The Effects of pH and Concentration on the Coloring of Eggs

Introduction

This is a fun exercise used to illustrate the effect that the pH of a solution and the concentration of dye has on the amount of dye that adheres to the surface of an egg. This laboratory exercise uses inexpensive and readily available material. It introduces the student to the concepts and definitions of acids, bases, pH, ionic bonding, and salt bridge formation. The laboratory exercise vividly illustrates the effect of pH on proteins and why this is important, as well as giving hands on experience in making solutions of differing pH and solute concentration.

Goals:

1.) Introduce and define acids and bases.
2.) Introduce and define molarity.
3.) Introduce and define pH.
4.) Discuss ionic bonding and salt bridge formation.
5.) Perform calculations based on pH.
   a.) Calculate the hydronium ion concentration knowing the pH.
   b.) Calculate the pH of a solution knowing the hydronium ion concentration.
6.) Prepare solutions of differing pH.
7.) Prepare solutions of differing concentration.
8.) Gain an appreciation of how pH affects proteins.

Definition of acids and bases

There are several definitions of acids and bases. Perhaps the most familiar definition is that of Arrhenius acids and bases. An **Arrhenius acid** is a chemical compound that increases the concentration of hydrogen ions, H^+, in aqueous solutions, while an **Arrhenius base** is a compound that increases the concentration of hydroxide ions, OH^-, in aqueous solution.

Arrhenius acids and bases are easily recognized from their chemical formula. An Arrhenius acid will have a replaceable or ionizable hydrogen atom(s) present in the correctly written chemical formula. A replaceable or ionizable hydrogen atom is one that will form a hydrogen ion in aqueous solution. For example, hydrochloric acid, HCl, has one ionizable hydrogen atom and will form one hydrogen ion, H^+, and one chlorine ion, Cl^-, in aqueous solution for every molecule of HCl present. Similarly, acetic acid, $HC_2H_3O_2$, has one ionizable hydrogen atom, and will form a hydrogen ion in an aqueous solution. The other ion formed will be the acetate ion, $C_2H_3O_2^-$. An Arrhenius acid may have more than one ionizable hydrogen atom present. For example, sulfuric acid, H_2SO_4, and phosphoric acid, H_3PO_4, have two and three ionizable hydrogen atoms, respectively.

Arrhenius bases can also be easily recognized from their correctly written chemical formula. Arrhenius bases contain the polyatomic hydroxide ion, OH^-. Sodium hydroxide, NaOH, is an example of an Arrhenius base. When dissolved in water, sodium hydroxide forms sodium ions, Na^+, and hydroxide ions, OH^-. Similar to Arrhenius acids, Arrhenius bases may have more than one ionizable or replaceable hydroxide ion. For example, barium hydroxide, $Ba(OH)_2$, and aluminum hydroxide, $Al(OH)_3$, have two and three replaceable hydroxide ions, respectively.

Acid and base strength is related to the ability of the acid or base to completely dissociate into its respective ions. For example, hydrochloric acid is considered a strong acid because it completely dissociates into hydrogen ions and chlorine ions in an aqueous solution, while acetic acid is considered a weak acid because it does not completely dissociate into hydrogen ions and acetate ions. In an aqueous solution of acetic acid, there are three species of ions and molecules present: hydrogen ions, acetate ions, and acetic acid molecules.

There are only seven strong acids:

Hydrochloric acid (HCl)
Hydrobromic acid (HBr)
Hydroiodic acid (HI)
Sulfuric acid (H_2SO_4)
Nitric acid (HNO_3)
Chloric acid ($HClO_3$)
Perchloric acid ($HClO_4$)

Strong bases include Group 1 metal hydroxides (LiOH, NaOH, KOH, RbOH, and CsOH) and the heavy group 2 metal hydroxides ($Ca(OH)_2$, $Sr(OH)_2$,and $Ba(OH)_2$). These strong acids and bases are completely dissociated or ionized into their component ions in an aqueous solution. All other acids and bases are considered weak acids and bases as they are only partly ionized in aqueous solutions.

The amount of hydrogen ions in solution is used to determine the pH of a solution. The pH scale commonly used goes from 0 to 14 with values of less than 7 acidic and values greater than 7 basic. A value of 7 is considered neutral.

Hydrogen chloride dissolved in water forms hydrochloric acid, which consists of hydrogen ions and chlorine ions in solution. The substance dissolved, in this case HCl, is called the solute. The dissolving medium, in this case water, is called the solvent. The resulting homogeneous mixture is called a solution. If the concentration of a solute, such as hydrogen ions, is measured in moles of solute per liter of solution, the concentration can be expressed in molarity, abbreviated with a capital M. If the hydrogen ion concentration is expressed in moles per liter of solution, the pH of the solution can be determined by finding the negative common logarithm of the hydrogen ion concentration:

$$pH = -\log[H^+]$$

The brackets surrounding H^+ ([ ]) denote concentration of the substance inside the bracket measured in moles per liter. For example, if the hydrogen ion concentration is 1×10^{-7} moles /liter, then the pH of the solution would be 7 because the negative common logarithm of 1×10^{-7} is 7. Thus, knowing the hydrogen ion concentration in moles per liter, the pH of the solution can be calculated.

