



Using Acetic Acid and Sodium Chloride to Convert Methylene Blue to Leucomethylene Blue

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ABSTRACT

Methylene blue (MB) is a redox-active dye that becomes colorless when reduced to leucomethylene blue. This study examined whether vinegar-based conditions could support MB reduction using vitamin C as the reducing agent without hydrochloric acid. Four factors were tested independently: vitamin C concentration, vinegar concentration, NaCl level, and temperature, while total volume and MB concentration were held constant. The fastest decrease in absorbance occurred at 65°C with 12.5 mg/mL vitamin C, 75% v/v vinegar, and 250 equivalents of NaCl. These results suggest that vinegar can support MB reduction under the tested conditions and may provide a more accessible, potentially safer option for small-scale redox experiments.

INTRODUCTION

❖ What is Methylene Blue (MB)

- redox-active thiazine dye that turns colorless when reduced to leucomethylene blue (LMB)
- It is used in redox chemistry, cell staining, and the treatment of methemoglobinemia.
- Vitamin C acts as the reducing agent, while acidity and ionic strength influence the rate of MB reduction.

❖ Factors of MB Reduction

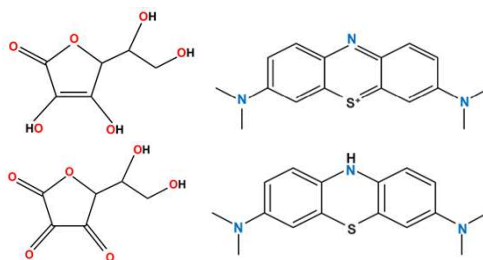
- Concentration of Reactant
- Temperature
- Salt Concentration
- Ionic Strength

❖ Problem of HCl

- Although HCl can facilitate the reaction, its corrosive nature makes it more difficult and hazardous to handle
- Storing, neutralizing, and disposing the acid are all complicated processes with lengthy safety protocols

OBJECTIVE

- ❖ Assess the feasibility of replacing HCl with vinegar
- ❖ Characterize the effects of vitamin C and NaCl on MB reduction
- ❖ Optimize reaction conditions by varying vitamin C concentration, vinegar level, NaCl equivalents, and temperature



Chemical structures of ascorbic acid, dehydroascorbic acid, methylene blue (MB), and leucomethylene blue (LMB).



Representative color changes observed during methylene blue reduction. Reproduced from Park et al. (2026).

METHODS

Prepare Stock Solution

- Prepare 0.1 mg/mL MB stock
- Prepare 2 mg/mL NaCl stock

Set Experimental Condition

- Vinegar: 2.0–7.5 mL
- Temperature: RT, 35–65 °C
- NaCl: 100–250 equiv.
- Vitamin C: 5–12.5 mg/mL

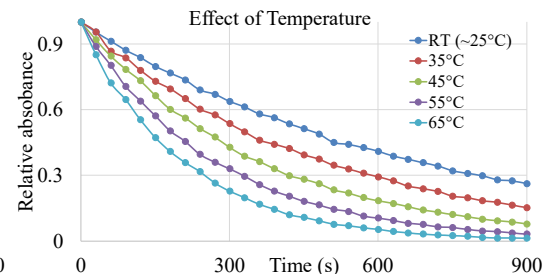
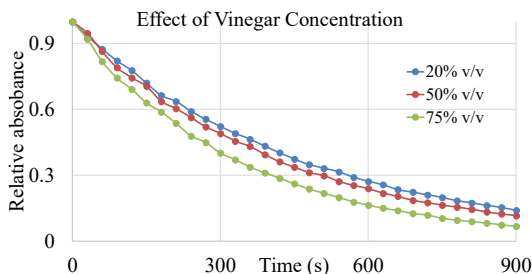
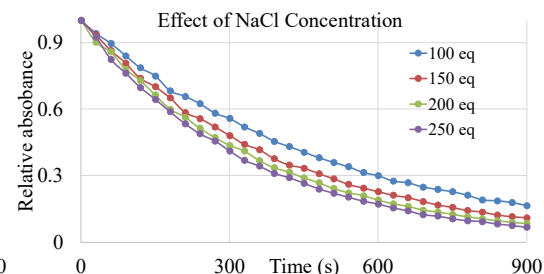
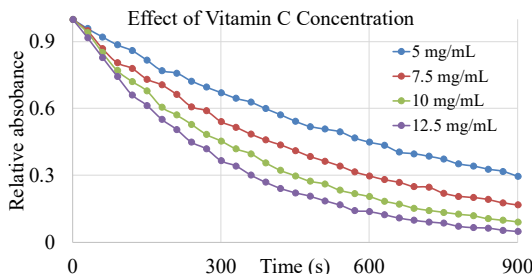
Initiate and Run Reaction

- Add vitamin C last
- Start timer immediately
- Maintain 10 mL total volume

Measure Reaction Outcome

- Measure Absorbance over time
- Record time to complete decolorization

RESULTS



❖ Key Findings

- Best tested condition: 12.5 mg/mL vitamin C, 75% v/v vinegar, 250 eq. NaCl, and 65°C.
- Increasing vitamin C and vinegar concentration accelerated MB reduction within the tested range.
- Higher NaCl concentration and temperature also accelerated MB reduction.

DISCUSSION

❖ Effect of Reaction Conditions

- Best tested condition: 12.5 mg/mL vitamin C, 75% v/v vinegar, 250 eq. NaCl, and 65°C.
- Increasing vitamin C and vinegar concentration accelerated MB reduction within the tested range.
- Higher NaCl concentration and temperature also accelerated MB reduction.

CONCLUSION

- ❖ Vinegar successfully supported the vitamin C reduction of methylene blue without HCl.
- ❖ These findings suggest that vinegar may provide a more accessible, potentially safer option for small-scale MB reduction experiments.
- ❖ Future studies should directly compare vinegar with HCl and test a wider range of reaction conditions.

❖ Interpretation

- Higher vitamin C concentration provides more reducing agent available for MB reduction.
- Increased ionic strength from NaCl may help stabilize the reaction process.
- Higher temperatures increase molecular collisions, accelerating the reaction.
- Vinegar produced faster reduction as its concentration increased within the tested range; further testing is needed to determine whether this trend continues beyond 75% v/v.

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