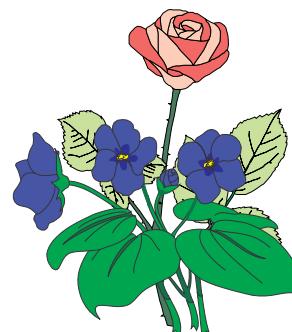


Natural Indicators

Natural Indicators and Household Substances

Introduction

Roses are red, violets are blue—or are they? Red roses, as well as many other flowers and fruits, contain natural indicators that are sensitive to acids and bases. The color of a natural acid–base indicator depends on pH. One of the most well known effects of natural indicators in plants occurs in the hydrangea or snowball plant. Hydrangea flowers are blue when grown in acidic soils and pink or red in basic soils. How do the colors of natural indicators vary with pH?



Concepts

- Acid–base indicators
- Weak acid vs. conjugate base
- Extraction
- pH Scale

Background

The purpose of this experiment is to extract natural indicators and design a procedure to investigate their color changes as a function of pH. A set of standard acid and base solutions of known pH (pH = 2–12) will be provided. The results will be used to construct color charts of the indicators. In Part B, the natural indicators will be used, along with other known indicator solutions, to analyze the pH values of unknown solutions.

Materials

Standard acid and base solutions of known pH (pH 2–12), 5 mL each	
Natural indicator sources	Water, distilled or deionized
Dried flowers, 2–3 g	Beakers, 100- and 150-mL, 1 each
Herbal tea	Color pencils, 1 set (optional)
Grape juice	Pipets, Beral-type, 5
Indicator solutions, 5 mL each	Reaction plate, 24-well
Thymol blue	Funnels and filter paper (optional)
Methyl orange	Hot plate (optional)
Bromthymol blue	Mortar and pestle (optional)
“Unknown” acids and bases, 5 mL each	

Safety Precautions

The standard acid and base solutions used in this experiment are body tissue irritants. Avoid contact of all chemicals with eyes and skin. Food-grade items that have been brought into the lab are considered laboratory chemicals and are for lab use only. Do not taste or ingest any materials in the lab and do not remove any remaining food items after they have been used in the lab. Wear chemical splash goggles and chemical-resistant gloves and apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

Preparation. Extraction of Natural Indicators

- For rose petals and hibiscus:
 - Obtain 2–3 g of dried flowers and place them in a 150-mL beaker with 50–60 mL of water.
 - Place the beaker on a hot plate or Bunsen burner setup and heat the flower/water mixture for 5–10 minutes.
 - Cool the mixture and decant or filter the liquid into a clean, 100-mL beaker. The natural indicator solution should be strongly colored but clear. If necessary, filter the mixture to obtain a clear solution.
- For teas: Place one tea bag in a 150-mL beaker, add 50-mL water, and heat to just below the boiling point for 10–15 minutes. Cool and decant the liquid into a clean, 100-mL beaker.
- For juices: Juices may be used directly “as is”, with no pre-treatment, in Parts A and B.

Part A. Indicator Color Changes

- Design a procedure using the standard acid and base solutions of known pH to determine the color changes for the natural indicator solution and the pH intervals in which the color changes occur.
- Construct a data table to record the results.
- Show the data table and discuss the proposed procedure with your instructor.
- Carry out the procedure and record the results.

Part B. Classifying Unknown Solutions

- Design a procedure using your natural indicator solution and at least one synthetic indicator to determine the pH values of unknown solutions. *Hint:* Choose indicators that will give you the narrowest range possible for the pH value of each unknown. The color charts for the available indicators are shown in Table 1.
- Construct a data table to record the results.
- Show your data table and discuss the proposed procedure with your instructor.
- Carry out the procedure and record the results.

Table 1.

pH Value	0	1	2	3	4	5	6	7	8	9	10	11
Thymol Blue	Red			Yellow							Blue	
Methyl Orange	Red					Yellow						
Bromthymol Blue	Yellow							Blue				

Disposal

Consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. All of the solutions may be flushed down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Tips

- Use buffer capsules to prepare standard acid–base solutions of known pH. Buffer capsules contain pre-weighed amounts of stable, dry powders that dissolve in distilled or deionized water to give solutions of known, constant pH. Dissolve each capsule in 100 mL of distilled or deionized water.
- Label unknowns with letter codes for student use. See also the *Supplementary Information* section for suggestions on using

household substances as unknowns in Part B.

