Cationic Partitioning Indicator Displacement Assay

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Background

- Indicator Displacement Assays (IDAs) are a form of molecular detection used across multiple disciplines for their convenience and effectiveness
- Molecules known as indicators are initially bound to a receptor called the host by intermolecular forces
- Competitive analytes known as guests, displace the indicator creating measurable visual changes (Rather and Ali 2021)
- Traditional IDAs have many pros including:
- Use of different indicators for each receptor to adjust sensitivity
- Can be used for water based and non-water-based solvents
- Some of the cons of traditional IDAs are:
- Low signal to noise ratio (desired signal is weak compared to background noise)
- High cost associated with research, modification, and production
- Blue dye interacts quickly with molecular aggregates (Bell et al. 2023)
- Previous research has shown an organic two-phase system using an analyte capable of partitioning and displacement in organic matter (Bell et al 2020)
- The purpose of this research is to enhance the IDA taking advantage of cationic partitioning by experimenting with various salt concentrations and droplet size and see how these interactions affect the IDA
- We hypothesized some salts, but not all salts would displace the methylene blue given the array of counterions as well as the smaller sized droplets would have increased displacement due to their volume to surface area ratio

Sensor Set-up

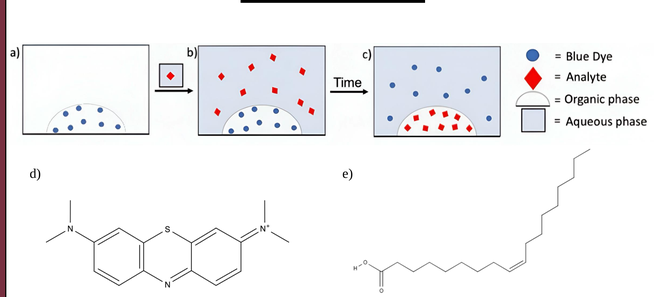

Figure 1. General schematic of two-phase sensor and chemical structures.
a) Indicator only is present in organic phase.
b) Analyte is added to aqueous phase.
c) Analyte partitions into organic phase displacing the indicator.
d) 2-D structural formula of Methylene Blue Dye
e) 2-D structural formula of Oleic Acid

Picture showing system is modified from: Tocci, V., Shiel, E., Zhou, H., Liu, S., Bell, T., Lenhert, S. (2025). Partitioning Indicator Displacement Assay. Submitted.

Methods: Salt

- Turn off room lights
- Place sensor on light microscope
- Set light microscope to obtain frames every 5 seconds for 15 minutes
- Flow 200µL of varying analyte solutions (lead nitrate, magnesium sulfate heptahydrate, copper, potassium, sodium) while using water as a control
- Record data on previously established settings
- Once picture frames obtained, analyze data using ImageJ and R script for absorbance values
- Absorbance values were used to demonstrate partitioning/ displacement

Methods: Size

- Turn off room lights
- Measure 1 mm on grid paper
- Photograph 1 mm grid paper under light microscope
- Place flow cell under light microscope
- Photograph blank
- Flow 200 µL of 2 pH HCL/Water solution over cell
- Set software to capture image for 30 minutes in 5 second intervals
- Repeat with three different flow cells
- Analyze data using ImageJ and R script
- Record absorbance and displacement values

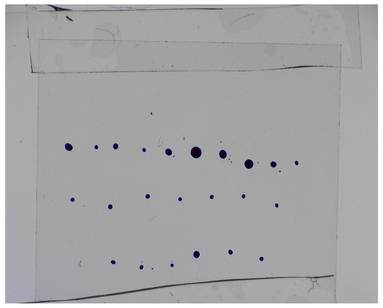

Figure 2. A droplet array before addition of the aqueous phase, involving the varying sized droplets.

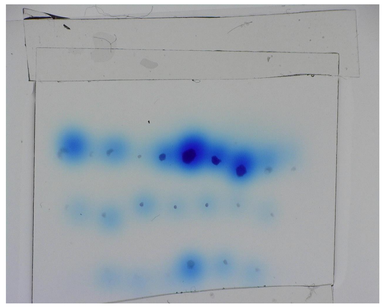

Figure 3. Droplets flowed with HCL solution resulting in methylene blue dye displacement.

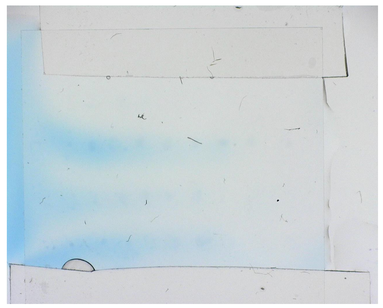

Figure 4. Droplets flowed with lead solution resulting in displacement.

