed. The remaining five electrodes performed well for the first half of this experiment. No significant drift between these electrodes was observed until around day 32. This drift continued until about day 42, and then began to decrease over the remainder of the experiment.

The two large pH fluctuations observed from days 30-42 in Figure 4-9 are due to the use of a new buffer and to evaporation. Around day 30 of the experiment it was discovered that an error had been made in preparing the original buffer (the salinity was estimated to be 38). Thus, a new buffer was prepared with a salinity of 35. The addition of this "new" buffer to the experiment is clearly seen in Figure 4-9 at day 31 when the pH dropped dramatically to 7.90 (the first pH fluctuation). Since the new buffer was not degassed, it was most likely saturated with CO_2 and, thus, had a lower pH due to carbonic acid formation (Equations 1-5, Chapter 1). As this buffer began to equilibrate with the atmospheric pCO2, however, the pH increased due to CO_2 outgassing.

The second pH fluctuation, from days 32-42, is most likely due to evaporation. This evaporation was a result of using a larger beaker to hold the new bicarbonate buffer. Previously, the buffer had been held in a 250 mL beaker. Since approximately 40 mL of buffer was extracted for each colorimetric pH measurement, the beaker had to be continually replenished. The new buffer, however, was held in a 500 mL beaker, eliminating the need to add more buffer after each colorimetric measurement (i.e. the frequency was reduced). The evaporation increased the TA, TCO_2 and pCO_2 of the buffer, causing the pH to slowly drop from days 32-39. The subsequent increase in pH at day 38 was due to the addition of buffer that had equilibrated with atmospheric CO_2.

Figure 4-10 shows the standard deviation of the six electrode average for this experiment. The linear increase in the standard deviation over the first 34 days indicates that one or more electrodes are drifting or malfunctioning. From Figure 4-9, it is quite evident that electrode #1823 is failing. The removal of this electrode should clearly improve the precision of the pH measurement.

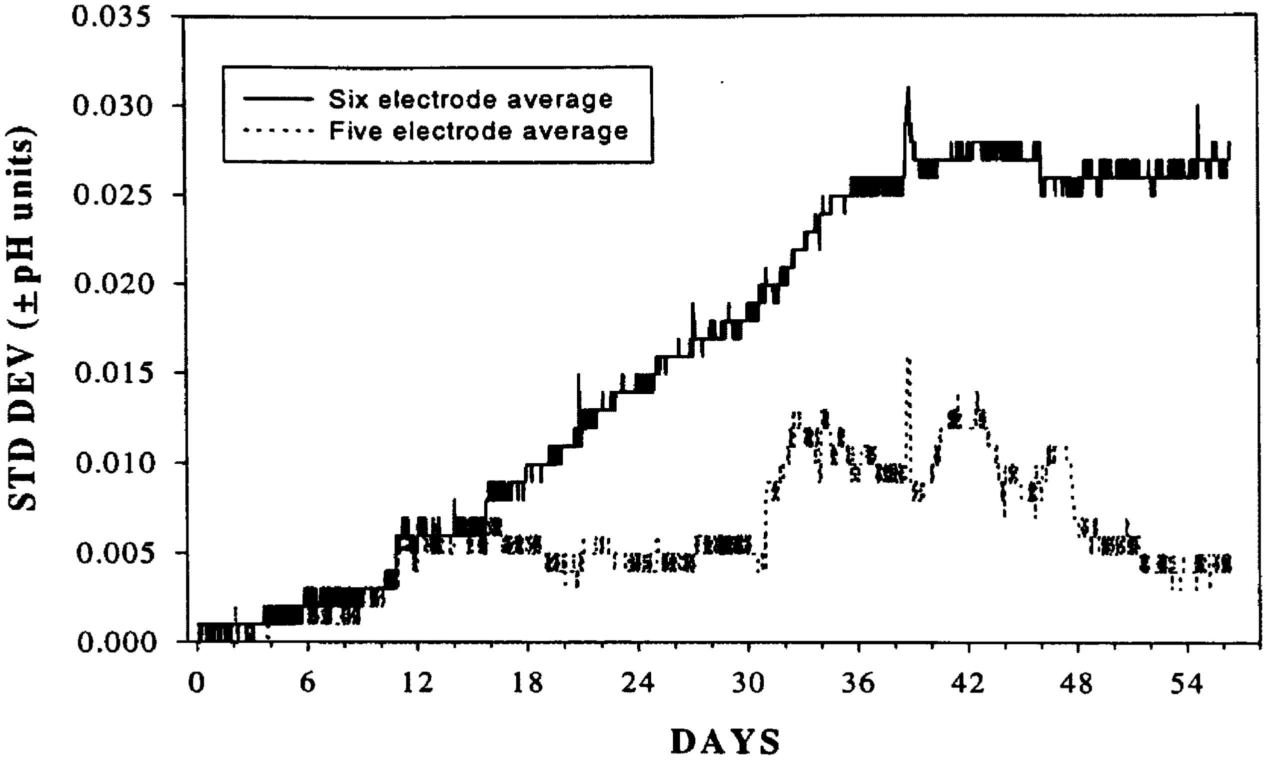

Figure 4-10: Standard deviation of the 5 and 6 electrode averages for the second bicarbonate experiment

Once again, the ASTM T_n test (Mandel, 1978) was used to verify that electrode #1823 could be rejected from the data set. At day 20, the average pH of the six electrodes was 8.089 while the pH measured by #1823 was 8.068. The standard deviation here was 0.011 pH units, resulting in a rejection quotient, T_n, of 1.91. Thus, electrode #1823 is most likely an outlier and can be eliminated from the average at day 20 with 97.5% confidence. By day 22 the rejection quotient increased to 2, indicating that electrode #1823 could be rejected with 99% confidence.

Figure 4-10 also shows the standard deviation of the 5 electrode average. This plot clearly shows the improvement in the precision once the outlier (electrode #1823) is rejected from the data set. This improvement would not be possible without using several electrodes. If only electrode #1823 had been used, for example, the pH measurement would have been inaccurate (>0.005 pH units) after day 10. By using more than one electrode the confidence of the pH measurement increases drastically since the failing electrodes are easily detected and eliminated. The linear increase in the standard deviation observed in Figure 4-10 when all six electrodes are included is no longer observed once electrode #1823 is rejected. Up to day 30 (prior to the use of the "new" buffer), the standard deviation of the five signals was ± 0.005 pH units. This is much improved over the standard deviation of ± 0.017 pH units when #1823 is included in the measurement. The resulting standard deviation for this data set after 56 days was ± 0.004 pH units.

At days 31-48, the standard deviation fluctuates dramatically for several reasons. The fluctuations from days 31-40 are most likely due to a changing salinity. Evaporation and the addition of a buffer with a lower (~3 %) salinity would change the liquid junction potential of each electrode due to the varying concentration of ions in the solution. Each electrode, however, will respond differently to this change in salinity and lead to the observed deviation between the five signals.

Although the fluctuations in SD from days 31-40 correlate well with the large pH swing (Figure 4-9) over the same time period, the fluctuations in SD from days 41-48 do not appear to correlate with any dramatic change in pH or salinity. In order to explain this fluctuation, the behavior of each electrode over this period was evaluated by comparing the pH of each electrode to each of the remaining 4 electrode signals. This evaluation revealed that electrode #2901 was fluctuating more significantly than the other 4 electrodes over this period. This was verified by eliminating electrode #2901 from the average pH and observing the SD of the 4 electrode average. The fluctuations in SD from days 31-40 remained, while the fluctuations from days 41-48 were significantly diminished. Therefore, it can be concluded that the fluctuations in SD from days 41-48 are due to one single electrode rather than all 5 electrodes.

Once electrode #1823 was eliminated from the measurement, the average pH of the remaining five electrodes was compared to the colorimetric pH measurements. Figure 4-11 is a plot showing the average pH and colorimetric measurements for the second bicarbonate experiment Once again, diurnal oscillations are readily seen in both the average pH and temperature plots. Over the course of this 56 day experiment, 26 colorimetric accuracy checks were made. The agreement between the accuracy checks and the average pH measurement is mixed. Through the first 32 days, as the pH (on average) was increasing, a majority of the accuracy checks appear to be slightly higher in pH than the average electrode pH. After the transition period when the new buffer was added (days 31-40), however, the accuracy checks seem to be slightly lower than the average pH measurement.

