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Optimization of the Control Line on a Lateral Flow Assay for Lead (II) Detection in Drinking Water

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Introduction

According to a recent survey by the United States Environmental Protection Agency (EPA), there are between 6 to 10 million lead service lines in the country.[1] Changes in the pH of water may cause pipe corrosion and increase the leaching of lead (Pb^{2+}). Consumption of lead may lead to brain and kidney damage.[2] Water samples can be tested for lead and other heavy metals in state-certified labs but range in price from $20-$100. Some states provide free water testing kits, but this issue persists globally as well.[5] Our goal is to create a simple, inexpensive paper-based Pb^{2+} assay to make lead testing accessible.

Lateral flow assays (LFAs) are rapid tests (5-30 mins to result) used to detect or quantify analytes in mixtures. LFAs are paper-based (inexpensive), user-friendly, and widely accepted by regulatory authorities. LFAs are made of a sample pad, a membrane with backing support, and an absorbent pad (Figure 1). Each LFA has a control line and a test line(s). The control line ensures the test is working while the test line only appears with detection of the analyte. The mixture runs through the sample using capillary action, which is aided by the presence of the absorbent pad.[4]

In our test, the control line is composed of polymer nanoparticles which are commonly used in medicine as drug delivery vehicles.[3] Our polymer nanoparticles encapsulate neutral red dye molecules. These neutral red nanoparticles (NRNP) are pH sensitive and display a colorimetric change with the addition of water.

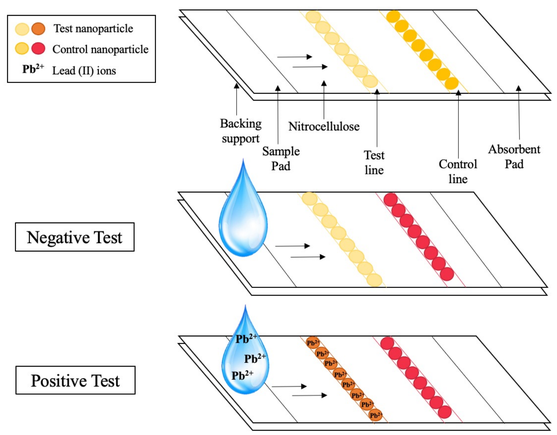


Figure 1: Layout of a lateral flow assay with positive and negative samples.

pH Sensitivity of Neutral Red

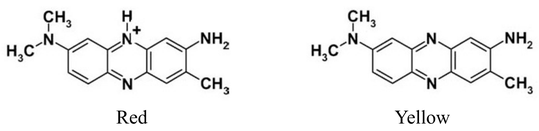


Figure 2: Structure of neutral red dye when red (pH ≤ 8) and yellow (pH ≥ 9)

- Colorimetric change was observed at alkaline pH

Synthesis of Control NRNP

- A solution of neutral red and PSMA in acetone and a 1% PVA solution were prepared separately
- The PVA solution was stirred as the neutral red solution was added dropwise
- The solution was sonicated, DI water was added, and the organic solvent evaporated.
- The nanoparticles dialyzed for 3.5 hours

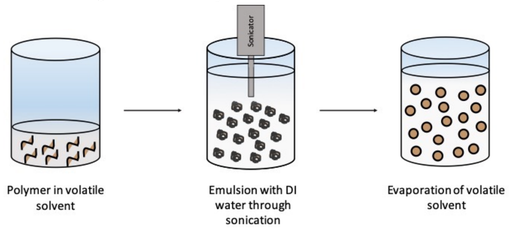


Figure 3: A diagram illustrating the synthesis of nanoparticles.