- Suitable unknowns for student use include dilute 0.1 M solutions of acetic acid, sodium bicarbonate, sodium carbonate, and sodium phosphate, monobasic.
- This experiment is designed as a fun, inquiry-based study of indicators and their uses. Students generally enjoy any kind of “natural product” chemistry and this lab is no exception. Because this experiment works well and the materials are not hazardous, the students can be given a great deal of freedom in designing their laboratory procedure. This approach usually gives students more pride and ownership of work. The experiment can reasonably be completed in one 50-minute lab period.
- To allow more time for measuring the indicator color changes and testing unknowns, consider setting up the extraction the day before lab. The natural indicators may be extracted overnight with water at room temperature. The amount of preparation time (to measure the dried flowers and place them in a beaker with water) is minimal—15 minutes should be sufficient. Cover the beaker with Parafilm™ or plastic wrap and allow the extraction to take place overnight. On the day of lab, filter or decant the mixture and use the resulting indicator solution in Parts A and B. Alternatively, extraction of the natural indicators with hot water may be completed as a homework assignment.
- Flowers and fruits may be extracted with hot water or 70% isopropyl (rubbing) alcohol at room temperature. There is no difference in the color charts of natural indicator solutions obtained using these different solvents. Use isopropyl alcohol only in a well-ventilated lab setting. The recommended minimum extraction time for isopropyl alcohol is 15 minutes regardless of the solvent used.
- Although the extraction step may be the most enjoyable part of this lab for students, it does tend to get a little messy, especially if the experiment is performed by multiple lab sections in the same room on the same day. Teachers may find it convenient to prepare the indicators ahead of time.
- A wide range of fruits and flowers contain natural acid–base indicators. The table on the following page summarizes the information obtained from a brief literature survey. The list is not meant to be exhaustive, but rather to demonstrate the variety of options suitable for classroom study.

Natural Indicators *continued*

Natural Fruit and Vegetable indicators	Natural Flower Indicators	Do Not Give Natural Indicators
Apple skin (red)	Dahlias	Daffodils
Beets	Daylilies	Daisies
Blueberries	Geraniums	Dandelions
Cabbage (red)	Hibiscus	Marigolds
Cherries	Hollyhocks	Mums (yellow)
Cranberries	Hydrangeas	
Grapes (red or purple)	Iris (blue)	
Onions (red)	Morning Glories	
Peaches	Mums (purple)	
Plums	Pansies	
Radish skin	Peonies	
Rhubarb skin	Petunias	
Strawberries	Poppies	
Tomato leaves	Roses (red, pink)	
Turnip skin	Violets	

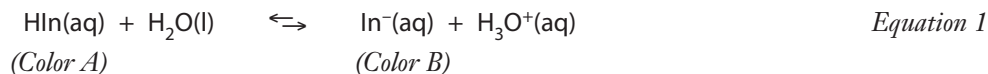
cators illustrates the definition of Brønsted acids (proton donors). Most natural indicators are further classified as weak acids (dissociate only partially in water and their reactions with water are reversible). The different colors observed for natural indicators thus reflect the position of equilibrium under different conditions. The color transitions are examples of LeChâtelier's Principle in action. Use Equation 1 to predict the direction the indicator equilibrium will be shifted as a result of increasing or decreasing the H_3O^+ concentration.

- A picture is worth a thousand words! Have students draw color charts of indicator color changes using colored pencils.

Discussion

Indicators are dyes or pigments that are isolated from a variety of sources, including plants, fungi, and algae. For example, almost any flower that is red, blue, or purple in color contains a class of organic pigments called anthocyanins that change color with pH. The use of natural dyes as acid–base indicators was first reported in 1664 by Sir Robert Boyle in his collection of essays *Experimental History of Colours*. Indeed, Boyle made an important contribution to the early theory of acids and bases by using indicators for the classification of these substances. The idea, however, may actually have originated much earlier—medieval painters used natural dyes treated with vinegar and limewater to make different color watercolor paints.

Acid–base indicators are large organic molecules that behave as weak acids—they can donate hydrogen ions to water molecules to form their conjugate bases (Equation 1). The distinguishing characteristic of indicators is that the acid (HIn) and conjugate base (In^-) are different colors.