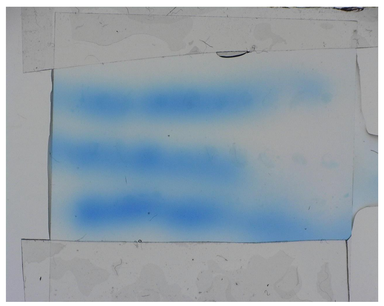

Figure 5. Droplets flowed with copper solution resulting in methylene blue dye displacement.

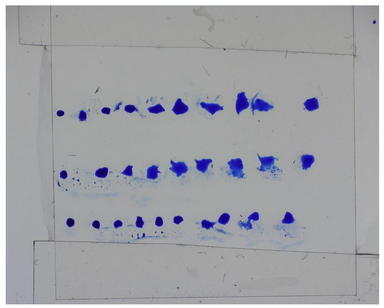

Figure 6. Flow of potassium portrays an example of droplet partitioning.

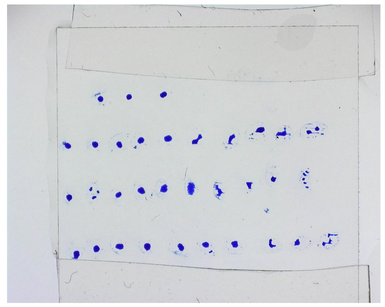

Figure 7. Flow of pure DI water represented as a control.

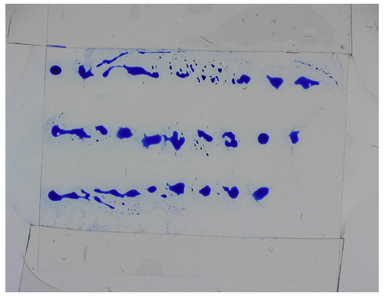

Figure 8. Flow of sodium portrays an example of droplet partitioning.

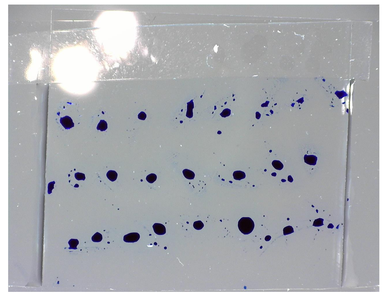

Figure 9. Flow of magnesium portrays an example of droplet partitioning.

Results: Salt

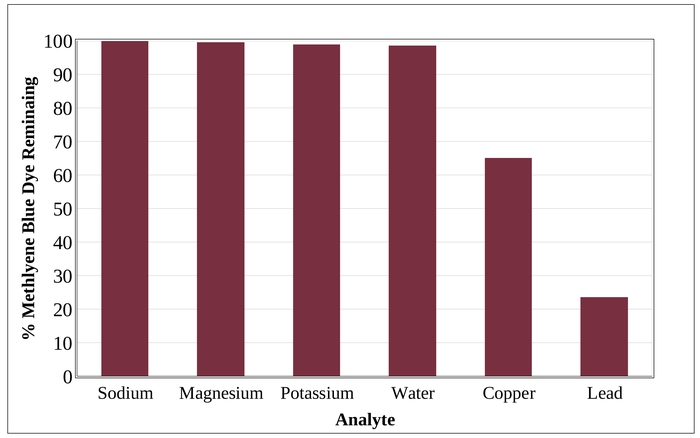

Figure 10. Portrays analyte selectivity to percent of methylene blue dye remaining.

Results: Size

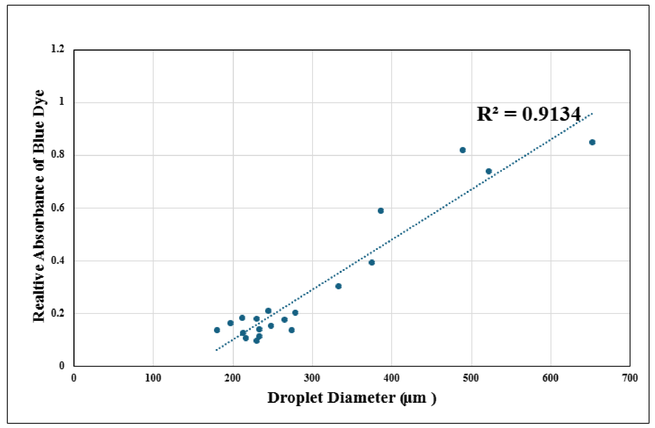

Figure 11: Relative absorbance at 15 minutes ($Absorbance_{15}$min / Max Absorbance) as a function of initial droplet diameter in a flow cell. This normalization allows for comparison across different droplet sizes.

Future Directions

- Further optimize the droplet composition and experimental conditions to selectively detect a range of metallic ions, enhancing the specificity of the IDA system.
- Work toward establishing a low-cost, easy-to-use water quality testing method that can be implemented in diverse settings, providing an accessible alternative to traditional testing methods.
- Investigate the limit of detection for various cations to determine the system's sensitivity and ensure it meets the requirements for real-world applications.
- Evaluate the stability and reusability of the droplet-based sensor over extended use to determine its practicality for continuous or repeated testing.

References