

Visual Redox Indicators as Low-Cost Tools for Environmental Water Safety and Public Health

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Abstract

This experiment specifically aims to address the lack of accessible methods for Oxygen detector by investigating methylene blue, a redox-driven color change agent, as a simple, low-cost, and visual tool to indicate oxygen levels, a plausible solution for hazardous water in Hinkley due to oxygen concentration.

In Hinkley, long-term exposure to hexavalent chromium (Cr(VI)), +6 oxidation state of the metal chromium posing serious health risks, especially cancer (lung, nasal, intestinal). Galvanic, Polarographic electrochemical sensors for detecting oxygen, which are more expensive and require specialized knowledge, limiting access and lead to a lack of awareness of the hazard posed by oxygen concentration.

Inspired by the health crisis in Hinkley due to oxygen concentration, this experiment specifically aims to address the lack of accessible methods for oxygen detectors by investigating methylene blue, a redox-driven color change agent, as a simple, low-cost, and visual tool to indicate oxygen levels.

Experimental results showed that exposure to oxidizing agents caused an immediate and visible color change from colorless to blue, confirming electron transfer and oxidation. Using methylene blue can signal the presence of oxidizing conditions in water, modeling a real-world oxidative pollution screening tool for oxidative water conditions, offering potential applications in environmental monitoring.

Goal

To design and evaluate a simple, low-cost, and visual redox indicator system using methylene blue that can signal the presence of oxidizing conditions in water, modeling real-world oxidative pollution scenarios such as those observed in the Hinkley groundwater contamination case.

Hypothesis

We hypothesize that after creating a leucomethylene blue from a methylene blue solution, exposure to oxidizing agents such as hydrogen peroxide or bleach will lead to a visible color change for detection. Oxidizing agents promote the loss of electrons from leucomethylene blue, which then oxidizes back to methylene blue, producing a visible color change. The detection of oxidizing conditions and redox mechanisms using the redox equilibrium of the Methylene blue dye is an indicator of oxygen concentration and a plausible indicator for ROS-generating water pollutants like hexavalent chromium.

Background/Research

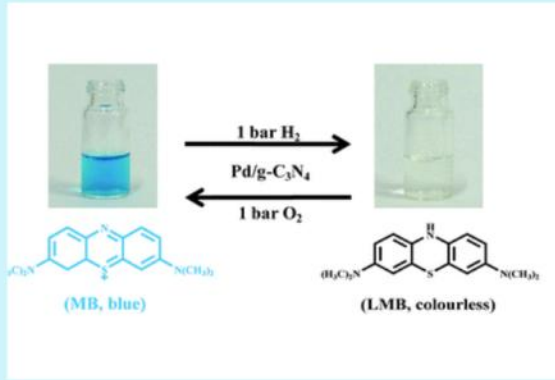
Groundwater contamination in Hinkley, California, where long-term exposure to hexavalent chromium (Cr(VI)) released by Pacific Gas and Electric Company led to severe health outcomes.

The toxicity of Cr (VI) is due to its higher oxidation state and solubility in water. The increase in cancer on people was considered the most serious problem, especially for people who used to drink or are exposed to polluted water regularly. The current study demonstrates that intraperitoneal administration of Cr(VI) to rats over 5 days can induce DNA damage in peripheral blood lymphocytes and oxidative stress in the liver and kidneys. Inside the body, it is gradually reduced to trivalent chromium, Cr(III), through a series of chemical reactions. The ROS, a byproduct of the reduction process, is a highly reactive molecule that can attack various parts of the cell, particularly DNA. They can break DNA strands, alter individual bases, such as by producing 8-oxo-dG, and disrupt the stability of entire chromosomes. When cells experience repeated or severe DNA damage, the repair systems may fail, causing mutations to accumulate over time. Accumulated mutations increase the risk of cancer because they can alter important genes that control cell growth and division, which significantly increases the risk of cancer.

