## LABORATORY EXERCISE NO. 1: QUALITATIVE TESTS FOR CARBOHYDRATES

NAME/S : ____________________________________________ COURSE/YEAR: ____________________

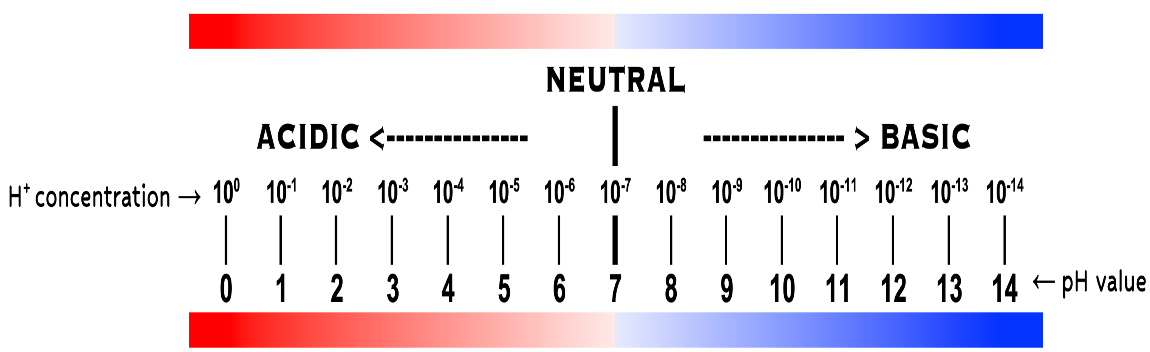
: ____________________________________________ DATE PERFORMED : ________________

: ____________________________________________ DATE SUBMITTED : ________________

### A. INTRODUCTION

All physiological processes within living cells are affected in a marked degree by the acidity or alkalinity of the medium in which they occur. $H^+$ concentration $[H^+]$ may affect the chemical structure (conformation) of substances and the concentration of molecular and charged species in solution. This in turn, may affect, for example, the transport of substances through membranes and/or the efficiency of enzymes. Therefore, it is very important to be able to measure accurately the $[H^+]$ in a solution, as well as to be able to regulate its concentration. The term "pH" stands for the power of the hydrogen ion, and referred to as the negative logarithm of the hydrogen-ion concentration $[H^+]$. Generally, the pH value of acids and bases are used to quantitatively define the relative strength of their "acidity." In this regard, a solution in which the $[H^+]$ is higher than $1{\times}10^{-7}$ M is "acidic," while a solution with $[H^+]$ lower than $1{\times}10^{-7}$M is "basic." It is cumbersome, however, to express the acidity of a solution by using the molarity (M) of $H^+$ because these quantities are generally very small. Thus, an easier system for indicating the concentration of $H^+$ called the pH scale was proposed by Søren Sørensen (1868-1939).

The pH scale, in general, is presented as running from 0 to 14, albeit it is possible to have a pH value of less than 0 or greater than 14. For example, a highly concentrated 3.0M of Hydrochloric acid (HCl) has a negative pH value. The figure given below illustrates the relationship between the concentration of $H^+$ and pH on a logarithmic scale. In context, solutions with pH of less than 7 are acidic, while solutions with pH values higher than 7 are basic.




In this laboratory exercise, you will learn how to determine the pH of a solution. There are two methods used to experimentally determine the pH value i.e., electrometric method and colorimetric method. In the electrometric method, an instrument called the pH meter is employed. The instrument operates based on the principle that the voltage of an electric current that passes through the solution being tested depends upon the pH value. The colorimetric method, on the other hand, is dependent upon the utility of indicators. In this regard, a weak organic acid or base, whose ionization is accompanied by a change in color, may be used as pH indicators. The most common pH indicator used in the laboratory is the litmus paper.

### B. INTENDED LEARNING OUTCOMES

At the end of this exercise, the student should be able to

1. Define the relevance of the pH concept in the chemistry;
2. Demonstrate how to use a pH meter and litmus paper to determine the pH of solutions; and
3. Classify the common substances either as acid or base based on their pH value.

## C. MATERIALS & METHODS

### SUPPLIES, MATERIALS, & APPARATUS

→ FOR THE WHOLE CLASS

- pH meter/pen
- Mid-point pH (6.86) calibration solution *
- 1L distilled water *
- 500 ml 20% (v/v) HCl solution
- 500 ml Brewed coffee
- 1L Zonrox bleach *
- 1L fresh milk *
- Low-point pH (4.01) calibration solution *
- High-point pH (9.18) calibration solution *
- 500 ml 20% (w/v) NaOH solution
- 500 ml 20% (v/v) $H_2SO_4$ solution
- 1L white vinegar *
- 1L soda (Coca-cola) *

→ FOR EACH GROUP/PAIR

- 1 pack pH test strips *
- 5 10-ml test tubes
- 6 50-ml beaker
- 1 alcohol lamp
- 10-ml graduated cylinder
- 3 pcs. fresh oranges *
- 3 pcs. fresh ripe tomatoes *
- 1 pc. bath soap *
- 1 roll cotton gauze *
- 1 test tube rack
- 1 timer *
- 1 lighter *
- 2 glass droppers *
- 3 pcs. fresh lemon fruits *
- 1 pack powdered detergent *
- 2 pcs. fresh eggs *