The abbreviation HIn represents an uncharged indicator molecule, and In^- an indicator ion after it has lost a hydrogen ion. The color changes of acid–base indicators illustrate an application of reversible reactions and equilibrium. Because indicators are weak acids, the reactions summarized in Equation 1 are reversible. Reversible reactions are easily forced to go in either

direction, depending on reaction conditions. The actual color of an indicator solution thus reflects the position of equilibrium for Equation 1 and depends on the concentration of H_3O^+ ions (and hence the pH) of the solution.

There are three possible cases. (1) Most of the indicator molecules exist in the form HIn and the color of the solution is essentially the color of HIn. (2) Most of the indicator molecules exist in the form In^- and the color of the solution is essentially the color of In^- . (3) The solution contains roughly equal amounts of the two forms and the resulting color is intermediate between that of HIn and In^- . The exact concentrations of H_3O^+ at which cases 1–3 will predominate depend on the structure of the indicator and the equilibrium constant for Equation 1. Different indicators change color in different pH ranges.

Natural indicator solutions are obtained by treating flowers and fruits with a solvent to dissolve the soluble components. This process, called extraction, is similar to the procedure used to make a cup of tea using a tea bag. The solid is crushed or ground and extracted with an appropriate solvent, such as boiling water, ethyl alcohol, or rubbing alcohol.

The color of an acid–base indicator depends on the concentration of H_3O^+ ions, which is most conveniently expressed using the pH scale. The mathematical relationship between pH and $[H_3O^+]$ is given in Equation 2.

$$pH = -\log[H_3O^+] \quad \text{Equation 2}$$

The H_3O^+ concentration in water ranges from 1 M in 1 M hydrochloric acid to 10^{-14} M in 1 M sodium hydroxide. In pure water, which is neutral (neither acidic nor basic), the H_3O^+ concentration is equal to 10^{-7} M. The logarithm of the concentration is the “power of ten” exponent in these concentration terms. Thus, the negative logarithms (Equation 2) of typical H_3O^+ concentrations are positive numbers from 0–14. The pH scale ranges from 0–14, with 7 being neutral. Acids have pH values less than 7, while bases have pH values greater than 7.

Within the pH range of acid solutions, either a more concentrated or a strong acid solution will have a lower pH than a less concentrated or a weak acid solution, respectively. Thus, the pH values of 0.1 and 0.01 M HCl solutions are 1 and 2, respectively, while the pH of 0.1 M acetic acid (a weak acid) is about 3. On the basic side of the pH scale, either a more concentrated or strong base solution will have a higher pH than a less concentrated or a weak base solution, respectively. Thus, the pH values of 0.1 and 0.01 M NaOH solutions are 13 and 12, respectively, while the pH of 0.1 M ammonia (a weak base) is about 11. Remember that the pH scale is logarithmic—a solution of pH 3 is ten times more acidic than a solution of pH 4, and 100 times more acidic than a solution of pH 5. Figure 1 summarizes the pH scale and the pH range of acids and bases.

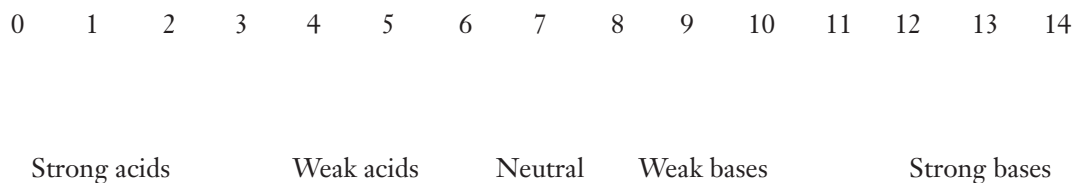


Figure 1. The pH Scale

Sample Data. Indicator Color Changes¹

Indicator Source	Roses (Red)	Cranberry Apple Tea	Hibiscus	Grape Juice
Color of Extract	Deep red	Red	Red	Purple
Color Changes as a Function of pH				
pH 2	Pink	Pink	Dark pink	Dark pink
pH 3	Light pink	Pink	Pink	Pink
pH 4	Pale pink	Light pink	Pink	Pink
pH 5	Very pale pink	Pale pink–colorless	Pale pink	Pale pink

Natural Indicators *continued*

pH 6	Colorless	Pale pink– colorless	Lavender	Pale pink
pH 7	Light brown	Light gray	Gray	Light gray
pH 8	Pale green	Pale green	Gray-blue	Gray
pH 9	Light green	Light green	Dark gray ²	Light green
pH 10	Yellow-green	Yellow-green	Brown ²	Green
pH 11	Yellow-green	Yellow	Brown ²	Green
pH 12	Gold	Gold	Green ²	Olive green

¹ Colors may vary due to concentration of the indicator solution.