This case demonstrates how oxidizing contaminants in water can harm through electron-transfer processes rather than through immediate toxicity alone, underscoring the importance of continuous monitoring of oxygen concentration.

Original Galvanic, Polarographic electrochemical sensors, original methods to detect oxygen are higher cost, require specialized knowledge of the equipment to use, and have a lack of portability by requiring long warm-up periods, and require sensitivity or susceptibility to interference from temperature, pressure, and some specific types of materials (hydrogen sulfide or strong acid).

Methylene blue offers a promising solution due to its well-characterized redox behavior. In its oxidized form, methylene blue is deep blue, while its reduced form is Leucomethylene blue (C16H19N3S). It is a colorless solution, but turns into a deep dark blue color when it is oxidized. It is one of the classes of phenothiazines where the ring hydrogens at positions 3 and 7 have been replaced by dimethylamino groups.



In this experiment, vitamin C (ascorbic acid) is used as a reducing agent to convert methylene blue into leucomethylene blue. Ascorbic acid readily donates electrons, efficiently driving the reduction reaction. The Hydrochloric acid provides the necessary acidic conditions to enhance the reduction process and stabilize the leucomethylene blue. The 2M concentration ensures efficient and reproducible reduction.

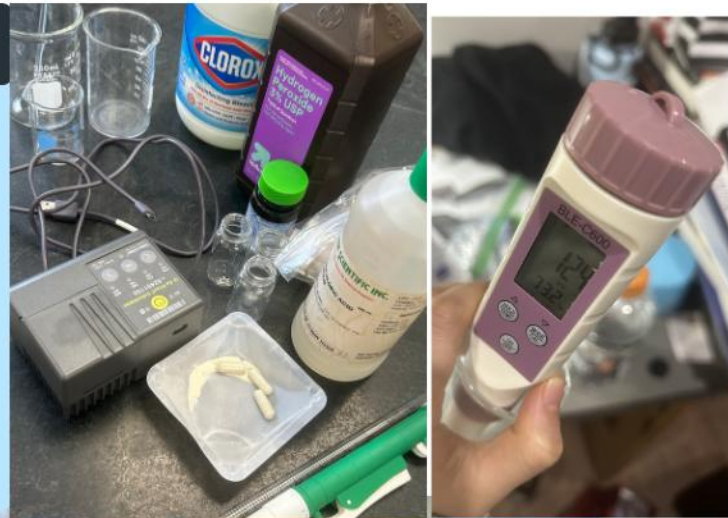
Preperation of Methylene Blue Solution

A methylene blue solution was prepared by dissolving 0.1 g of methylene blue (ALDON, 25 g, model #IS22075) in 500 mL of water, resulting in a 0.00062529 M solution. From this stock, 4 mL was transferred into a 20 mL plastic cap vial (Cadibbe, model #20 mL plastic cap vial-50pcs) containing 1 mL of water. The subsequent dilution (4 mL solution + 1 mL water) enhances the consistency of the reaction through every experiment that proceeded further on. Using a 20 mL vial provides a controlled environment, promoting uniformity and reproducibility between trials.



Materials

Methylene blue solution
Vitamin C (Ascorbic acid) (0.2g each)
Distilled water
Hydrochloric acid (2 M HCl)
Hydrogen peroxide (3ml)
Household bleach (3ml)
Plastic cap vials (20 mL)
Dropper
Burette
Beaker
UV-Vis spectrophotometer
Burette
Colorimeter
ORP detector



(left to right) 0.02g of vitamin c, 0.01g of vitamin c, 0.005g of vitamin C, no vitamin C