**** students should bring these materials to class***

### LABORATORY PROCEDURE

### PART A: Calibration of the pH Meter

The pH meter has three parts: a pH probe, a reference pH electrode, and the pH meter itself. It is the electrodes inside the probe that measure the hydrogen-ion activity in a solution. The probe records a voltage from the hydrogen ions, then the pH meter converts/digitize the voltage into a readable value by measuring the difference between the internal electrode and the reference electrode. The pH probe is connected to the display panel of the instrument which shows the measured pH value. Nowadays, most probes contained in a pH meter comprise both a glass hydrogen ion-sensitive electrode and a reference electrode, known as combination electrodes.

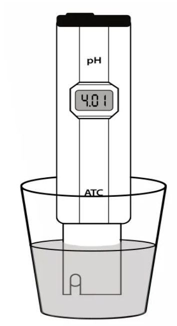
**TASK: Label the typical structures of a pH probe on the space provided in the worksheet.**

Calibration is an important (albeit mostly overlooked) step in pH testing. This is necessary because the electrodes found inside the pH probe change over time, which can cause errors. Therefore, instrument calibration is essential to be able to determine an accurate pH level.

To properly calibrate a pH meter, at least a 2-point calibration is required, utilizing two different buffer solutions of known pH level (i.e., pH 4 and pH 7). For high-accuracy pH measurements, however, a 3-point calibration (pH 4, pH 7, and pH 10) is used. The utility of the calibration solutions ensure that you obtain the correct pH values when the pH meter reads the solution. Before measuring the pH of a solution, always check the buffer/calibration solutions are within date and are fresh.

The sequential steps to properly calibrate the pH meter are straightforward. In the interest of time (and resources), however, your lab instructor will demonstrate to the class how the 3-point calibration is performed. We lean towards 3-point calibration as the same provides higher accuracy over a full pH range. Prior to the calibration proper, the standard pH solutions are dissolved in deionized (or distilled) water according to the manufacturer's instruction. The step-wise procedure for the 3-point calibration is generally done as follows at room temperature (~25ºC):

**STEP 1: MID-POINT CALIBRATION.** The mid-point calibration must be done at the first step, as it clears the other calibration points. To do this, rinse the pH probe well with distilled water prior to dipping in the pH 6.86 calibration solution. Place the probe inside the bottle (as shown in the figure below), and let it stay in the solution until the pH readings stabilize (this usually takes 1-2 minutes). Once the pH readings have stabilized, issue the mid-point calibration command: cal, mid, 6.86.




← **Proper way of dipping the probe of the pH meter into the test sample solution.**

**STEP 2: LOW-POINT CALIBRATION.** Rinse the pH probe with distilled water pat dry with a paper towel before calibrating the low-point. Insert the probe inside the acidic buffer solution (pH 4.01), then wait for the pH readings to stabilize. After the pH reading has stabilized, issue the low-point calibration command: cal, low, 4.01.

**STEP 3: HIGH POINT CALIBRATION.** Again, rinse the probe with distilled water then pat dry prior to placing it in the basic buffer solution (pH 9.18). Wait for a while until the pH readings stabilizes, then issue the calibration command: cal, high, 9.18.

Note that the accuracy of the standard pH calibration solutions only lasts for about 20 minutes, as the same are subject to contaminants once exposed to the open air. Hence, these are no longer suitable as standard reference in the succeeding pH meter calibration attempts.

Once the pH meter has been properly calibrated, you can now practice using the instrument. To do this, obtain 4 unknown samples (A-D) and place 10 ml of each it in a 50-ml beaker. Then, measure the pH of each unknown sample using the pH meter. Note that the probe must be rinsed first with distilled water (and pat dry with a paper towel) before the next reading is done. Place the unknown samples in boiling water bath for 2 minutes, then re-measure the pH. Record the pH of each unknown sample (on the space provided in the worksheet section. Heat the unknown samples

### PART B: Measurement of pH Level

Place 10 ml each of the test solutions listed below in clean test tubes. To determine the approximate pH range of the samples using a rapid pH indicator, place 1-2 drops of the test solution directly on the paper test strip, then wait for at least 5-10 seconds for any color change to occur. Interpret the results by comparing the color of the strip obtained for each test solution to the standard reference. Read the result within 1 minute soon after the solution is dropped on the paper strip, otherwise the test becomes invalid. Record your observations on the space provided in the worksheet section. Using the same test solutions, transfer 10 ml each into 50-ml beaker and measure the pH with a pH meter. Make sure that the pH meter is properly calibrated prior to measurement the pH.

| | | |
|---|---|---|
| NaOH solution | Zonrox bleach | Bath soap solution * |
| Detergent solution * | Egg white | Distilled water |
| Fresh milk | Brewed coffee | Tomato juice ** |
| Orange juice ** | Lemon juice ** | Soda (Coca-Cola) |
| White vinegar | HCl solution | H2SO4 solution |

* Prepare the test solution by dissolving 10 g of the sample material to 100 ml of distilled water
** Squeeze the fruit then filter out the pulp through a cotton gauze to obtain the test sample

### PART C: Mathematics of the pH Concept

Traditionally, solutions were labeled as being acidic or basic based on their taste and texture. Those that tasted sour were known to be acidic, while solutions that tasted bitter and were slippery to touch were considered to be basic. Thus, common substances such as lemon juice and vinegar were identified as acids; and solutions with lye and caustic soda as bases. Several definitions have been proposed for acids and bases. Depending upon the situation, one or more definition is applicable.

