



# Colorimetric detection of zinc in water samples using paper-based microfluidic analytical devices.

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## ABSTRACT

This study entails the colorimetric detection of Zn<sup>2+</sup> on paper-based microfluidic analytical devices and also optimizing the reaction conditions with zincon immobilized with Polydiallyldiamethyl ammonium chloride (PDDA). The results of the colorimetric assays on the paper devices were captured with a camera phone and downloaded into a computer for image analysis with the Image J software using the RGB colour mode. A wide range of optimization tests was performed in terms of volumes, substrate concentrations, and reaction time. Calibration curves for the data sets of standard concentrations at varying concentrations were fixed with Zincon, immobilized with PDDA were obtained. Series of experiments were also performed with a - cafetiere style filter system; the presence of zinc was detected in 100mL of each freshwater sample immobilized with PDDA on the paper membrane and a visible colour change (from brown to blue) was observed. The arithmetic mean of the colour intensity within each test zone was used to quantify the colorimetric response at various Zn<sup>2+</sup> standard solutions between 8-100µM. The EPA has stated that drinking water should not exceed 5mg of zinc per liter of water (76.5 µM) which was found to lie within the detectable range of the paper-based microfluidic reactions.

**Key Words:** *colorimetric assay, freshwater, Microfluidic, polydiallyldiamethyl ammonium chloride, Zinc.*

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## INTRODUCTION

· Clean water and sanitation is one of Sustainable Development Goals, intimately intertwined with the goals of good health and well-being.

· The contamination of water by toxic metals (As, Pb, Cu, Cr, Co, Ni, Zn) is one of the most ubiquitous forms of pollution across the globe, but in particular in developing countries [UNEP GEMStat] where industrial, agricultural and domestic waste leach into river and groundwater and where water management frameworks are often not in place [RSC Pan-Africa Report].

· There is a widespread recognition that regular monitoring of water pollution is required, however quick and effective monitoring is needed.

· Tests typically cost around \$100 per sample, making it impossible to frequently measure in multiple locations.

· Reports on the measurement of water pollution are being published, they give, at best, a sporadic picture of very dynamic environments (e.g. with flow changes) with comparison at best on an annual basis. What is needed are frequent measurements (up to daily).

· To tackle this challenge, paper-microfluidic dip-stick devices with colour based readout chemistry that can simultaneously measure several toxic metals in many locations, *in-situ*, undertaken by volunteers and members of the community with simple, instrument-free devices.

· This research aims to develop a novel approach for addressing the key challenge of monitoring pollution, by using paper-microfluidic analytical devices for *in-situ* measurements of heavy metals, Zinc as the case study.

## MATERIALS AND METHODS

- Fabricated paper-based microfluidic analytical devices
- Zincon monosodium salt
- Zinc sulfate heptahydrate,
- Polydiallyldiamethyl ammonium chloride