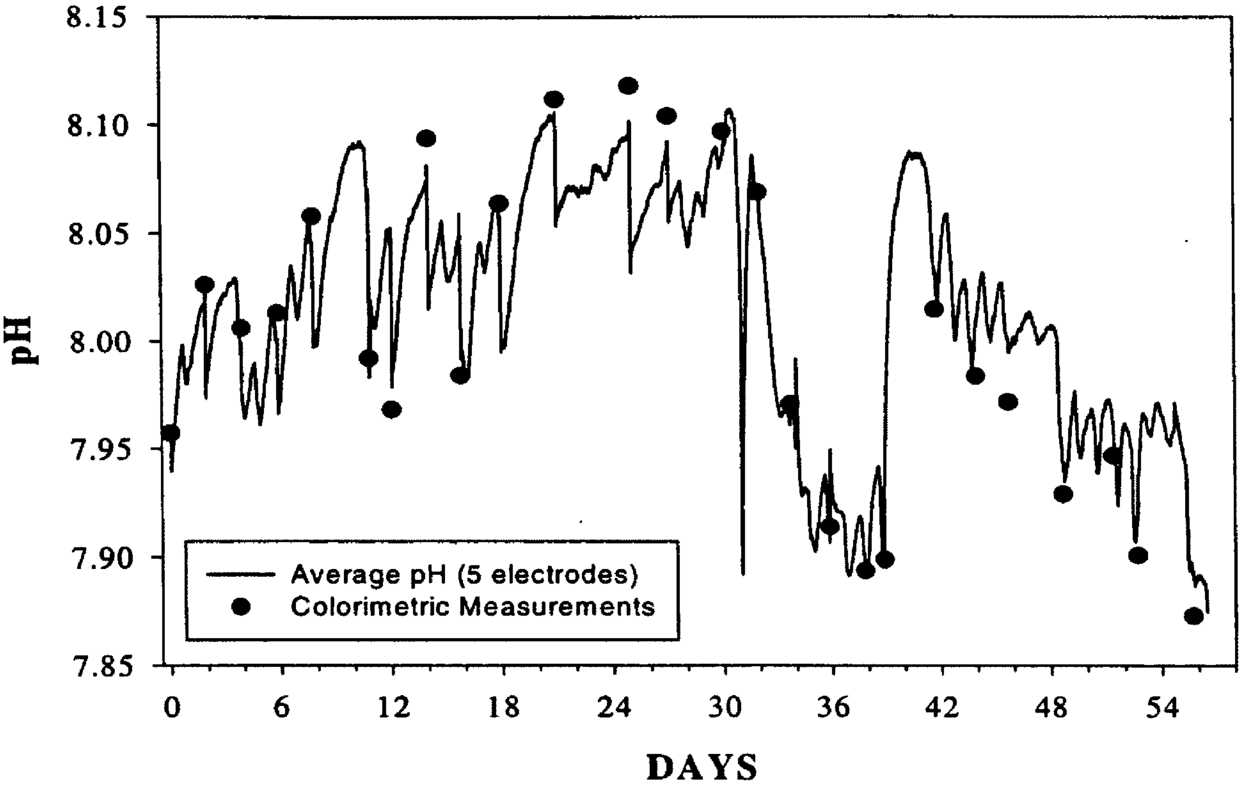

Figure 4-11: Average pH and colorimetric accuracy measurements of the second bicarbonate experiment

Figure 4-12 shows the difference between the average pH of the five electrodes and the colorimetric accuracy checks. Through the first eight days of the experiment the deviation became more negative, indicating that the colorimetric measurements were higher than the average electrode pH (the difference at day 8 was –0.017 pH). The three large positive deviations at days 11, 12 and 16 will be discussed later in this section. From Figure 4-11 it can be seen that the average pH was trending upward over these first 16 days. By day 18, however, the large pH oscillations observed over the first 16 days diminished. During this period where the pH oscillations were reduced, the agreement between the average pH and colorimetric accuracy checks generally improved (with the exception of day 25). The difference at day 30, the final day of this "stable period", was –0.011 pH units.

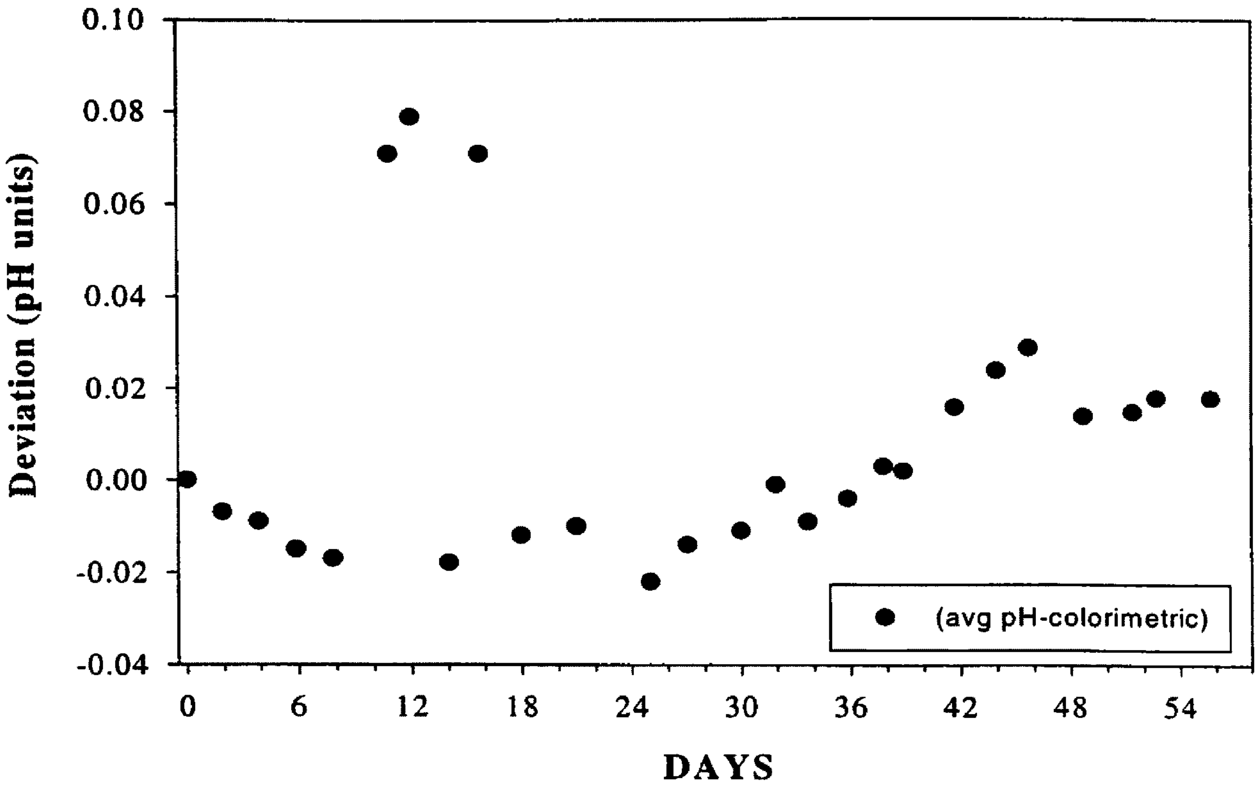

Figure 4-12: Difference between the average pH and the colorimetric accuracy checks for the second bicarbonate experiment

Once the accuracy check was made on day 30, the buffer was replaced with the fresh buffer of different salinity. The first accuracy check using this buffer (made at day 32) resulted in a good agreement of –0.001 pH. At days 34-46, however, the difference began to increase until a maximum of 0.029 pH was reached at day 46. From day 48 through the remainder of the experiment the accuracy improved once again. At day 49 the difference was 0.014, while the difference at day 56 was 0.018 pH units. The improvement in the accuracy at day 48 might be explained by examining the overall trend of the average pH over this period.

From Figure 4-11 it can be seen that the average pH from days 32-39 and 42-47 is in a downward trend. The accuracy over these days degraded (see Figure 4-12). The downward pH trend, however, ended at day 48. The average pH from day 48 through the end of the experiment was not in an upward or downward trend, and as a result the accuracy improved.

From the preceding discussion, it appears that the accuracy of this experiment was best when the average pH of the buffer was not in an upward or downward trend. In order to test this hypothesis, the agreement between the colorimetric measurements and average electrode pH was intensely examined. In this experiment, eleven accuracy checks were made over an eight hour period and compared to the average electrode pH.

