Blue Bottle Experiment

A colorless solution partially fills a flask, is shaken, and the solution becomes blue. Upon standing, the solution returns to colorless. Further shaking regenerates the blue color. Repeating the shaking/standing procedure allows multiple observations. Other indicators produce different color changes.

Overall Goal

Demonstration, investigation and discussion of the reaction reinforces and demonstrates the application of scientific method to determine the kinetics of the reaction and thus the mechanism.

For advanced students, the reaction rate law can be found by measuring how long it takes for a solution of known concentration to go colorless. Additionally, the activation energy can be calculated using a normal Arrhenius plot - natural logarithm of the de-coloration time [ln(t)] against the reciprocal of absolute temperature [1/T]. This can be done because the rate of the slow step is independent of the oxygen concentration, and thus the time, t, which is required for the total oxygen to disappear, is directly related to the rate constant, k. A plot of ln(t) against 1/T results in a straight line.

The rate law for the reaction is: $Rate = k[MB_{ox}][CH][OH^-]$

where MB_{ox} is the oxidized (blue) form of methylene blue and CH is the carbohydrate, dextrose.

Background Information

To understand the demonstration, students should previously be familiar with the following topics:

- Scientific Method
- Indicators
- pH
- Concentrations and Reaction Rates

Before the demonstration, students should be able to:

- Identify the general pH range indicators produce by reading a table.
- Advanced students should be able to quantify reaction rates based on concentration.
- Advanced students should be familiar with and able to graph using log scales.

This demonstration should follow the discussion, qualification and quantification of chemical reactions, as well as concepts of pH and indicators.

Blue Bottle Experiment

Equipment
1 or more 500-mL Florence (or Erlenmeyer) flask(s)
Rubber stopper to fit flask(s)

Reagents
- 300 mL distilled water
- 8 g potassium hydroxide, KOH (6g sodium hydroxide may be substituted)
- 10 g dextrose, $C_6H_{12}O_6$ (glucose)
- 1 or more of the following indicators (for each sequence of color changes demonstrated):
    - 6–8 drops methylene blue indicator solution (To prepare a stock solution, dissolve 0.2 g of methylene blue in 100 mL of distilled water.)
    - 6-8 drops resazurin indicator solution (To prepare a stock solution, dissolve 0.1 g of resazurin in 100 mL of distilled water.)
    - 20 drops indigo carmine indicator solution (To prepare a stock solution, dissolve 0.25 g indigo carmine in 25 mL of distilled water. This solution must be prepared within a few hours of use.)

Preparation
Prepare the materials for this demonstration within 15 minutes of its presentation. The solution is not stable for longer periods.

- Pour 300 mL of distilled water into the 500-mL flask and add 8 g of KOH.
- Swirl the flask to dissolve the KOH.
- When the KOH has dissolved, add 10g of dextrose to the flask and allow the sugar to dissolve completely.
- Add 6–8 drops of methylene blue (or other listed amount and type of) indicator solution to the flask and swirl it.
- Allow the flask to rest until the solution becomes colorless.

Upon standing, all solutions turn yellow-brown and cannot be reused. Even a solution that contains only dextrose and potassium hydroxide develops this color within 10-15 minutes.

Procedure
1) Give the colorless solution in the flask a few quick shakes, until a color change is visible.
2) The blue color that appears will then slowly fade. The time required for the color to fade depends on how much the flask is shaken.
3) The regeneration and fading of the blue color may be repeated a number of times by shaking the flask and allowing it to rest.

Hazards Potassium hydroxide is very caustic and causes severe burns. Prevent eye or skin contact with the solid or solutions. Always clean up any spills!

Sodium hydroxide and potassium hydroxide release significant heat when dissolved in water.

Disposal Flush solutions down the drain with adequate amounts of water.

Tips and Suggestions

Variations on this demonstration are possible, from differing color changes to varying rates of reaction. Try other listed indicators to develop other colors.

If resazurin indicator solution (6-8 drops) is used in place of methylene blue indicator solution, the alkaline dextrose solution will initially be fluorescent red-blue. After about 2 minutes (depending on how much oxygen is dissolved in the water), the color changes to fluorescent red and gradually fades to colorless. If the flask is given a gentle shake, the fluorescent red reappears and then fades. Shaking the flask vigorously restores the color to the original fluorescent red-blue, which fades to fluorescent red and then to colorless. The demonstration may be repeated several times.

If indigo carmine indicator solution (20 drops) is used, the solution is initially yellow-green. In 3-5 minutes the color changes to orange and finally to yellow. A gentle shake restores the orange color, and, upon more vigorous shaking, the green reappears. Because this indicator solution is not very stable, this mixture, as well as all mixtures containing indigo carmine solution, cannot be used repeatedly.