Experiment 1: Reduction of Methylene Blue

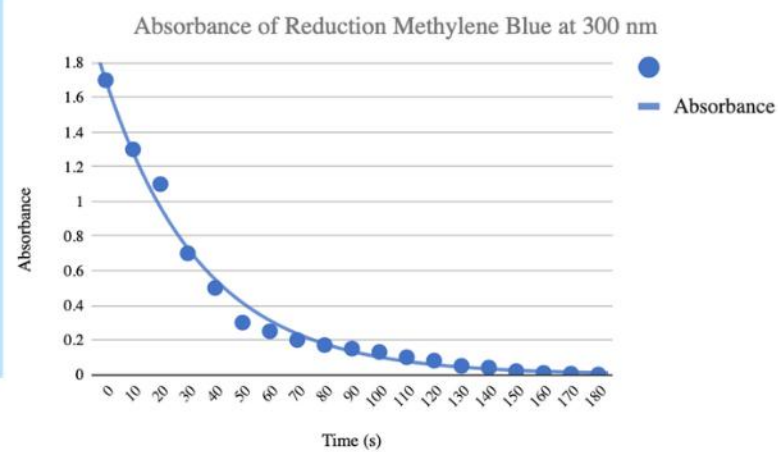
VARIABLE	dependent Variable	Control
Independent Variable vitamin C(It's Just!, model #JVTC), (Ascorbic Acid) 0.005 g of vitamin C, 0.02 g of vitamin C, 0.01g of vitamin C, no vitamin C. Vitamin C was chosen as it acts as a reducing agent that facilitates the conversion of methylene blue to leucomethylene blue. Its ability to donate electrons makes it effective for this reduction reaction (the volume of hydrochloric acid was held constant at 1 mL of 2 M HCl)	Absorbtion of the 300nm wavelength	Control 5 mL methylene blue solution(0.1 g of methylene blue (ALDON, 25 g, model #IS22075)+500ml of distilled water) in the vial 1 mL of 2M Hydrochloric acid (ALDON, model #IS17019)*4 0.02 g of Vitamin C (It's Just!, model #JVTC)*3 20 mL plastic cap vial (Cadibbe, model #20 mL plastic cap vial-50pcs) *2

PROCEDURE

0 g, 0.005 g, 0.01 g, and 0.02 g of Vitamin C was added to each vial containing 5ml of methylene blue solution with 1ml of water, and 2ml of Hydrochloric acid

based on the color change, compare the color change and determined the best leucomethylene blue experiment of each concentration was tested for 3 times, for a consistent reaction

UV-Vis spectroscopy was used to monitor absorbance changes in the solution over time



RESULT

98% of MB was reduced.

0.02g of Vitamin C was selected to standardize the amount of methylene blue available for reduction.

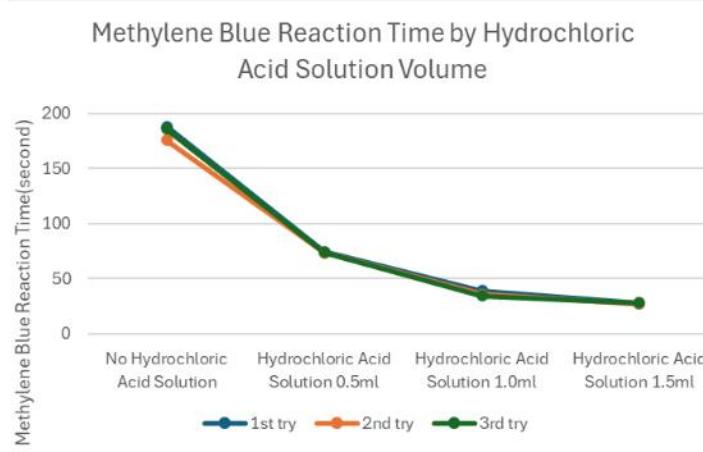
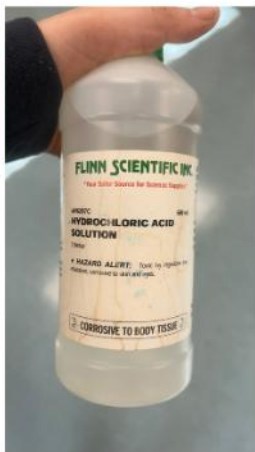
Experiment 2: Effect of Acid Concentration