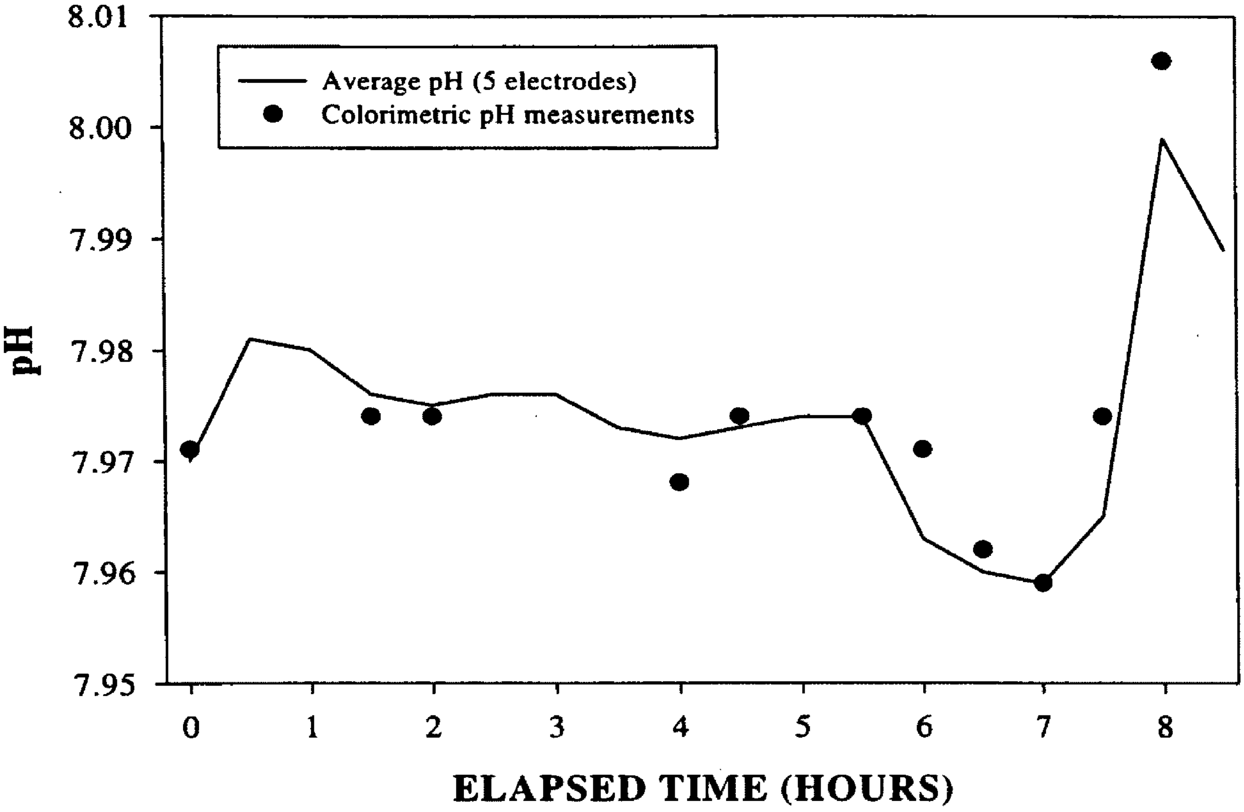

Figure 4-13: Reproducibility of the colorimetric accuracy checks for the second bicarbonate experiment (from day 30, Fig. 4-12)

Through the first 5.5 hours of this experiment the pH remained quite stable (Figure 4-13), resulting in good agreement between the average pH and the accuracy checks. The largest difference through this period was –0.004 pH units at hour 4. The remaining five accuracy checks had a difference of 0.002 pH or less. After the sixth accuracy check was made, additional buffer was added to the beaker to introduce a pH change (notice the large pH fluctuation at hours 6-8). Five accuracy checks were made through this pH fluctuation, but the results were not as good as those observed during the first 5.5 hours of the experiment. The first accuracy check (at hour 6) made during this fluctuation period resulted in a difference of 0.008 pH units. The largest difference (0.009 pH units) was at hour 7.5. The final accuracy check at hour 8 yielded a difference of 0.007 pH units from the average electrode pH. From these results it can be concluded that the average pH measurements match up much better with the colorimetric accuracy checks when the pH of the buffer remains stable. This effect may be due to the slow response of the electrodes. During large pH swings, the electrode response may be too slow to accurately track the pH change.

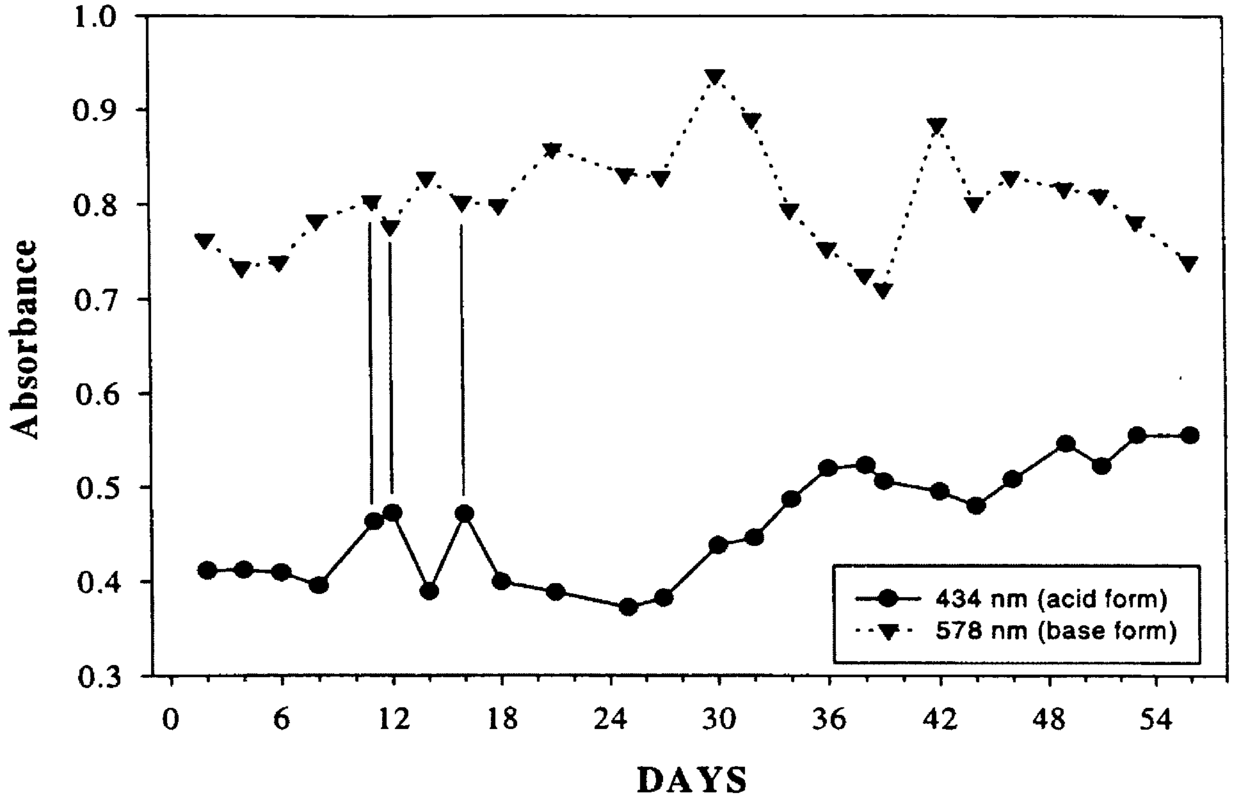

Figure 4-14: Acid and base form absorbances of the indicator for each colorimetric measurement of the second bicarbonate experiment

Although the preceding discussion may explain some of the smaller deviations between the average pH and the colorimetric accuracy checks, the larger deviations at days 11, 12, and 16 of Figure 4-12 require a more in-depth investigation. In order to explain these three apparent outliers, the absorbances of each colorimetric pH measurement were examined. Figure 4-14 is a plot of the absorbance of the acid (434 nm) and base form (578 nm) of the indicator for each colorimetric pH measurement. The three outliers are highlighted by a line connecting the acid and base form absorbances of each.

In a colorimetric measurement, the pH is determined by the absorbance of the acid and base forms of the indicator. A higher pH (more basic) will give a larger absorbance in the base form and a smaller absorbance in the acid form. As the pH drops, the absorbance of the acid form will increase while the absorbance of the base form will decrease. A true pH change, then, will show a positive deviation in one form and a negative deviation in the other form of the indicator. From Figure 4-14, however, it is obvious that the absorbance at 434 nm increased drastically for each of the three outliers while the absorbance at 578 nm decreased minimally. The molar absorptivity is higher for the base form so the absorbance at 434 nm could not increase more than the absorbance at 578 nm. This inconsistency indicates that the large pH drop observed in the colorimetric measurement was not a true pH change.

The large increase in absorbance at 434 nm for the three outliers was most likely caused by error in the colorimetric measurement. Although the source of this error is not yet known, several possibilities have been investigated. One possible source that was considered was biological growth in either the seawater buffers or the cell. Chlorophyll is known to absorb at around 430 nm and would explain the increased absorbance in the acid form of the indicator. The cell used in these experiments, however, was rinsed and dried thoroughly after each use to prevent any type of biological growth. Also, no visible indication of biological activity was ever noticed in the seawater buffers. Thus, biological contamination is highly unlikely.