## EXPERIMENTAL PROCEDURES

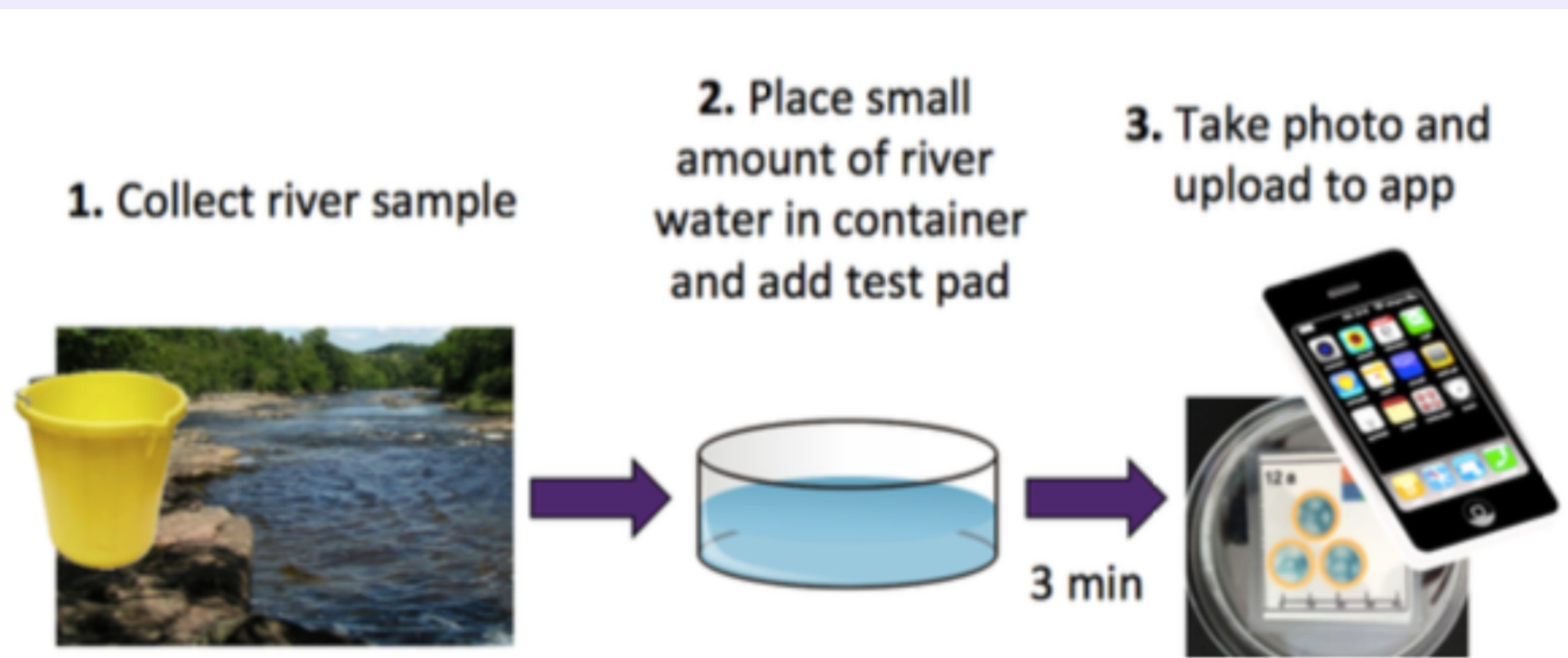


**Fig 1: Experimental procedures for paper based microfluidic analysis**

- In this study, the colorimetric detection of Zn<sup>2+</sup> on paper-based analytical devices by optimizing the reaction conditions with Zincon immobilized with PDDA.
- Zinc- Zincon reactions on paper microfluidic devices immobilized with PDDA at optimized reagent volumes and concentrations also applied to the cafetiere-style system interfacing with the µPAD.