VARIABLE	dependent Variable	Control
Independent Variable 2M Hydrochloric acid (ALDON, model #IS20892) Each 0.5ml, 1ml, 1.5ml of 2M Hydrochloric acid*2	reaction time of the solution of Leucomethylene Blue	Control 5 mL methylene blue solution(0.1 g of methylene blue (ALDON, 25 g, model #IS22075)+500ml of distilled water) in the vial 0.02 g of Vitamin C (It's Just!, model #JVTC)*3 20 mL plastic cap vial (Cadibbe, model #20 mL plastic cap vial-50pcs) *2

PROCEDURE

1) Add each 0ml, 0.5ml, 1ml, 1.5ml of 2M Hydrochloric acid (ALDON, model #IS17019) to each vial that contains 5ml of methylene blue solution with 0.02g of vitamin C, and 1ml of water.

2) Repeat the process for 3 times, and time the reaction with a smartwatch for every reaction that is measured.



RESULT

Variation in hydrochloric acid Concentration produced minimal differences in the final redox state of the solution. However, increased acidity significantly reduced reaction time by facilitating electron transfer and stabilizing the leucomethylene blue intermediate.

Experiment 3: Effect of Acid Concentration

VARIABLE	Dependent Variable	Control
Independent Variable 2ml hydrogen peroxide 3% USP(up&up, #245-07-0177)*1 2ml household Bleach(Clorex, #30966)*1	color change, absorbance in 475nm wavelength in the Methylene Blue Solution	Control 2 sets of original methylene blue solution[5 mL methylene blue solution(0.1 g of methylene blue (ALDON, 25 g, model #IS22075)+500ml of distilled water) in the vial, 0.02 g of Vitamin C (It's Just!, model #JVTC), 1 mL of 2M Hydrochloric acid (ALDON, model #IS17019)]X4

PROCEDURE

due to indirect usage of Chromium Oxide(Cr(IV))chemical, the experiment was conducted using alternative oxidizing agents such as bleach and hydrogen peroxide.

1) A control sample containing leucomethylene blue without an oxidizing agent was measured to account for spontaneous air oxidation.

2) Add 1 mL of hydrogen peroxide, 1ml of household Bleach to each original Leucomethylene Blue solution(experiment 2) in a 20mL plastic vial

3) place each of the mixtures into the plastic cuvettes and insert them into Go Direct Colorimeter slot to determine the absorbance of the color change at 475 nm wavelength

4) measure the ORP (oxidation-reduction potential) of the mixture of hydrogen peroxide, and the mixture of household Bleach with Leucomethylene blue, using a water quality tester.

5) Add additional 1ml of hydrogen peroxide and and 1ml of household Bleach to the solution done in #3

6) run the colorimeter again

7) Measure the ORP (oxidation-reduction potential) of the mixture again for each solution.

Calculation (Example for 1 mL of H₂O₂)
The oxygen-equivalent of 1 mL of 3% H₂O₂ was determined based on the stoichiometric relationship 2H₂O₂ → 2H₂O + O₂.



Go Direct Colorimeter was used to determine the absorbance of the color change at 475 nm wavelength, and 635nm wavelength

	without oxidation agent	1ml of Household bleach	1ml of Hydrogen peroxide
	0g O2	0.0312g O2	0.0142gO2
475nm			
635nm			
ORP	-196mV	727mv	567mv

91.16074% oxidized back to methylene blue by adding 1ml of bleach
82.22% oxidized back to the methylene blue solution by adding 1ml of hydrogen peroxide solution
91.704% oxidized back to the methylene blue solution by adding 2ml of Hydrogen Peroxide
98.67% of leucomethylene blue oxidized back to methylene blue and then suddenly turned colorless result of dye destruction by hypochlorite.