The spectrophotometer is another possible source of error. Since this instrument was shut down after each use, it was hypothesized that an error would arise if the colorimetric measurement was made before the instrument was completely warmed up. Thus, an experiment was performed in order to monitor the behavior of the instrument during a three hour "warm-up" period. In this experiment, the cell was filled with the bicarbonate buffer and placed in the cell holder of the instrument. The instrument was then turned on and a blank scan was immediately run. The absorbances at 434, 578 and 730 nm were then measured every 10 minutes over a three hour period. It was thought that error might arise if the intensity at one of these wavelengths varied differently than the others during this "warm-up" period. However, the results indicated that the absorbance characteristics of all three wavelengths behaved similarly over the duration of the experiment. Nonetheless, the 3 outliers are considered to be incorrect even though the source of the error, at present, remains unresolved.

As part of the original hypothesis, it is important to illustrate that the accuracy from using multiple electrodes will be better than any one single electrode. This improvement is illustrated by Table 2. In this table, the pH of each electrode (1=#2901, 2=#2929, 3=#2899, 4=#2876 and 5=#2907) was compared to the pH measured colorimetrically on day 32 of this experiment. The average pH of the 5 electrodes was also compared to the colorimetric pH. These results show that the 5 electrode average was much closer to the actual pH. Thus, it is obvious that using multiple electrodes can be more accurate than using any one single electrode.

Table 2: *Accuracy improvement from using multiple electrodes*

Electrode #	Electrode pH	Colorimetric pH	Δ (elec.-color.)
2901	8.058	8.069	-0.011
2929	8.061	8.069	-0.008
2899	8.064	8.069	-0.004
2876	8.077	8.069	0.008
2907	8.079	8.069	0.010
Average (5)	8.068	8.069	-0.001

4.4 Conventional Electrode Experiment

To test the hypothesis that Ross electrodes would perform better (i.e., drift less) in long-term seawater pH measurements, an experiment using conventional combination electrodes was carried out for comparison with the Ross electrodes. Experimental details are given in Section 3.3d. Figure 4-15 is a plot of the four pH signals recorded over this 7 day experiment. From this plot it is obvious that the four electrodes began to drift away from one another almost immediately, although no single electrode deviated significantly from the others. When comparing these results to Figure 4-9, it can be seen that the Ross electrodes have essentially no drift between the 6 electrodes over the same amount of time (7 days). This is also illustrated by comparing the standard deviation between the two experiments.

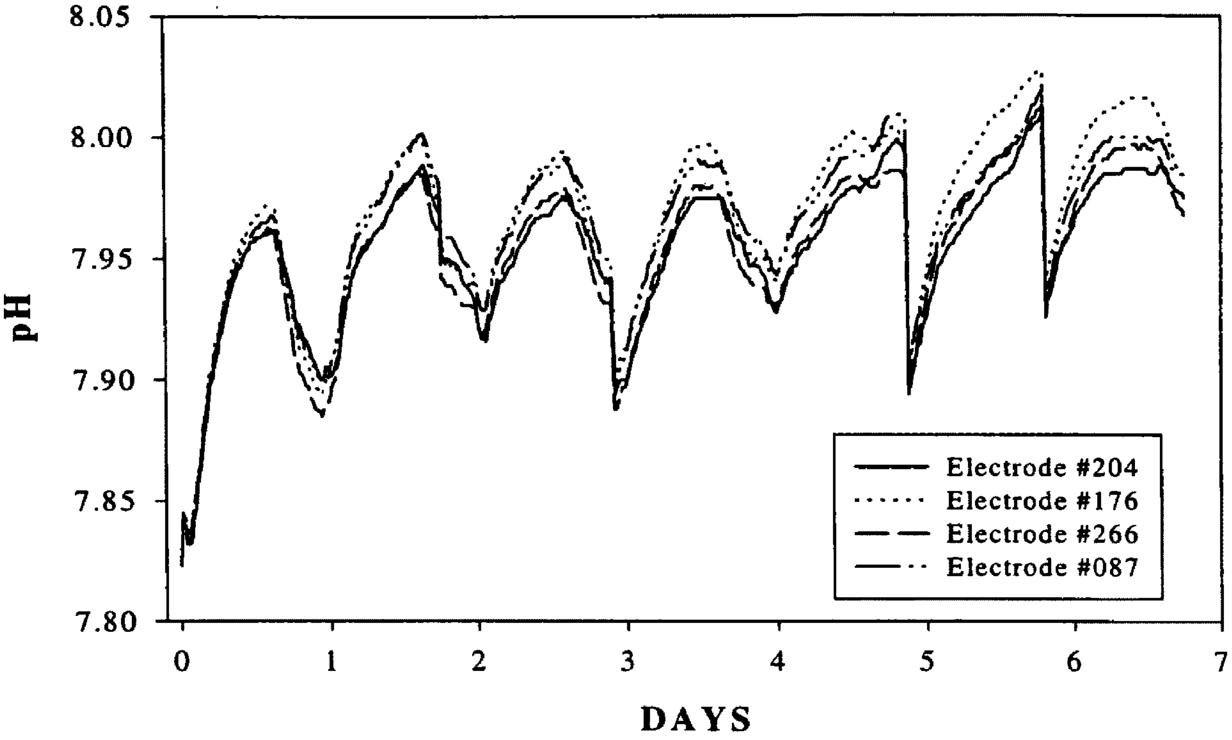

Figure 4-15: Four pH signals of the conventional electrode experiment

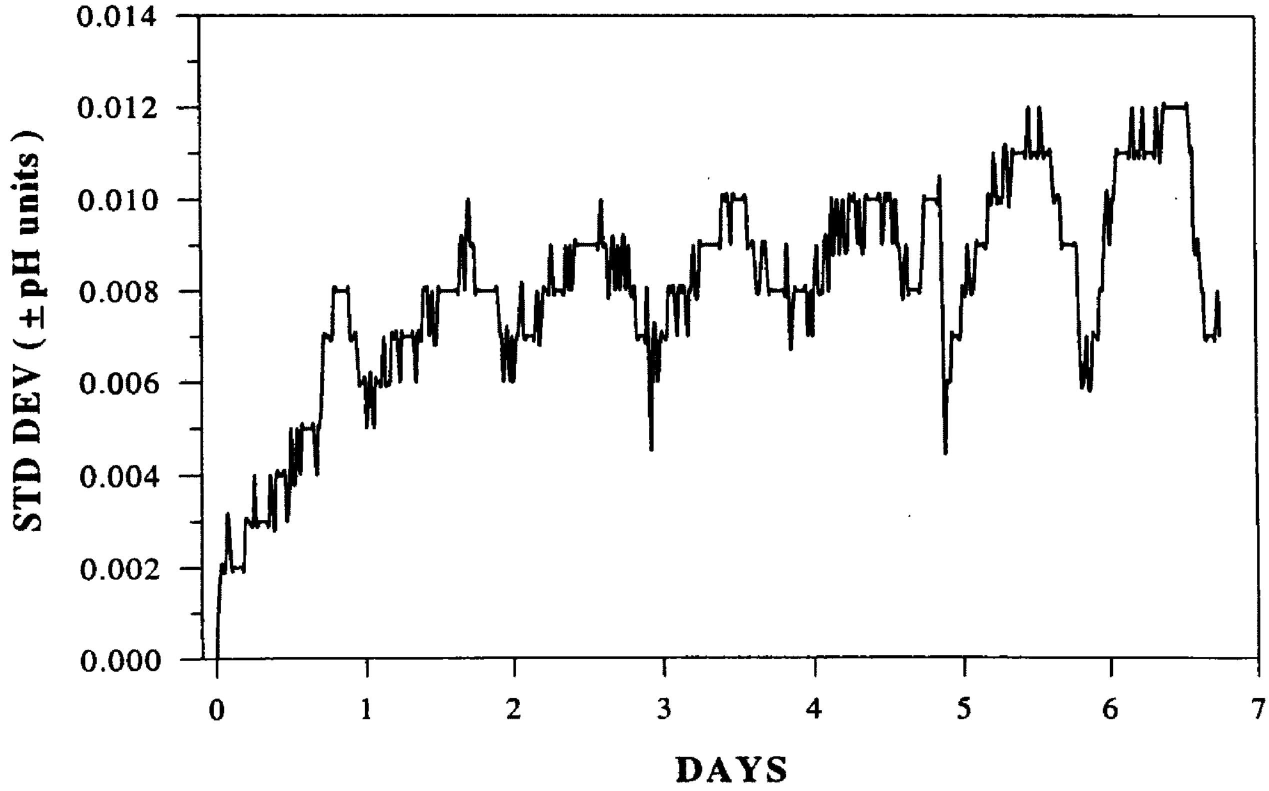

Figure 4-16: The standard deviation of the four electrodes used in the conventional electrode experiment

Figure 4-16 is a plot of the standard deviation of the four electrodes used in this experiment. After the first day of this experiment the standard deviation had already reached 0.006 pH units. The SD continued to increase over the remainder of the experiment. By day 4 it was 0.010 pH, and the final deviation was 0.012 pH units. Using Figure 4-10 for comparison, it is obvious that the conventional Ag/AgCl reference electrodes used in this experiment drift much more significantly than the Ross electrodes. Through the first 7 days of the second bicarbonate experiment the SD of the Ross electrodes is only 0.002 pH units, compared to 0.012 pH units for the conventional electrodes. It should be noted, however, that the conventional electrodes used in this experiment were not new and, thus, may not have given a true representation of the capabilities of Ag/AgCl reference electrodes.