² Fades to greenish-brown.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

Constancy, change, and measurement

Content Standards: Grades 5–8

Content Standard A: Science as Inquiry

Content Standard B: Physical Science, properties and changes of properties in matter

Content Standards: Grades 9–12

Content Standard A: Science as Inquiry

Content Standard B: Physical Science, structure and properties of matter, chemical reactions

Answers to Post-Lab Questions

1. Assume that the pH 2 color of the natural indicator represents its most acidic form (HIn).

a. What is the pH range in which the most acidic form predominates?

b. Calculate the lowest H_3O^+ concentration at which the indicator still exists in this form.

*For the red rose indicator as an example, the most acidic form of the indicator is pink in color. This acidic form of the indicator appears to be the predominant form of the indicator in solution at pH values less than or equal to 4. The lowest H_3O^+ concentration at which the acidic form predominates is therefore 1×10^{-4} M. **Note to teacher:** At pH 5–6, the solution is still pink, but it becomes very pale in color; and finally almost colorless at pH 7. The very pale pink color thus appears to be a “transition” or intermediate color.*

2. Assume that the pH 12 color of the natural indicator represents its most basic form (In^-).

a. What is the pH range in which the most basic form predominates?

b. Calculate the highest H_3O^+ concentration at which the indicator still exists in this form.

For the red rose indicator as an example, the most basic form of the indicator is yellow in color. This basic form of the indicator appears to be the predominant form of the indicator in solution at pH values greater than or equal to 10. The highest H_3O^+ concentration at which the basic form predominates is therefore 1×10^{-10} M.

3. For one of the unknown acid–base solutions that you tested, explain why you chose the combination of indicators you did to determine the pH value of the solution. What is the advantage of using multiple indicators, rather than a single indicator, to determine the pH of a substance?

In the case of Unknown C (sodium bicarbonate), the natural red rose indicator gave a pH estimate of 8–9. Bromthymol blue was selected as an alternate indicator to confirm or narrow the pH range. Bromthymol blue was green, suggesting that the pH was in the 7–8 range. The overlap of these two pH estimates suggests that the pH of the solution is very close to 8. Using multiple indicators, rather than a single indicator, often makes it possible to obtain a more precise (narrow) estimate of the pH of a substance.

4. Construct a Results Table to summarize the properties of the unknowns.
 - a. Estimate the pH value of each unknown.
 - b. Classify each solution as acidic or basic.
 - c. Within each class of unknowns—acids and bases—arrange the solutions in order from least acidic to most acidic and least basic to most basic, respectively.

Analysis of Household Substances

The following household substances provide convenient unknowns for classifying acid–base solutions using indicators.

Alka-Seltzer®	Grapefruit juice
Ammonia, household	Hair spray
Antacid tablet	Hand lotion
Aspirin tablet	Laundry detergent
Baking powder	Lemon-lime soda
Baking soda	Lemon juice
Bleach	Milk
Club soda	Mouthwash
Cola	Shampoo
Contact lens solution	Tea
Cream of tartar	Toothpaste
Drain cleaning solution	Vinegar
Fruit Fresh®	Vitamin C tablet
Ginger ale	Windex®

Natural Indicators Worksheet

Post-Lab Questions

1. Assume that the pH 2 color of the natural indicator represents its most acidic form (HIn).
 - a. What is the pH range in which the most acidic form predominates?

 - b. Calculate the lowest H_3O^+ concentration (highest pH) at which the indicator still exists in the HIn form.

2. Assume that the pH 12 color of the natural indicator represents its most basic form (In^-).
 - a. What is the pH range in which the most basic form predominates?

 - b. Calculate the highest H_3O^+ concentration (lowest pH) at which the indicator still exists in the In^- form.

3. For one of the unknown acid–base solutions that were tested, explain why you chose the combination of indicators you did to determine the pH value of the solution. What is the advantage of using multiple indicators, rather than a single indicator, to determine the pH of a substance?

4. Construct a Results Table to summarize the properties of the unknowns.
 - a. Estimate the pH value of each unknown.
 - b. Classify each solution as acidic or basic.
 - c. Within each class of unknowns—acids and bases—arrange the solutions in order from least acidic to most acidic and least basic to most basic, respectively.