If the pH of a solution is known, the concentration of the hydrogen ion can be determined because the antilog of a common logarithm is 10 raised to the number. Therefore, the hydrogen ion concentration of a solution of a given pH is 10 raised to the negative numerical value of the pH:

$$[H^+] = 10^{-pH}$$

For example, a solution with a pH of 4 would have a hydrogen ion concentration of 1×10^{-4} moles/liter.

In this laboratory exercise, eggs will be dyed using various solutions of food dyes. Dyes used in foodstuffs are approved by the Food and Drug Administration (FDA). Darker colors, such as red no. 4 and blue no. 1, best illustrate the effect of the varying pH and dye concentration, however, any color will work. Egg shells are composed of calcium carbonate, $CaCO_3$, surrounded and protected by a thin layer of protein called the cuticle. It is this protein to which the dye binds through the formation of a salt bridge which colors the egg. A salt bridge forms between the negatively charged dye particle and the positively charged protein. The protein is composed of long chains of amino acid residues, some of which possess amino groups ($-NH_2$). Under acidic conditions, when the hydrogen ion concentration is high, some of the amino groups gain an extra hydrogen ion, thus becoming positively charged ($-NH_3^+$). These positively charged cations attract negatively charged anions forming a bond between the oppositely charges ions. This type of bond is called an ionic bond, which is a bond that results from the attraction of a positively charged cation and a negatively charged anion. Another way of describing the bond is to say that a salt bridge is formed between the cation of the protein and the anion of the dye.

Procedure

Effect of pH on the adsorption of dye:

Prepare a stock solution of French's red food color by adding 90 mL of dye to 1290 mL water. Prepare twelve solutions using 80 mL of the red dye stock solution and adjust the pH of each solution by addition of either HCl(aq) or NaOH (aq), as needed, so that the resulting solutions span the pH range of 1 to 12 differing by one pH unit. Add one hard-boiled egg to each solution and allow to stand at room temperature for 10 minutes. After soaking for 10 minutes, remove the egg, rinse with water and allow to dry at room temperature. A significant graduation in color should be observed as the pH of the dye solution changes from one through twelve with the darker colored eggs at the lower pH.

Effect of dye concentration on color of the egg:

Using the stock solution prepared above, prepare twelve solutions by successive serial dilution so that each new solution has one-half the dye concentration as the previous solution. For example start with 160 mL of the stock solution. Take 80 mL of this solution and label it solution one. To the remaining 80 mL add 80 mL water and mix thoroughly. After mixing remove 80 mL and label this solution two. It will have one-half the dye concentration as solution one. To the remaining 80 mL, add 80 mL water and repeat the procedure. Each successive solution will have one-half the concentration as the previous solution.

After all twelve solutions have been prepared, add one egg to each solution and proceed as above. After drying, the color of the eggs should show a significant graduation of color intensity with solution one the darkest and solution twelve the lightest.

Discussion

French's red food dye is an anionic or acidic dye due to the carboxylate group ($-CO_2^+$) present on the molecule. This anionic carboxylate group directly binds to the cationic amino group of the cuticle when it is ionized (becomes protonated: $-NH_2 \rightarrow -NH_3^+$) at lower pH. As the pH of the dye solution increases, fewer and fewer amino groups are protonated, resulting in fewer and fewer sites for the formation of a salt bridge between the cation of the protein and the anion of the dye. The observer result is a decrease in color intensity as the pH is increased.

The results of the second experiment should be as predicted. The gradual decrease in color intensity as the concentration f the dye solution is reduced is a direct result of fewer ions of dye available to form salt bridges with the existing protonated amino groups of the cuticle.

Comments

This should be a fun lab where students learn about acid, bases, and pH. Students can gain experience making solutions, adjusting the pH of a solution, and making dilutions. The results obtained should reinforce concepts discussed in pre-lab discussions. Pre-lab discussions can be expanded to include a discussion of the Bronsted-Lowry and Lewis concept of acid and based. Also, the self-ionization of water can be introduced and the discussion expanded to include the relationship between the pH and pOH. Additional problems relation pH and pOH can be included to reinforce this concept. The discussion can be further expanded to include a related topic, the ability of a solution to conduct electricity, by relating strong, weak, and non-electrolytes to the solutions used in the laboratory.

The actual laboratory exercises can be expanded to include observations of the effect that varying the amount of acetic acid present in solution has on dye adsorption, dying marble chips which are pure calcium carbonate (no protein present), dying eggs which had the cuticle removed by treatment with 5% EDTA, pH 8.0, and studying the effect of ionic strength of the dye solution on dye adsorption.

Reference

Mebane, R. C. and Rybolt, T. R. 1987. Chemistry in the dyeing of eggs. J. Chem. Ed. 64: 291-293