4.5 Freshwater (Low Ionic Strength) Experiment

As with seawater, freshwater pH measurements are quite susceptible to electrode drift. The extremely low ionic strength of freshwater results in large differences in the liquid junction potential when using NIST buffers for calibration. The change from high ionic strength to low ionic strength media leads to a slowly drifting signal, making accurate pH measurements difficult (Dickson, 1993a). Since the Ross electrodes showed very little drift in the saline media of the second bicarbonate experiment, a drift experiment using freshwater was performed in order to evaluate the performance of Ross electrodes in a low ionic strength media. Details of this experiment are given in Section 3.3e. Since electrode drift was the focus of this particular experiment, the pH's were not calculated and colorimetric accuracy checks were not made. Drift was monitored by plotting the change in potential from the initial potential for each electrode. Figure 4-17 is a plot of the six signals for this seven day experiment.

From this plot it is obvious that the new electrodes (#4051 and #4034) drifted substantially from the remaining four electrodes. At day 3.5 of this experiment, the potential of electrode #4051 had dropped 1.7 mV from the initial potential while electrode #4034 had increased by 9.6 mV. The average potential of the remaining four electrodes was 2.5 mV. By the final day of this experiment, electrode #4051 recorded a difference of –7.8 mV from the initial potential while #4034 recorded a difference of 10.3 mV. The remaining four electrodes had an average difference of –3.5 mV.

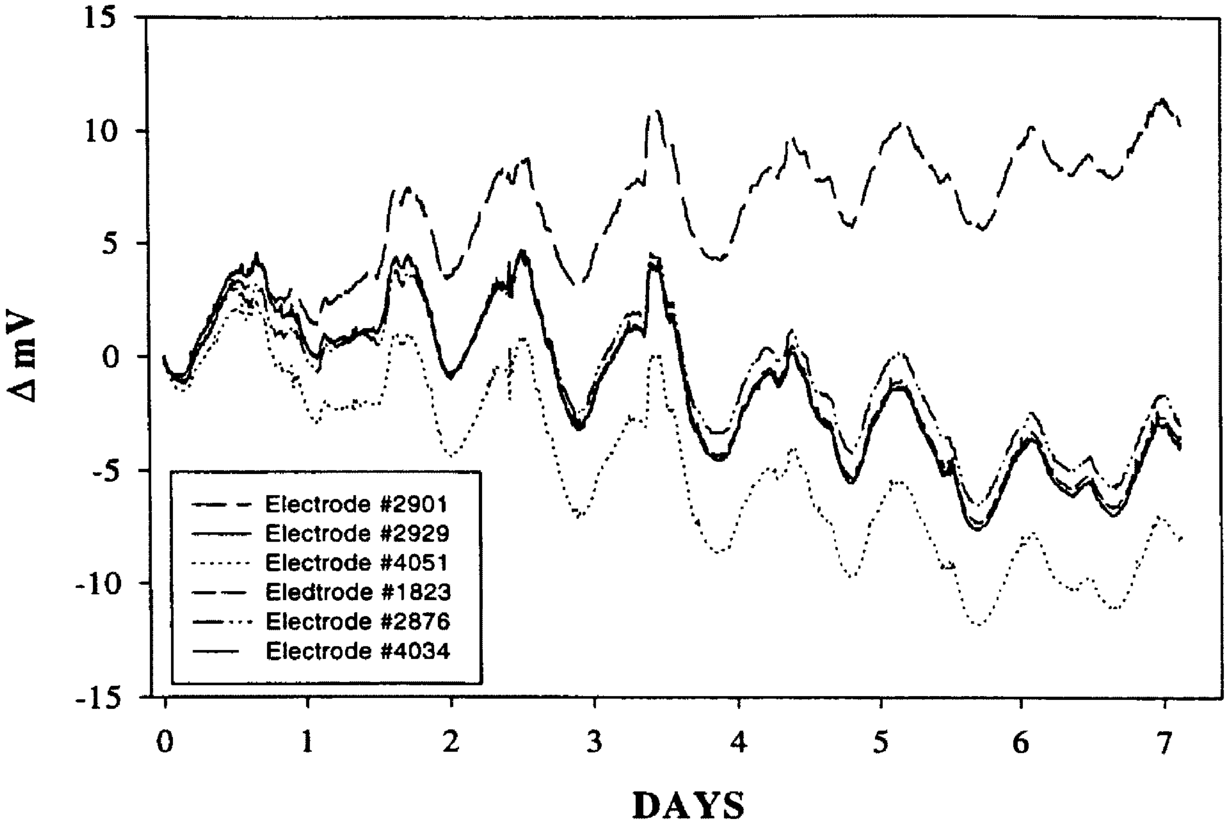

Figure 4-17: Deviation of the six signals from the initial potential for the freshwater experiment

Figure 4-18 is a plot of the standard deviation of the six and four (less #4051 and #4034) electrode averages for this experiment. The linear increase in the standard deviation of the six electrode average verifies that electrodes #4051 and #4034 were drifting away from the remaining 4 electrodes. These two electrodes were subsequently removed from the average and the improvement is obvious. By day three of this experiment the standard deviation of the six electrode average was ± 2.9 mV compared to ± 0.4 mV for the four electrode average. The final day gave a standard deviation of ± 5.0 mV and ± 0.4 mV for the six and four signal averages, respectively.

The most reasonable explanation for the relatively poor performance of the "new" electrodes of this experiment would be the conditioning of the electrodes. Although the older electrodes had been used exclusively in saline media for past experiments, these electrodes were soaked in a low ionic strength storage solution for approximately two weeks prior to this experiment. This procedure most likely conditioned the electrodes for the low ionic strength of the lake water and reduced the possibility of drift. The new electrodes, however, were stored dry and conditioned for less than one day prior to this experiment. Thus, it is promising to observe that Ross electrodes may perform well (i.e. with minimal drift) in low ionic strength waters with prior conditioning.

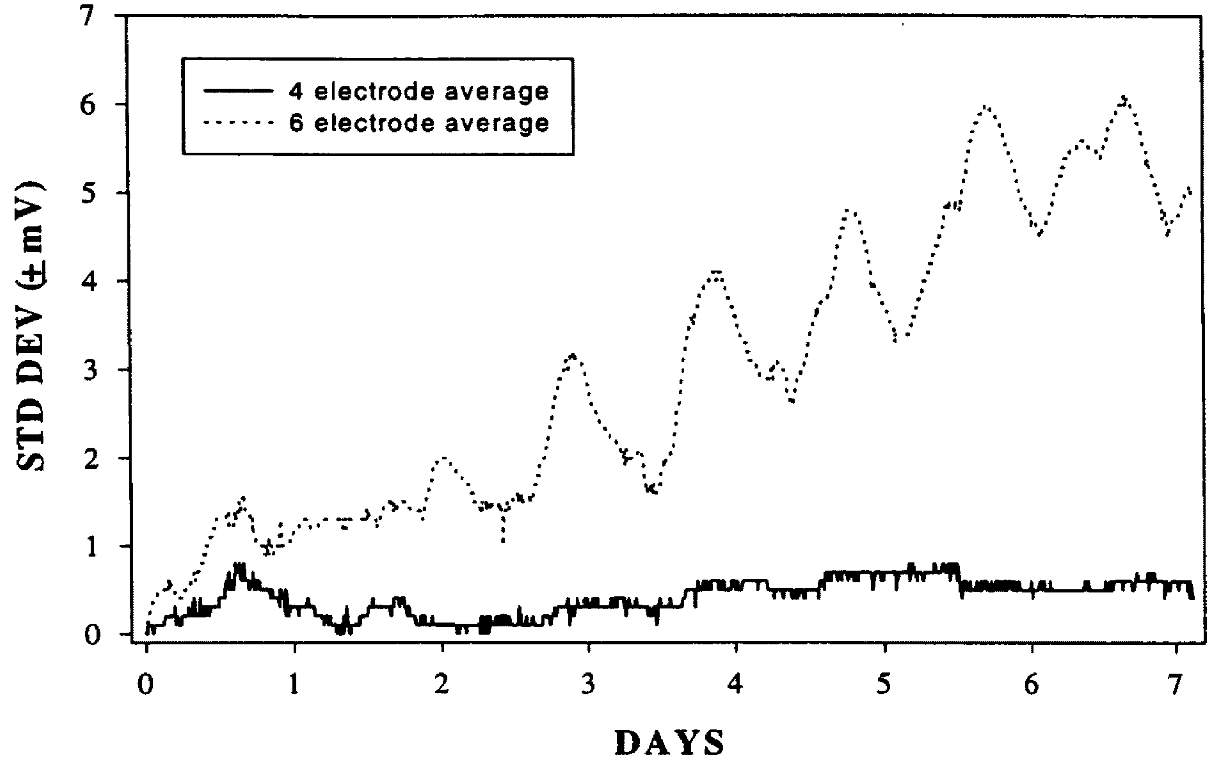

Figure 4-18: The standard deviation of the four and six electrode average for the freshwater experiment

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Analysis of Results

The significance of the results presented in Chapter 4 are evaluated by comparison with the results from previous research. For this comparison, however, only the results from the second bicarbonate experiment will be analyzed due to the electrode problems (discussed in Chapter 4) encountered with the tris and first bicarbonate experiments. The electrodes used in the second bicarbonate experiment were new and most likely gave the best representation of the potential capabilities for a multiple electrode and signal averaging approach to reducing electrode drift.

