Characterization of Anaerobicity in Microfluidic Loops

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Introduction

Studying anaerobic biological and chemical samples is challenging at LCLS due to difficulty maintaining **anaerobic conditions** during data collection. This limits the potential of LCLS for users with anaerobic samples.

The problem extends beyond any single sample delivery method, as many rely on **microfluidic loops**. By investigating and improving the anaerobicity of these loops, we can enable anaerobic studies across instruments like LCLS and SSRL.

Therefore, we aim to advance **anaerobic sample delivery** broadly to increase capabilities for users with oxygen-sensitive samples.

Methods

We investigate **anaerobicity** using a **chemical oxygen sensor** called **methyl viologen** that changes from blue to clear as it is oxidized. The transient change in oxidation state induced by oxygen is **monitored** within a microfluidic loop using a **UV-Vis spectrometer**. The change can be correlated to oxygen content.

As there are various possible components used in sample delivery, the project focuses on the anaerobicity of a **subset of standard parts**, i.e.,

- Pumps
- Tubing
- Reservoirs

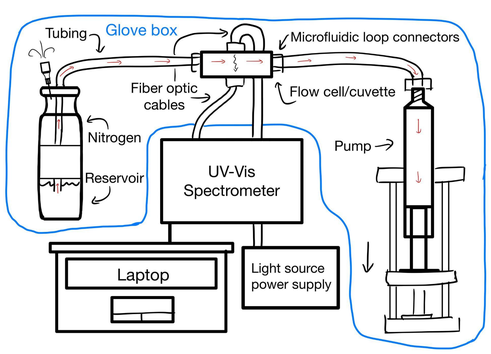
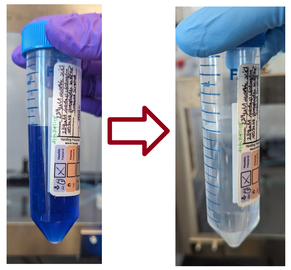


Fig. 1: The experimental set up with methyl viologen flowing through a microfluidic loop and a UV-Vis spectrometer observing changes in oxidation.

Methyl Viologen as an Oxygen Sensor

Changes in Oxidation States



Reduced
No Air
Oxidation State: 1+

Oxidized
Excess Air
Oxidation State: 2+

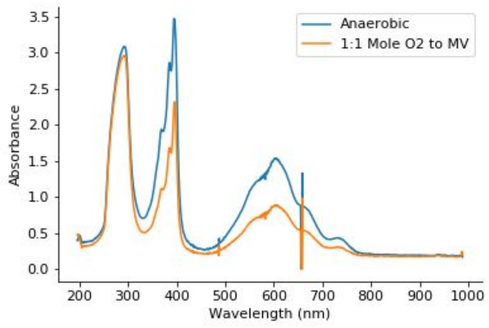


Fig. 2: Changes in oxidation state from 1+ to 2+ are evident visually and in UV-Vis absorption spectra measurements. As the methyl viologen is oxidized, it changes from a deep blue color to transparent. The UV-Vis absorption spectra shows a decrease in absorbance as oxygen concentration increases.

Potential Reducing Agents

Ascorbic Acid:
- ➢ **Mild reducing agent**
- ➢ Reduces only methyl viologen
- ➢ Even in <1ppm glovebox, was **unable to reduce** methyl viologen

Sodium Dithionite:
- ➢ **Strong reducing agent**
- ➢ Reduces methyl viologen *and* oxygen
- ➢ In 1:1 mole ratio, was **able to reduce** in the mini glovebox

Beer-Lambert Law

Beer's Law relates the attenuation of light to the properties of the material is it traveling through. For the **UV-Vis spectrometer**, the light being used falls in the 200-1000nm range.

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon l c$$

Extinction Coeffecients:
257nm: $\varepsilon = 2.07 \cdot 10^4 M^{-1} cm^{-1}$
396nm: $\varepsilon = 4.21 \cdot 10^4 M^{-1} cm^{-1}$
606nm: $\varepsilon = 1.37 \cdot 10^4 M^{-1} cm^{-1}$

A = Absorbance
ε = Extinction Coefficient (varies based on wavelength)
l = Optical Path Length (1cm)
c = Concentrations Used
I_0 = Background Intensity of the Solvent
I = Measured Intensity

Calibration Curve

In order to **correlate changes** in UV-Vis absorption spectra to oxygen content, a calibration curve between methyl viologen and oxygen must be made.

To do so, **known volumes of gaseous oxygen** are introduced into the methyl viologen solution, and the **corresponding changes in absorption spectra** recorded.

This calibration correlates the UV-Vis spectra of methyl viologen measured in the microfluidic loop to the oxygen content and allows assessment of the **anaerobicity of the loop.**

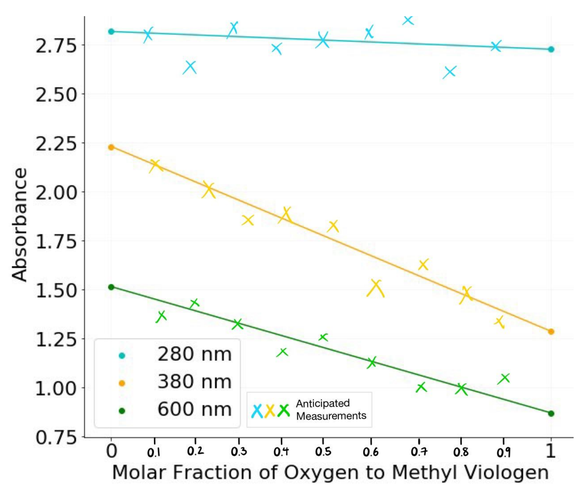


Fig. 3: Ongoing measurements for a calibration curve demonstrating the change in absorption values as oxygen is introduced to the methyl viologen solution. We calibrate to a 1:1 molar ratio between methyl viologen and oxygen. X's show expected measurements according to procedure in [2]

Discussion

Reducing methyl viologen proved challenging as there are a multitude of potential points of failure. For example, the glovebox where many of the experiments were conducted at times reached **19% oxygen**. Additionally, there are varying extremes of degassing components, a range of concentrations to choose from, and long preparation time scales that may impact the effectiveness of certain solutions.

Despite such factors, it was determined that the ascorbic acid was unable to reduce methyl viologen. The experimental approach then pivoted towards the **use of sodium dithionite**, which **showed efficacy.**

The initial calibration curve with preliminary data relating methyl viologen absorbance to oxygen concentration **demonstrates the sensitivity of this chemical to oxygen levels**. Additional measurements to further populate the calibration curve would improve accuracy and reliability when using methyl viologen to quantify oxygen content.

Moving forward, **an experimental procedure** and analysis tools have been **developed** for using methyl viologen as an oxygen sensor to conduct further investigation into the characterization of anaerobicity in microfluidic loops.

References

[1] Tadashi Watanabe and Kenichi Honda, "Measurement of the extinction coefficient of the methyl viologen cation radical and the efficiency of its formation by semiconductor photocatalysis" The Journal of Physical Chemistry 1982 86 (14), 2617-2619
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[2] Stephanie Muggler, LCLS Intern 2022

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