Characterization of Nanoparticles

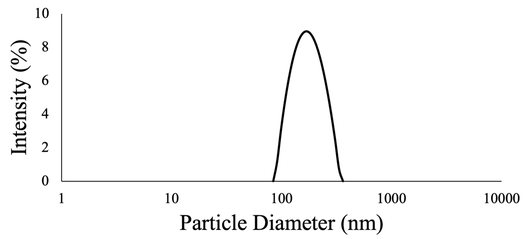


Figure 4: Size distribution of dialyzed NRNP

- Dynamic Light Scattering (DLS) confirmed successful nanoparticle synthesis
- Hydrodynamic diameter (D_h): 180 ± 30 nm
- Polydispersity Index (PDI): 12 ± 5%

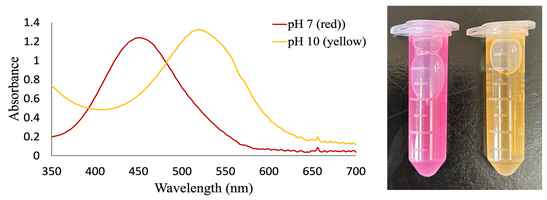


Figure 5: The absorbance spectrum of dialyzed NRNP at pH 7 and 10 (left). Dialyzed NRNP at pH 7 and 10 (right).

- Maximum absorbance changes from 450 nm to 520 nm with pH increase

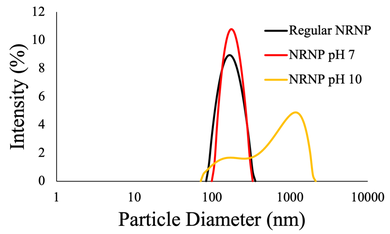


Figure 6: The size distribution of dialyzed NRNP at different pH

- NRNP at pH 10 shows particle aggregation at 160 and 920 nm
- Regular NRNP and NRNP at pH 7 are 180 nm

Quantification of Neutral Red

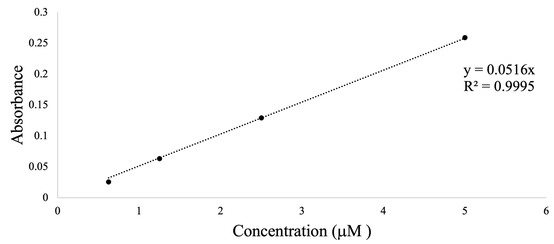
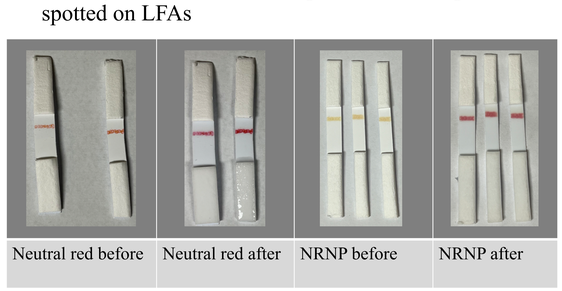


Figure 7: Beer's Law plot made with neutral red in acetone at concentrations of 0.625, 1.25, 2.5, 5, 10 μM.

- Concentration (μM) of neutral red in nanoparticles was determined using Beer's Law
- Concentration: 40 ± 10 μM

LFA Optimization

- 20 spots per test strip, each spot 0.4 μL
- pH 7 buffer was dried onto sample pad and aided colorimetric change
- A 50 μM NRNP solution gave a clear signal when spotted on LFAs



Neutral red before	Neutral red after	NRNP before	NRNP after

Figure 9: LFAs comparing before and after of neutral red dye to NRNP

- Interactions between dye and nitrocellulose membrane are decreased with use of nanoparticles, allowing them to dry yellow on LFA
  - Increases signal-to-noise

LFA Time Trial

LFA before tap water was added.	LFA 5 mins after tap water was added. Color of the control line has fully changed.	LFA 15 mins after tap water was added. The control line has some streaking.

Figure 8: Lateral flow assays spotted with neutral red nanoparticles.

- The control line was most distinct between 5-15 mins after the water sample was introduced

Conclusions

- Neutral red control polymer nanoparticles with a size of 180 nm were synthesized
- Colorimetric change of the control line was observed with pH 7 buffer
- The control line signal on the lateral flow assay was best 5 minutes after tap water was added

Future Work

- Shelf life of lateral flow assay
- Use of control nanoparticles with lead nanoparticles on lateral flow assay

References

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