As discussed in Section 1.3, electrode drift was monitored in this work by observing the standard deviation of the signals. The standard deviation of the second bicarbonate experiment is given by Figure 4-10. Evaluation of the SD for outlier detection is discussed in Section 4.3 (pp. 40-42). These results clearly show that using multiple electrodes and signal averaging will enable the detection of a drifting electrode and subsequently reduce the drift in the resulting pH calculation.

Once the electrodes that were obviously failing were removed from the data set, the drift in the remaining electrodes was monitored using the colorimetric accuracy checks. If the electrodes were in fact drifting, the drift could be monitored by noting the difference between the average pH and the colorimetric accuracy checks (Figure 4-12). It was hypothesized that a drifting electrode would cause a unidirectional drift in the accuracy. Alternatively, if the difference between the average pH and the accuracy checks remained fairly constant, it would be a good indication that the electrodes were not drifting. From Figure 4-12, however, it is difficult to make any definite conclusions. Although there was no obvious unidirectional drift, there was a wandering drift throughout the experiment. This wandering drift may be due to unexplained experimental errors in the colorimetric pH measurements (as discussed in Section 4.3).

Using the SD of the 5 electrode average from Figure 4-10, the precision of this experiment was found to be ± 0.004-0.005 pH units. This range is typical over the period when the electrode behavior is stable (i.e., days 0-30 and days 48-56), as discussed in Chapter 4. From days 31-47, the precision of this experiment degraded to ± 0.010 pH units due to the introduction of the new buffer with a lower salinity. Using the average standard deviation of the 22 accuracy checks (the 3 outliers were omitted) of Figure 4-12, the accuracy of this experiment was found to be ± 0.015 pH units.

The accuracy and precision found in this research is comparable to the results reported by researchers making shipboard potentiometric seawater pH measurements. Fuhrmann and Zirino (1988), for example, reported an at-sea precision of ± 0.003 pH units and an accuracy of ± 0.010 pH units using calomel reference electrodes. Mackey et al. (1989) reported a precision of ± 0.002 pH units and an accuracy of ± 0.008 pH units using Ross electrodes. Copin-Montegut and Avril (1995) also used Ross electrodes and found a precision of ± 0.001 pH units and an accuracy of ± 0.010 pH units.

Although the results of this research seem reasonable in comparison to the accuracy and precision reported by shipboard researchers, one important point must be emphasized. The electrodes used in ship-based potentiometric pH measurements are frequently calibrated. Thus, the reported accuracy and precision of these measurements is over the course of a day or two at most. No calibrations were made in this experiment, so the reported accuracy and precision is over the course of 56 days. This is significant because it shows that relatively good accuracy and precision can be achieved with a simple calibration-free approach.

5.2 Future Work and Applications

The results of this research indicate that a multiple electrode / signal averaging approach can be used to make long-term, calibration-free potentiometric seawater pH measurements with relatively good accuracy and precision (compared to shipboard measurements). The next step in this research is to apply this approach to the development of an autonomous *in situ* potentiometric seawater pH instrument. In order for this approach to be successful in an autonomous instrument, however, the electronic design of the instrument must first be considered.

Although the instrumental setup used in this research eliminated the common electrical problems often associated with making potentiometric pH measurements (e.g. noise and ground loops), this setup would not be practical for an autonomous instrument. An electronic circuit capable of isolating multiple electrodes from one another would need to be developed for the instrument. One such circuit was built and tested in this research (details are given in Appendix III). Although preliminary results indicate that this design may be successful, more rigorous testing needs to be made in order to verify that the electrodes do not influence one another.

An autonomous *in situ* pH instrument would have a wide variety of applications in natural waters. Besides offering a better understanding of the processes which control CO_2 fluctuations in seawater, long-term pH measurements might someday be used to monitor the impact of the greenhouse effect on the oceans. Further improvements in the accuracy and precision of pH measurements would allow oceanic pH to be combined with either *in situ* $p\mathrm{CO}_2$ or TA measurements to quantify the small changes in TCO_2 that result from the influx of atmospheric CO_2. This type of study might help researchers assess the impact human activities have on the ocean environment.

Autonomous calibration-free pH measurements would also be beneficial for freshwater applications. Since the problems associated with making calibrations (as discussed in Section 4.5) would be avoided with this approach, the resulting pH measurements would be more accurate and precise. This may lead to a better understanding of the freshwater processes which control, or are controlled by, pH. The damaging effects of humans (such as acid-mine drainage) on rivers and lakes might then be better understood and, consequently, more easily remediated.