Two-color changes may be observed by using both resazurin indicator and methylene blue indicator solutions. Add 6—8 drops of resazurin indicator solution to the alkaline dextrose solution. When the solution has become colorless, add 6—8 drops of methylene blue indicator solution. After the solution has again become colorless, a gentle shake will restore the fluorescent red of the resazurin. A second, more vigorous shake produces a blue solution. Upon resting, the solution then changes from blue, to violet, to red, to colorless, and the demonstration may be repeated.

If 6-8 drops of methylene blue indicator solution are added to a flask containing 20 drops of indigo carmine indicator solution and the alkaline dextrose solution, the color change will be from green, to orange, to yellow.

If 6-8 drops of resazurin indicator solution are added to a flask containing 20 drops of indigo carmine indicator solution and the alkaline dextrose solution, the color change will be from red to yellow.

Finally, combining all three indicator solutions with the alkaline dextrose solution produces color changes from violet, to red, to yellow. The shaking procedures may be repeated only a few times, because the indigo carmine indicator solution is not very stable.

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Explanation of Why the Demonstration Works

The color change results from the reversible oxidation-reduction reaction of the methylene blue indicator. In alkaline solutions, glucose is oxidized to D-gluconic acid or alpha-D-gluconolactone:

$$\underset{\text{(Dextrose)}}{HOCH_2(CHOH)_4CHO} + 3OH^- \longrightarrow \underset{\text{Gluconic Acid}}{HOCH_2(CHOH)_4CO_2} + 2H_2O + 2e^-$$

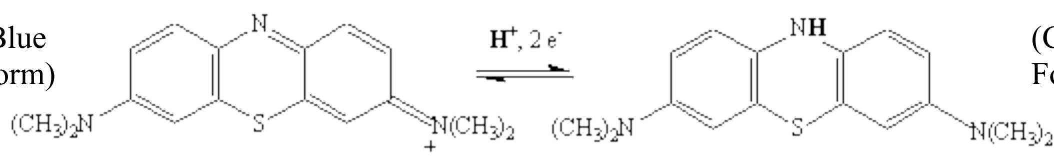
$$O_2 + \underset{\text{(Colorless)}}{MB} \longrightarrow \underset{\text{(Blue)}}{MB_{ox}} \text{ (Fast Reaction)}$$

$$\underset{\text{(Blue)}}{MB_{ox}} + \underset{\text{Reduced Form}}{CH} \longrightarrow \underset{\text{(Colorless)}}{MB} + X^- \text{ (Slow Reaction)}$$

Where CH is the carbohydrate (in this case, dextrose), MB is the reduced (colourless) form of methylene blue, MB_{ox} is the oxidized (blue) form, and X- represents the oxidation products from glucose (gluconic acid – different carbohydrates form different acids like arabinoic, formic, oxalic and erythronic acids).

The combination of the above can be represented by the following equation:

(Blue Form)



(Clear Form)

The left-to-right reaction proceeds due to the presence of the carbohydrate. The right-to-left reaction proceeds due to the presence of oxygen.

Questions for Students

- Pick up the bottle and shake it. What happens?
- Set the bottle down and watch it for a while. What do you observe?
- What are some hypotheses about why this reaction takes place?
- What else is present in the flask besides the solution?
- Is there anything else the solution might be reacting with?
- Does the amount of agitation change the reaction?
- How many times do you think the equilibrium can be shifted before it stops working? Why?
- Is the color change due to a chemical reaction or an indicator? Why do you think so? How might we confirm your hypothesis?
- Based on your previous hypotheses, how might we change some of the variables to see what effect those variables have on the system?
- What does the indicator indicate about the solution?

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Additional Suggestions

One variation allows students to see the effect of concentration on the rate of reaction.

- Half-fill two 1 L flasks with distilled water.
- Put 2.5 g of dextrose into one flask (A) and 5 g of dextrose into the other (B).
- Dissolve 2.5 g of sodium hydroxide in flask A and 5 g of sodium hydroxide in B.
- Add 1 mL of 0.2% methylene blue solution into each flask
- Stopper both flasks and shake to dissolve the indicator.
- When solutions are colorless, shake the flasks until a blue color develops.
- Set aside the flasks and observe as the blue color gradually disappears at a different rate in each flask.

The flask with the higher concentration takes about half the time for the color to disappear, using up the dissolved oxygen twice as fast. You should point out to students that, having gone colorless, a blue zone remains close to the surface of the solution. This is a result of oxygen diffusing from the air space within the flask into the solution.