RESULT

Control samples of leucomethylene blue that were not exposed to oxidizing agents, but were exposed to the air showed no significant absorbance change or visible color shift, confirming that oxidation did not occur spontaneously, and that external oxidizing conditions drove the observed responses.

Spectrophotometric data collected at 470 nm and 675nm showed a clear increase in absorbance following oxidant exposure, quantitatively validate the observed visual color changes. Both oxidation reactions proceeded in less than 20 seconds, demonstrating the rapid responsiveness of the methylene blue redox system and its suitability as a fast visual indicator.

Bleach produced a stronger oxidation response, with absorbance of the wavelength in the wavelength range of, and reacted less than about 8 seconds.

Hydrogen peroxide reflects a far slower and more gradual reaction

Bleach reflects a greater oxidative strength and higher redox potential in the graph

However, at high hypochlorite concentration, the oxidant no longer stopped at the regeneration of the dye and instead degraded the methylene blue chromophore, leading to irreversible loss of color.

After hypochlorite oxidized leucomethylene blue back to methylene blue, excess bleach further oxidized the methylene blue itself, destroying its light-absorbing conjugated structure. As a result, the solution turned colorless due to oxidative bleaching of the dye. (2ml of Bleach)

Conclusion and Discussion

The leucomethylene blue solution was prepared using varying concentrations of methylene blue, Vitamin C, and hydrochloric acid to determine the optimal conditions for rapid and complete reduction.

The optimal combination to create the Leucomethylene solution using the methylene blue dye was 5 mL methylene blue solution(0.1 g of methylene blue (ALDON, 25 g, model #IS22075)+500ml of distilled water) in the vial, 0.2 g of Vitamin C (It's Just!, model #JVTC), and 1 mL of 2M Hydrochloric acid (ALDON, model #IS17019)

By adding two different Oxidative agents: 2ml 3% USP hydrogen peroxide (up&up, #245-07-0177)*1, and 2ml household Bleach (Clorex, #30966) to two different vials containing the Leucomethylene blue solution, Spectrophotometric data collected at 470 nm and 635nm showed a clear increase in absorbance following oxidant exposure. Visual color concentration has been depicted by the absorption, oxidative Bleach consistently produced a stronger oxidation response than hydrogen peroxide. Although bleach causes irreversible degradation of the dye, the reaction still remains useful for detecting oxidative conditions because it produces a clear and observable color change.

This study successfully demonstrated that the methylene blue-leucomethylene blue redox system can function as a simple, low-cost, and rapid visual indicator of oxidizing conditions in water by showing the visible color changes as a result of the reduction-oxidation behavior

These findings indicate that the redox equilibrium of methylene blue can be exploited as a practical proxy for detecting oxidative environments in water. It effectively signals conditions that favor the generation of reactive oxygen species (ROS), which are central to the toxicity of many environmental pollutants. This approach is particularly relevant when modeling oxidative pollution scenarios such as the groundwater contamination observed in Hinkley, where hexavalent chromium drives oxidative stress through redox cycling and ROS formation.

Overall, this research demonstrates that a methylene blue-based redox indicator can serve as a visual, accessible screening tool for oxidative water conditions, offering potential applications in environmental monitoring and educational demonstrations of redox-driven toxicity. The results validate the hypothesis and establish a foundation for further development toward oxidative pollution indicators

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Future Plan

1) Due to the toxicity of Chromium Oxide(Cr(IV))chemical, The experiment was conducted using alternative oxidizing agents such as bleach and hydrogen peroxide. Future results will include testing data using testing result with application in real life.

2) While the current experiment focuses on oxidative aquatic environments, future work could extend this approach to detecting low oxygen concentrations. This could enable low-cost monitoring of both hypoxic and oxidative water conditions.

3) Instead of hydrochloric acid, determining the concentration a more accessible acid such as vinegar could make the system more practical and user-friendly for the general public.