APPENDIX I

QUICKBASIC PROGRAM

```
'*****************************************************************************
'*             QUICK BASIC PROGRAM FOR pH/TEMPERATURE DATA AQUISITION
'*                           --VERSION 1.0--
'*       J.C. Seiter          4/09/96
'*****************************************************************************
' VERSION 1.0:
'   -Requires Keithley adc16 A\D Board (for pH and temp. aquisition)
'
'*****************************************************************************


' This file contains all function DECLARAtion supported by the driver.
'       $INCLUDE: 'Q4IFACE.BI'

'-----------------------------------------------------------------------------
' STEP 2 : Define ALL local variables required by the program here.  NOTE
' that you must avoid declaring and using QuickBASIC variables on the
' fly.

DIM NumOfBoards AS INTEGER
DIM DERR AS INTEGER
DIM DEVHANDLE AS LONG
DIM CHAN AS INTEGER
DIM GGAIN AS INTEGER
DIM temp AS LONG
DIM BoardNum AS INTEGER
DIM adcode AS LONG
DIM adcodes AS LONG
'-----------------------------------------------------------------------------
' STEP 3: Initialize the internal data tables according to the
' information contained in the configuration file ADC16.CFG.
'
    A$ = "ADC16.CFG" + CHR$(0)
    DERR = ADC16DEVOPEN%(SSEGADD(A$), NumOfBoards)
    IF DERR <> 0 THEN BEEP: PRINT "ERROR "; HEX$(DERR); " OCCURRED DURING
'     ..DEVOPEN'": STOP

    BoardNum = 0
    DERR = ADC16GETDEVHANDLE%(BoardNum, DEVHANDLE)
    IF (DERR <> 0) THEN BEEP: PRINT "ERROR, DEVICE HANDLE IS null. . .": STOP


'------------------------Open Communication Ports------------------------------
CLS
LOCATE 2, 15
PRINT "******WELCOME TO THE QB pH\TEMPERATURE DATA AQUISITION PROGRAM******"
LOCATE 10, 1
PRINT "SAVE DATA AS(Include .DAT Extension)";
INPUT NAME$
OPEN "COM1:9600,N,8,1,DS1000" FOR RANDOM AS #1
OPEN "COM2:9600,N,8,1,DS1000" FOR RANDOM AS #2
PRINT "SAMPLING INTERVAL(in sec.)";
INPUT SAMPLTIME!
```

```
'---------------------------CLEAR OUT-----------------------------------------
CLS
LOCATE 2, 1

PRINT #1, "KP"              'clears out initial blank line output of meter 1
INPUT #1, H$: PRINT H$
INPUT #1, G$: PRINT G$


PRINT #2, "KP"              'clears out initial blank line output of meter 2
INPUT #2, J$: PRINT J$
INPUT #2, I$: PRINT I$
'---------------------------COLLECT DATA FROM METER #1------------------------
DO
PRINT #1, "KP"
INPUT #1, A$: PRINT A$         'input from channel a
PRINT #1, "KH"                 'switch to ch. b

BEGINT! = TIMER                'delay
DO
OVERT! = TIMER
LOOP WHILE ABS(OVERT! - BEGINT!) < 6

PRINT #1, "KP"
INPUT #1, B$: PRINT B$         'input from channel b
PRINT #1, "KH"                 'switch to dual channel

FIRST! = TIMER                 'delay
DO
SECONDT! = TIMER
LOOP WHILE ABS(FIRST! - SECONDT!) < 2
PRINT #1, "KH"                  'switch back to ch. a

'---------------------------COLLECT DATA FROM METER #2-----------------------

PRINT #2, "KP"
INPUT #2, C$: PRINT C$         'channel a
PRINT #2, "KH"                 'switch to channel b

NOWT! = TIMER                  'delay
DO
THENT! = TIMER
LOOP WHILE ABS(NOWT! - THENT!) < 6


PRINT #2, "KP"
INPUT #2, D$: PRINT D$         'channel b
PRINT #2, "KH"                 'switch to dual channel

 SOONT! = TIMER                 'delay
 DO
 LATERT! = TIMER
 LOOP WHILE ABS(SOONT! - LATERT!) < 2
 PRINT #2, "KH"                  'switch back to ch. a

'---------------------------Record mV from 5th Electrode----------------------

 FOR J = 1 TO 150
  DERR = KADRead%(DEVHANDLE, 7, 1, adcode)          'read A/D code
  IF DERR <> 0 THEN BEEP: PRINT "ERROR "; HEX$(DERR); " OCCURRED DURING
'KADRead'": STOP
 x! = x! + adcode
```

```
 NEXT J
   Y! = x! / 150               'average of 150 signals
  Z! = Y! * (-1) / 100           ' convert A/D codes to mV
  PRINT TAB(9);
  PRINT USING "###.#"; Z!
  x! = 0

'--------------------------Record mV from 6th Electrode------------------------
FOR K = 1 TO 150
  DERR = KADRead%(DEVHANDLE, 5, 1, adcodes)          'read A/D code
  IF DERR <> 0 THEN BEEP: PRINT "ERROR "; HEX$(DERR); " OCCURRED DURING
'KADRead'": STOP
 p! = p! + adcodes
 NEXT K
   q! = p! / 150                   'average of 150 signals
  s! = q! * (-1) / 100             ' convert A/D codes to mV
 PRINT USING "###.#"; s!
  p! = 0

'----------------------------Read temperature---------------------------------

DERR = KADRead%(DEVHANDLE, 6, 0, temp)     'get temperature
IF DERR <> 0 THEN BEEP: PRINT "ERROR "; HEX$(DERR); " OCCURRED DURING
'KADRead'": STOP

  E! = ((temp - 32768) / 10000)        'convert to volts
  r! = (20 * (2.5 - E!)) / (2.5 + E!)   'convert to resistance
  T! = -.007713# * (r! * r! * r!) + .37362 * (r! * r!) - 7.6457 * r! + 71.802
       'converts to temp. (deg. C)
PRINT USING "##.#"; T!
'------------------------SAVE TO FILENAME-------------------------------------
OPEN NAME$ FOR APPEND AS #3
PRINT #3, A$
PRINT #3, B$
PRINT #3, C$
PRINT #3, D$
PRINT #3, USING "##.#"; T!
PRINT #3, TAB(9);
PRINT #3, USING "###.#"; Z!
PRINT #3, USING "###.#"; s!
CLOSE #3
'--------------------------DELAY TIMER----------------------------------------
STARTT! = TIMER
DO
STOPT! = TIMER
LOOP WHILE ABS(STOPT! - STARTT!) < SAMPLTIME!
'--------------------------END OF PROGRAM-------------------------------------
LOOP WHILE INKEY$ <> CHR$(27)
CLOSE #1
CLOSE #2
CLOSE #3
END
```

APPENDIX II

ELECTRODE CHARACTERISTICS

***TABLE A2-1*: Electrode Slope and Temperature Response**

Electrode	Slope (mV/pH)	Temp. Response in Tris (mV/ °C)	Temp. Response in $NaHCO_3$ (mV/ °C)
E3	59.57	1.498	0.572
E4	59.43	1.474	0.631
E5	59.29	1.444	0.652
E6	58.62	1.442	N / A
E8-CP	57.94	N / A	0.633
#2907	58.89	N / A	0.655
#2929	59.02	N / A	0.650
#2899	59.02	N / A	0.649
#2876	59.16	N / A	0.654
#1823	58.89	N / A	0.646
#2901	58.75	N / A	0.652

APPENDIX III

ELECTRONICS RESULTS

The two largest problems associated with making long-term potentiometric seawater pH measurements have been electrode drift and various electronics problems such as noise and ground loops. These electronics problems usually arise when using more than one electrode. If the electrodes are not electronically isolated (i.e., connected to the same floating ground point) from one another, the previously mentioned problems will become apparent. Since our approach to using multiple electrodes and signal averaging has shown promising results in reducing electrode drift, an attempt was made to eliminate the electronics problems associated with potentiometric pH measurements. The elimination of these electronics problems would further enhance the development of an autonomous *in situ* potentiometric seawater pH instrument.

The approach used for solving these electronics problems consisted of building an electronic circuit and then performing several tests in order to verify that the electrodes were electronically isolated from one another. Figure A3-1 is a schematic of the circuit design (obtained from Denver Instruments) tested in this research. The operational amplifier (National Semiconductor, model LMC6464 BIN) is capable of handling input from up to four electrodes (through BNC connectors) and is powered by a 9 V battery. Since most dataloggers accept only positive inputs, the ground of each electrode is tied to a +2.5 V reference (Maxim, model MAX872CPA). The signal to the datalogger, then, would be equal to the potential of the electrode plus 2.5 Volts.

Since ground loops are the most common electronic problem in potentiometric pH measurements, two experiments were conducted with this circuit in order to show that the electrodes were not influencing one another. The first experiment consisted of connecting two electrodes to the circuit, placing each in a separate beaker of pH 7 buffer and recording the potential of each. Over a fifteen minute period, small additions of pH 4 buffer were added to one beaker while the other remained at pH 7. The potential of each electrode was recorded after each addition of the pH 4 buffer. The results of this

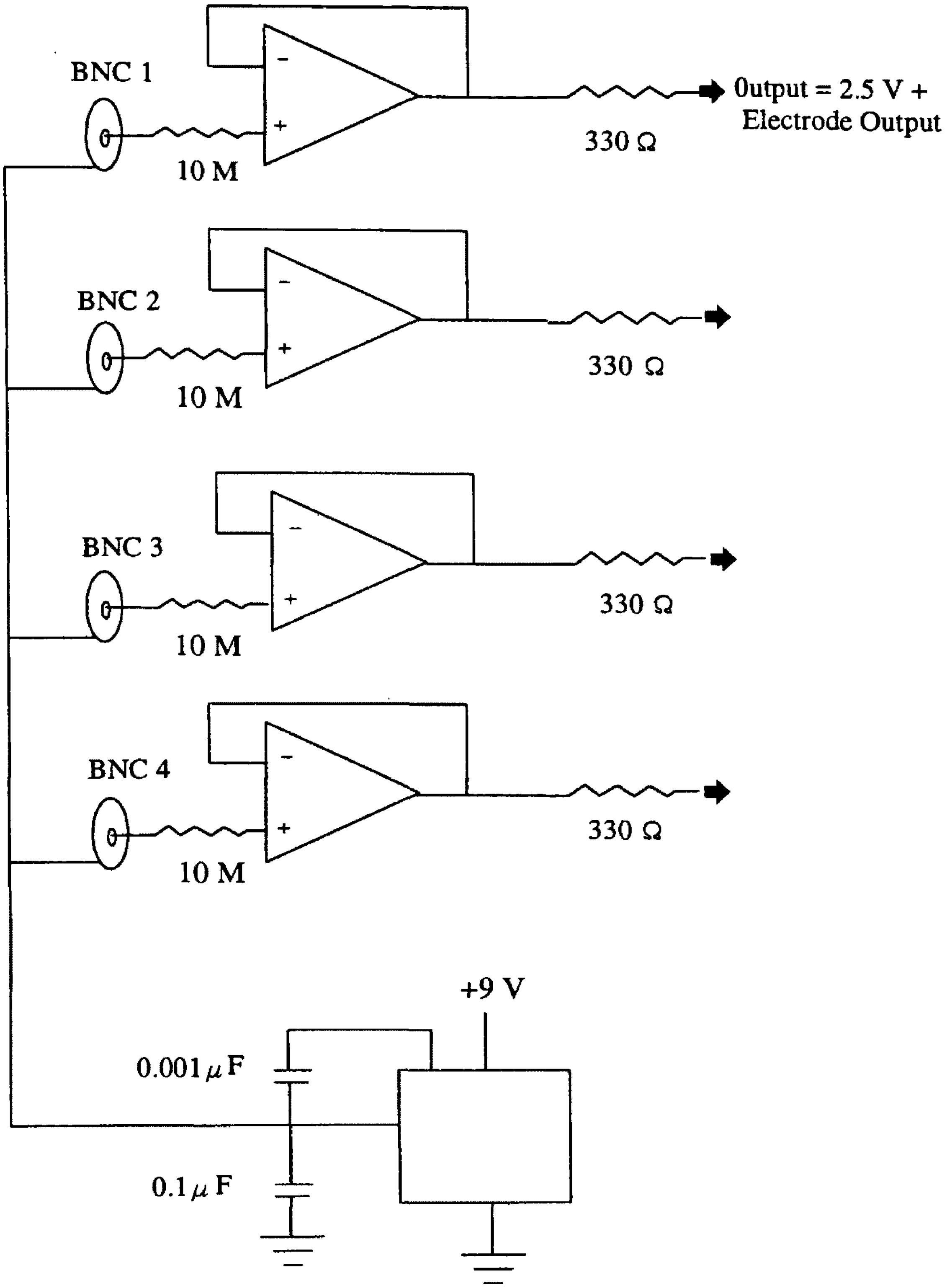

Figure A3-1: Electronic Circuit Design experiment are given by Figure A3-2. This figure shows that the electrodes were behaving independently and did not influence one another.

