EXPERIMENT #1

Pipette Calibration, pH Measurement and Statistics

OVERVIEW

The purpose of this experiment is to become familiar with the lab by making some simple measurements with some of the tools we will use for other labs. The statistical concepts we have discussed in lecture (mean value, standard deviation, confidence intervals, etc.) will be applied to the calibration of micropipettes and to the pH measurement of several samples.

EQUIPMENT NEEDED

Lab Notebook, Safety Glasses, Lab Coat, gloves
Balance
Weighing boats
Teflon spatula
Deionized water
Thermometer
pH Probe and Orion 250A/290A pH Meter
Wash bottle with deionized water
100 ml volumetric flask
Glass Funnel
Micropipettes and pipette tips
Test tubes and rack for holding samples
Dilute sulfuric acid sample (provided by teaching assistant)
Cloud/fog samples (provided by teaching assistant)
Table of density of water (provided by teaching assistant)

PROCEDURE

Calibration of micropipettes

The Finnpipette micropipettes used in this lab are a convenient way to measure and dispense small volumes of liquids. Disposable polyethylene tips are used with these pipettes. The teaching assistant will demonstrate how to attach the tips, how to pick up liquids and dispense them, and how to discard the used tips. In later lab exercises it will be important to be able to use these pipettes correctly in order to properly measure solutions and prevent contamination of samples.

Check that the analytical balance you will use is level. Set the 40 - 200 µl pipette to 200 µl and use it to pick up a clean tip. Place a clean weighing boat on the balance and tare the reading to zero (depending on the balance you are using, you may have to open the glass doors in order to place things on the balance; it's a good idea to close the doors when taking readings to prevent interference from air currents). Add 200 µl of deionized water (DI water) and record the reading. Repeat this for a total of five measurements (either remove the weighing boat, empty it, dry it, and re-tare it, or continue adding water

to the weighing boat and re-tare it in between measurements -- which method do you think is more accurate?). Set the 200 - 1000 µl pipette to 1000 µl and record 5 measurements. Record the temperature of the DI water. The teaching assistant will have a chart of the density of water as a function of temperature. Use this chart and record the density of the water you have used for these measurements.

Calibrate pH probe

In order to start the calibration, first set the temperature. Turn on the pH meter and use the arrow keys to enter the temperature of the water you measured above. Then press the button on the bottom right hand of the meter marked "2nd" and then the button marked "cal". The display will show some numbers and then "P1", which means the meter is ready for the first calibration point. Rinse the probe with pH 7.00 buffer and place it in the test tube with the pH 7.00 solution. You should always use the pH 7 buffer for the first point of the calibration. Wait for the reading to stabilize (some of the meters beep when the reading is stable, flash the reading value and display the word "ready" next to the reading).

These pH meters are set to recognize standard buffer solutions of 4.01,7 and 10. Once the reading stabilizes, you should only have to press the "yes" key twice. If a reading other than the one desired is shown, press the “yes” key once and then use the arrow/”yes” keys in combination to select the appropriate values. The display should flash the value of the buffer solution at the temperature you've set (see the side of the large pH buffer bottles to see how the buffer pH changes with temperature). The display should now show "P2", which means the meter is ready for the second calibration point. Rinse the probe with pH 4.01 buffer and place it in the test tube with the pH 4.01 solution and wait for the reading to stabilize and then beep. Again, you should only have to press the "yes" key twice. The two-point calibration is complete, and the display should show a slope value (disappears after a few seconds). The model 290A meter may ask for a third calibration point (P3). Press the "measure" key to skip the third point. Once calibration is completed, the meter will display a slope reading. This value should be at least > 90. If not, calibration should be repeated until a high enough slope is obtained (should be close to 0.99). If this takes more than two tries, consult the TA.

When the battery is replaced, the meters automatically re-set themselves to shut off 10 minutes after the last button is pressed. This should not affect the calibration, but you should double-check the calibration by placing the probe back into a buffer to check after the meter has been turned back on.

If something went wrong with the calibration, an error code will appear (e.g. E-23) accompanied by several beeps and you will have to perform the calibration again. If the problem persists, consult the TA.

Checking the calibration periodically is a good idea. If one of the buffer solutions does not read within a few one-hundredths of the calibration value, you should recalibrate.

General electrode care: In between measurements (or when the pH portion of this experiment is completed), leave the probe soaking in the pH 4.01 buffer. For storage, fill the electrode cap half-way with storage solution (KCl) and cap the electrode. To recondition a dried or drifting electrode, store in a test tube with storage solution for an hour. Do not leave the probe for an extended period of time in DI water.

pH Measurement of the Diluted Acid Solution

Now that the pH probe has been calibrated, you can measure the pH of the diluted acid solution the teaching assistant has provided. Rinse the pH probe with 0.5 ml of the acid solution and then place it in a test tube with approximately 1 ml of the acid solution. Take three measurements of the pH of this so-

lution. Use the same tube with a fresh portion of acid each time, rinsing the probe with the dilute acid solution between each measurement.

You now want to make three measurements of a 1/10 dilution of this acid solution. Prepare enough diluted acid (mix well!) to make three pH measurements, remembering you need to rinse the electrode with solution prior to each measurement. Make the pH measurements and record the results in your lab notebook (you should be recording all procedures and results there).

pH of Deionized Water

Measure the pH of the DI water (three times) and record the result in your lab notebook. Recheck your calibration using fresh pH 7 and pH 4.01 buffers.

pH of Cloud/fog Samples

The teaching assistant will provide you with actual cloud/fog samples collected from two different locations. Measure the pH of each sample three times, using the same procedures you've used above with 0.5 ml for rinses and a fresh 1ml for the measurements.

Data Exchange

Before you leave the laboratory, be sure to review the list of topics below to discuss in your lab write-up. Exchange data with other groups as needed.

DISCUSSION/LAB WRITE-UP

Begin this, and each lab writeup, with a brief summary of the experiment in your own words. State the purpose of the experiment. Answer each of the questions below and address the discussion points. Consider these questions/discussion items as a starting point. Expand your analysis/discussion to include other relevant or interesting points as you see fit.

Statistical Evaluation

Using the statistical methods we've discussed in class and the material in Chapters 2-4 of the *Fundamentals of Analytical Chemistry* text by Skoog, West and Holler, answer the following questions:

1. What are the means and the 95% confidence limits for the pipette measurements?
2. What is the 95% confidence limit of your pH measurements for cloud/fog samples with pH < 4? Use pooled statistics for data from your group and other AT 560 groups to obtain a pooled standard deviation.
3. Are the pH measurements of the two cloud/fog samples you measured different at the 95% confidence level? Be sure to perform a hypothesis test; don’t just check whether the confidence intervals overlap.
4. Compare your measurements of the cloudwater sample pH values with those obtained by other lab groups. Are your results statistically different?

Discussion

- Comment on your experience with any difficulties measuring the pH of individual samples.
- Did you find it difficult to measure the deionized water pH value? Did your reading match your expectations?
- Compare the pH measurements of the original and diluted acid solutions. Is the pH difference what you expected?