

3.1 Standard operating procedure (SOP) for Methylene Blue reaction

Reaction Background

The reaction involves four chemicals: Dextrose ($C_6H_{12}O_6$), Potassium Hydroxide (KOH), Oxygen (O_2) and Methylene Blue ($C_{16}H_{18}ClN_3S$). The reactions involved are shown below:

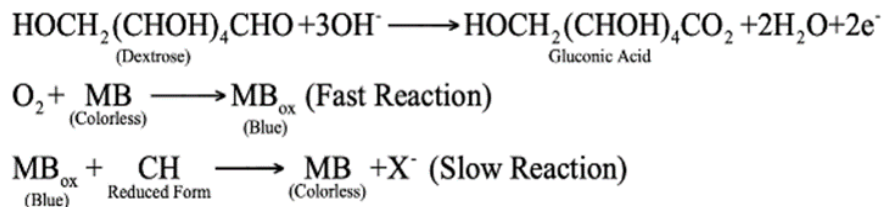


Figure 1. Chemical reactions involved in the experiment.

To initiate the reactions, a combination of two solutions is required. The first solution consists of 10 mL of clear and colourless aqueous KOH solution. The second solution is a mixture of Dextrose and a small quantity of MB, imparting a blue hue to it. The reaction commences by adding the KOH solution to the dextrose and MB mixture. As the reactions commence, the mixture assumes a blue colour owing to the presence of MB. Subsequently, the colour undergoes minimal changes until the reaction reaches its endpoint, marked by the transformation from blue to colourless. If no Oxygen is introduced into the solution, it maintains its colourless state.

The rate of the reaction is contingent upon the concentrations of MB, Dextrose, dissolved Oxygen, and hydroxide ions. An augmentation in the concentration of any of these constituents, except Oxygen, leads to an accelerated reaction rate, causing the blue colour to fade more rapidly. A higher concentration of dissolved Oxygen elongates the reaction time.

Constraints

- The reaction vessel is a 100 mL glass beaker that is only allowed to hold **at most 60 mL** of liquid at any given point
- The injection syringe has a capacity of 12 mL but is only allowed to hold **at most 10 mL** of liquid at any given point
- The **minimum** volume of the liquid in the reaction vessel with all chemicals is **30 mL**
- The reaction time should not exceed **2 minutes**
- No solution should contain a KOH concentration higher than **6 M**

Suggested Chemical Concentration Range

Based on data collected by UTCV, the following concentration combination is likely to make the reaction last between 50 seconds to 120 seconds:

Methylene blue: 0.008793 M

Dextrose: 0.3770 M

KOH: 0.4010 M - 0.8912 M

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Chemicals Provided

- 1) Methylene blue solution (1.5 wt%)
- 2) Dextrose (100% pure, powder)
- 3) Potassium Hydroxide solution (45 wt%)

Procedure

The quantities of the chemicals and capacity of glassware specified in the procedure are only for reference and should not be used in real procedures. Please adapt the procedure with your own quantities. However, you should follow the procedure for choosing the correct type of glassware/apparatus. If you can not find appropriate glassware for the quantity of the chemicals you need, report to the lab supervisor on site before you move on.

1) Preparation of MB/Dextrose Solution

1. Using the analytical balance and a weighing boat, measure out 1.0000 g of dextrose. Record the actual mass of the dextrose for later calculation.
2. Transfer the dextrose into a 50 mL beaker, and use distilled water to rinse off the remaining solids in the weighing boat, making sure all solids are transferred to the beaker.
3. Label the flask with the dedicated labelling tape assigned to your team, and explicitly record the content, concentration (“X g Dex/100mL”), date and your name.
4. Use approximately 3 times the volume of the dextrose powder of water to dissolve the dextrose in the beaker, and transfer the solution into a 100 mL volumetric flask. Rinse the beaker with distilled water 3 times to make sure all dextrose is collected in the flask. Use minimum water to avoid exceeding 100 mL
5. Using a 0.2 mL manual pipette and a disposable pipette tip, pipette 10 μ L of methylene blue solution into the 100 mL volumetric flask. Avoid pipetting methylene blue onto the flask walls.
6. Fill the flask with distilled water until the water level reaches approximately 1 cm below the calibration line.
7. Cap the flask and mix the solution thoroughly by inverting it several times.

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8. Use a dropper to adjust the final volume of the solution. Add distilled water drop by drop as you approach the calibration mark. Read the meniscus at eye level to ensure accuracy.

2) Preparation of KOH Solution

1. Calculate the required mass of the KOH stock solution. Keep in mind the final KOH solution should not exceed 6 M in KOH concentration.
2. Label a 50 mL volumetric flask with the dedicated labelling tape assigned to your team, and explicitly record the chemicals, concentration (XXXg/100 mL), date and your name.
3. Fill 20% of the volumetric with distilled water.
4. Use the analytical balance and a 1 mL manual pipette to weigh the required KOH solution in a weighing boat.
5. Transfer the KOH solution from the weighing boat to the labelled volumetric flask. Use a funnel if needed. Rinse the weighing boat with distilled water 3 times to make sure all KOH is collected in the flask. Use minimum water to avoid exceeding 50% of the capacity of the volumetric flask.
6. Fill the flask with distilled water until the water level reaches approximately 1 cm below the calibration line.
7. Cap the flask and mix the solution thoroughly by inverting it several times.
8. Use a dropper to adjust the final volume of the solution. Add distilled water drop by drop as you approach the calibration mark. Read the meniscus at eye level to ensure accuracy.

3) Injection Procedure - *ex-situ* Trials (in beaker)

It's recommended to test the same concentration combination at least twice.

1. Obtain a clean and dry 100 mL beakers. Do not label them.
2. Label a clean 250 mL Erlenmeyer flask and transfer some MB+Dex solution from the volumetric flask containing the MB+Dex solution to the Erlenmeyer flask. The Erlenmeyer flask should not contain more than 200 mL of liquid at any given point.
3. Label a clean 50 mL beaker and transfer some KOH solution from the 100 mL flask containing the diluted KOH solution to the beaker.

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4. Use a 50 mL volumetric glass pipette to transfer 50 mL of MB/Dextrose solution from the Erlenmeyer flask into a 100 mL beaker.

If you are using a 10 mL micropipette:

5. Use a 5 mL glass pipette to transfer 7 mL of KOH solution into a clean **15 mL** Falcon tube. The falcon tube can be reused as long as it's used for the same solution of the same concentration.
6. Attach a clean 10 mL pipette tip to the micropipette. Adjust the pipette volume to slightly exceed the required volume (usually +50 μ L). Using the pipette to draw liquid from the Falcon tube slowly and carefully to avoid spill. Do not insert the tip to the bottom of the falcon tube too fast. Instead, draw the liquid while you insert the pipette into the liquid.

In case of a spill of the KOH solution:

A. Make sure no one is spilled with the chemicals. If so, resort to the proper decontamination procedure outlined in the safety plan.

B. Report to the on-site lab supervisor and notify other people around the scene.

C. Use paper towel to absorb the spilled solution. If a large quantity is spilled, use the spill pad on the bench.

D. Wait for the lab supervisor before proceeding.

If you are using a syringe:

7. Use a 7 mL glass pipette to transfer 5 mL of KOH solution into a clean **50 mL** Falcon tube. The falcon tube can be reused as long as it's used for the same solution of the same concentration.
8. Attach a clean metal tip to the syringe. Draw all the liquid in the Falcon tube using the syringe. Draw more volume than you need to ensure no liquid is left in the metal tube.
9. Remove the metal tube from the syringe. The metal tube can be reused as long as it's used to draw the same solution with the same concentration.