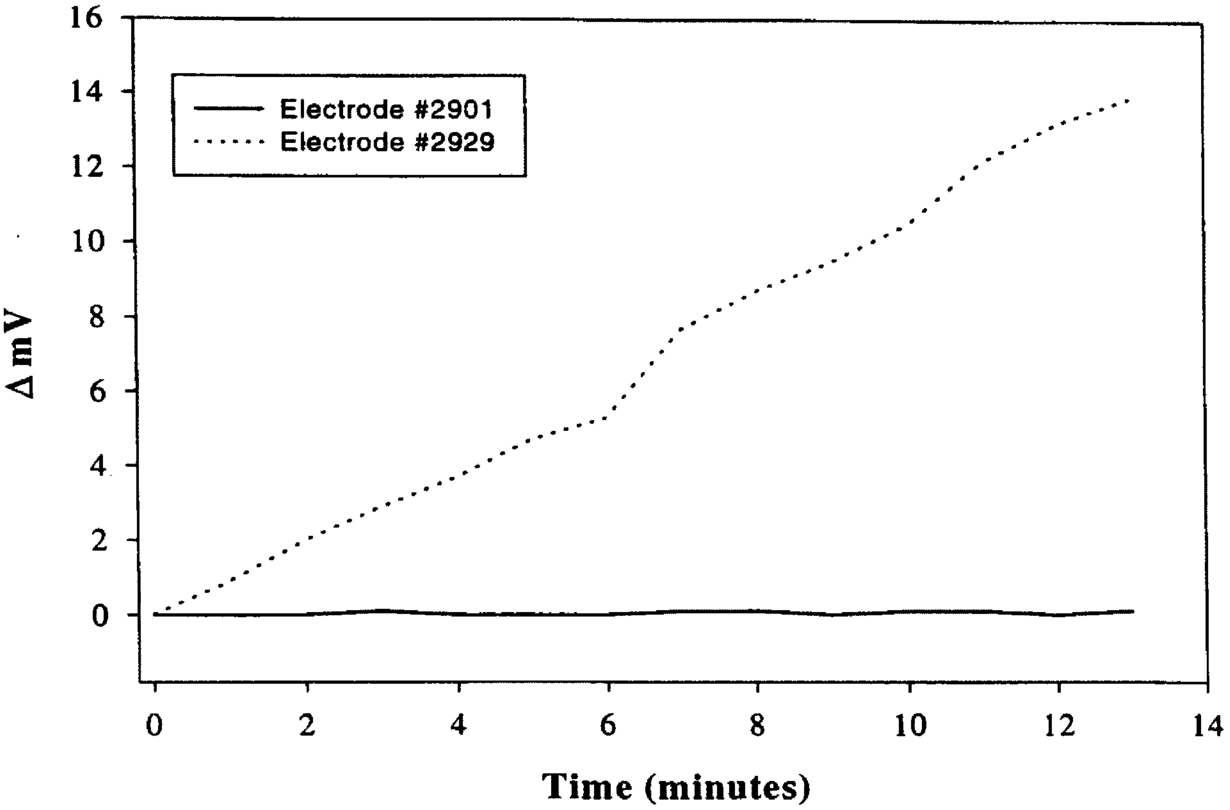

Figure A3-2: Independent behavior of two electrodes

The second experiment monitored the influence of the electrodes on each other over a longer period of time. Here, four electrodes were connected to the circuit and placed in a beaker containing approximately 300 mL of synthetic seawater buffered with 2.0 mM $NaHCO_3$. The QuickBasic program was started and the potential of each electrode was recorded every 10 minutes over 4.5 days. The deviation from the initial potential for each electrode was then plotted over this period.

Figure A3-3 is a plot of the four signals recorded for this experiment. From this plot it is obvious that electrode #2929 was slowly drifting away from the other three electrodes. This observation also indicates that the electrodes were behaving independently. Had the electrodes not been electronically isolated from one another, one or more of the remaining electrodes would have drifted with #2929.

These preliminary experiments indicate that the circuit design (Figure A3-1) tested in this research can be used in an autonomous *in situ* potentiometric pH instrument. The ground loops which are commonly observed when measuring pH potentiometrically with more than one electrode seem to be eliminated with this design. Although these results look promising, more experiments (i.e., longer in duration and more rigorous) need to be made in order to determine if this circuit truly isolates the electrodes from one another.

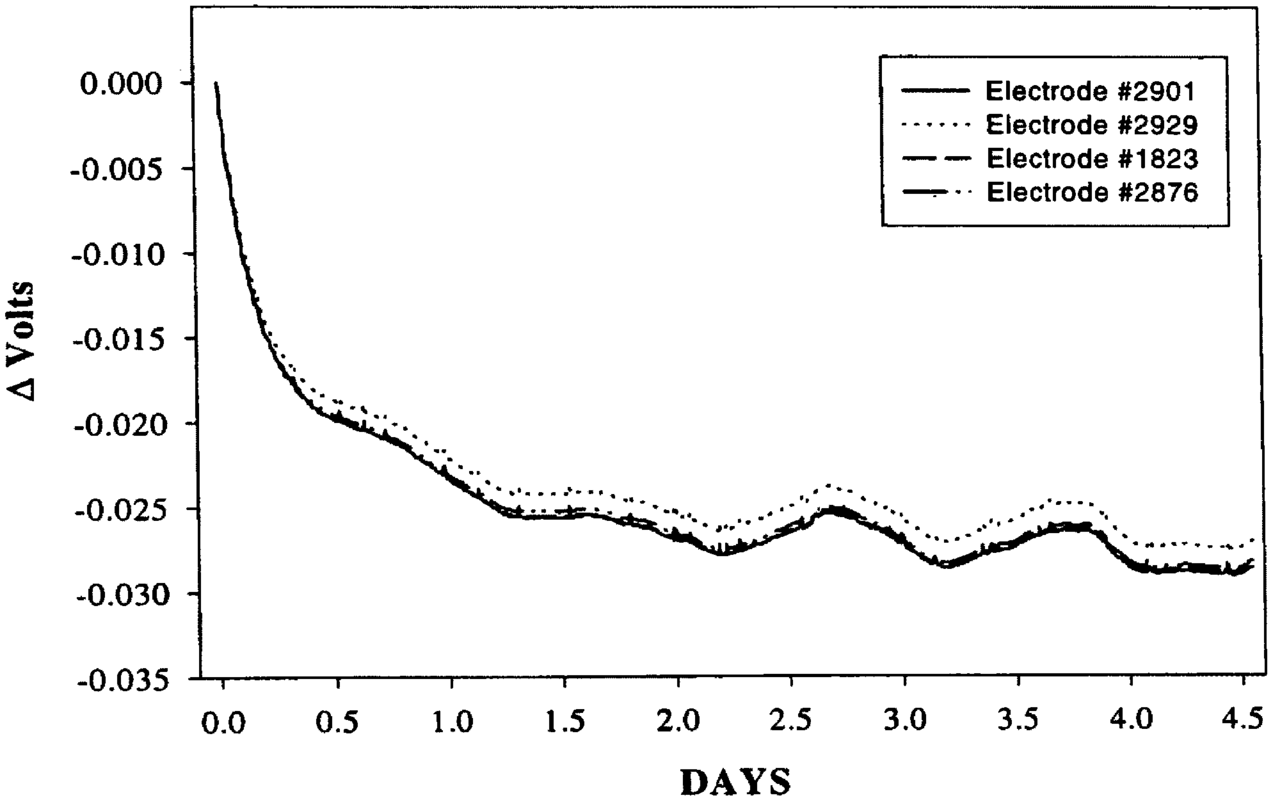

Figure A3-3: Independent behavior of four electrodes in synthetic seawater buffered with 2.0 mM NaHCO3

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