In this case, the hydronium ion, $H_3O^+$, forms when a proton, $H^+$, is transferred from one $H_2O$ molecule to another. The other species that result from this acid-base reaction is the hydroxide ion, OH-. Hence, while one water molecule (i.e., the proton acceptor) functions as the base, the other assumes the role of the acid. The ability of one water molecule to accept a proton (H+) from another water molecule or any acid, is due to the two lone pairs of electrons on the oxygen atom of water.

The autoionization results in equal molar amounts of $H_3O^+$ ions and $OH^-$ ions and hence the solution is neutral. In a sample of pure $H_2O$, the concentrations of $H_3O^+$ and $OH^-$ at 25 ºC are $1.0 \times 10^{-7}$ M (Eq. 1). The concentration of hydronium ion $[H_3O^+]$ for a given solution is commonly expressed in terms of the pH of the solution, which is defined as the negative logarithm of $[H_3O^+]$ or $[H^+]$ in the solution (Eq. 2). Thus, using Eq. 3, the $[H^+]$ can be obtained from the pH of the solution. Similarly, the $[OH^-]$ for a given solution is mostly expressed in terms of the pOH of the solution, which is defined as the negative logarithm of $[OH^-]$ (Eq. 4); while the $[OH^-]$ can be obtained from the pOH of the solution using Eq. 5. Additionally, the pH and pOH of any aqueous test solution are related, as are the $[H^+]$ and the $[OH^-]$. The relevant equations are given in Eq. 6 and Eq. 7, respectively.

**TASK: Using these equations, solve the practice problem sets below. Show your calculations and answers on the space provided in the worksheet.**

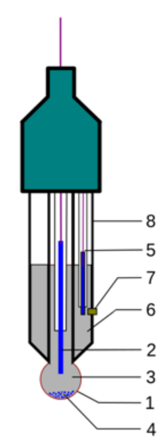
**Eq. 1** $[H_3O^+] = [OH^-] = 1.0 \times 10^{-7}$ M
**Eq. 2** $[H_3O^+] = 10^{-pH}$
**Eq. 3** $pOH = - \log [OH^-]$
**Eq. 4** $[OH^-] = 10^{-pOH}$
**Eq. 5** $[H^+] [OH^-] = 1.0 \times 10^{-14}$
**Eq. 6** $pH + pOH = 14$

**Problem 1:** What is the pH and pOH of a solution that contains 3.50 x 10-5 M hydronium ions?
**Problem 2:** Calculate the [H3O+] and $[OH^-]$ of a test sample solution that has a pOH equal to 4.40.
**Problem 3:** The pH of a solution is 12.4. Calculate the a. [H3O+], b. pOH, and c. $[OH^-]$.

## D. WORKSHEET

### PART A: The pH Meter

⇊ Label the typical structures of a pH probe.




| | |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |

⇊ Record the pH level of the 4 unknown solutions (heated and non-heated) on the corresponding given space.

| | | | | |
|---|---|---|---|---|
| NON-HEATED | | | | |
| HEATED | | | | |
| | Unknown A | Unknown B | Unknown C | Unknown D |

PART B: pH Level of Common Substances

⇣⇣ Indicate the pH level of the different test samples measured using a pH strip and a pH meter.

| Sample | pH strip | pH meter |
|---|---|---|
| NaOH solution | | |
| Zonrox bleach | | |
| Bath soap solution | | |
| Detergent solution | | |
| Egg white | | |
| Distilled water | | |
| Fresh milk | | |
| Brewed coffee | | |
| Tomato juice | | |
| Orange juice | | |
| Lemon juice | | |
| Soda (Coca-cola) | | |
| White vinegar | | |
| HCl solution | | |
| $H_2SO_4$ solution | | |

PART C: Problem Sets

⇣⇣ Show your calculations and answers to the practice problem sets.

| Problem 1: | Problem 2: |
|---|---|
| | |

| Problem 3: | | |
|---|---|---|
| a. | b. | c. |

## E. QUESTIONS TO ANSWER

1. Can the colorimetric method be used to determine the pH of water? milk? blood? Why?

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2. How would the pH of fertile soil differ from the pH of a depleted soil? Explain.

3. Glucose in aqueous solution has a pH of 7. What does that tell you about the number of $H^+$ and $OH^-$ ions it has? Briefly elaborate your answer.

4. Why is it that you need to use both red and blue litmus paper when testing for acids and bases?

## F. CONCLUSION

## G. REFERENCES