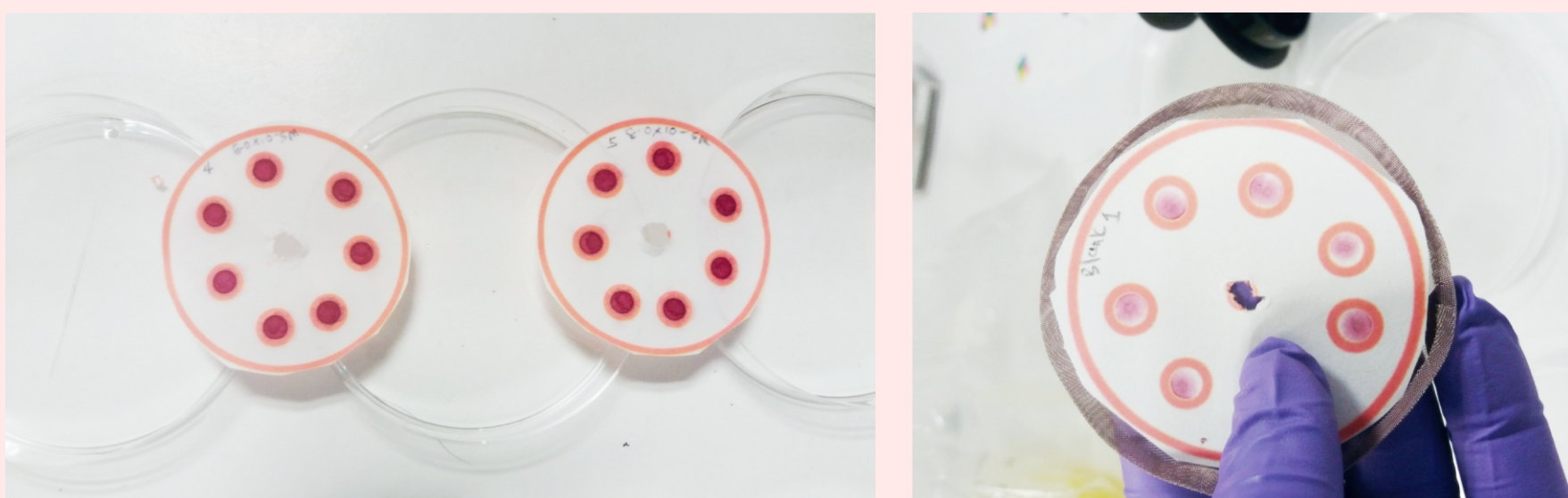
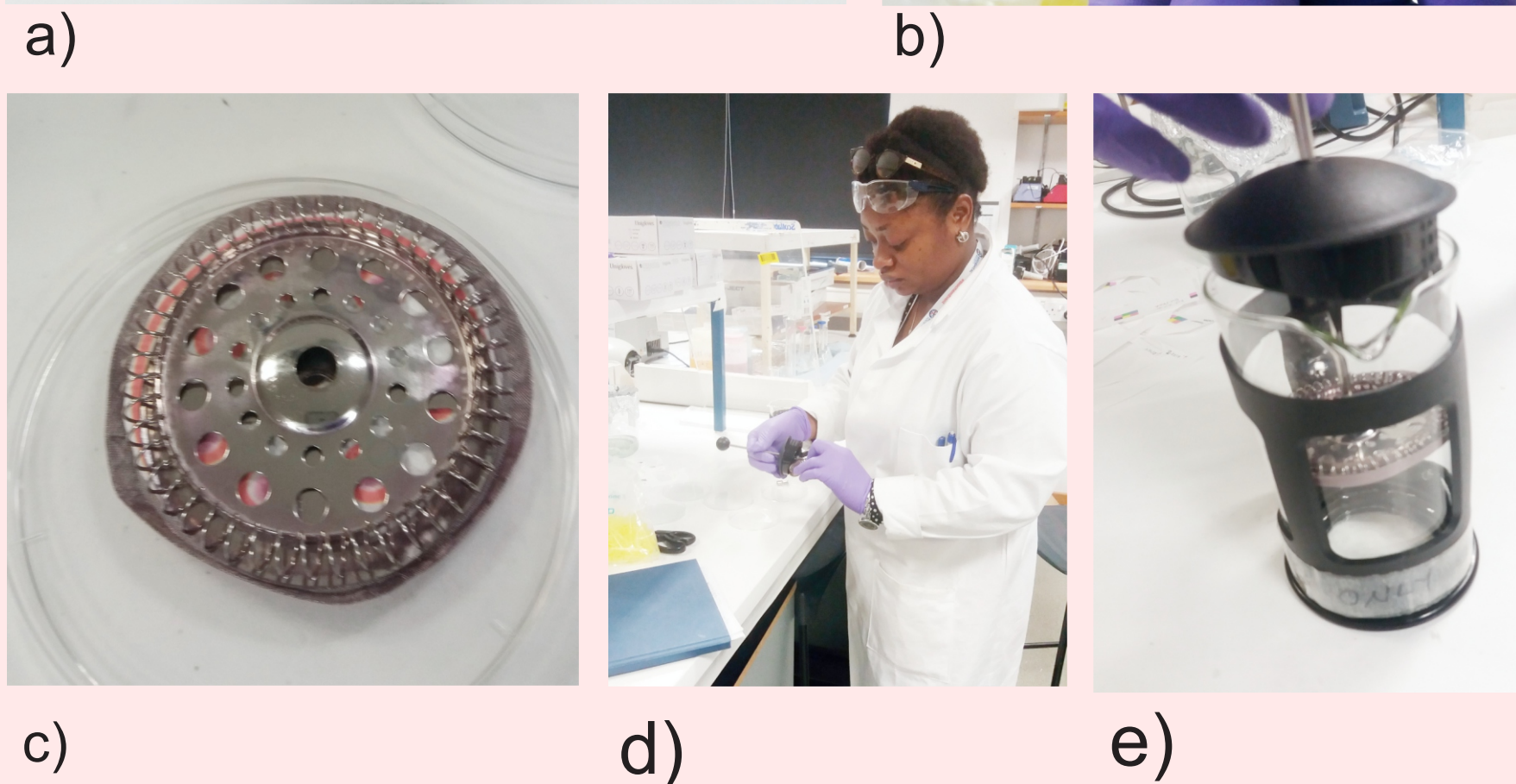


**Fig 2 (a-e) Shows fitting procedures using the cafetiere style push-down system for paper-based microfluidic assay.**

· The time reaction of the colour change was monitored, the colour change is was not instant, within 10-30minutes the blue colour become visible. Images of Zinc-Zincon reactions was captured every 30mins with an android camera phone and the data analyzed with image J software

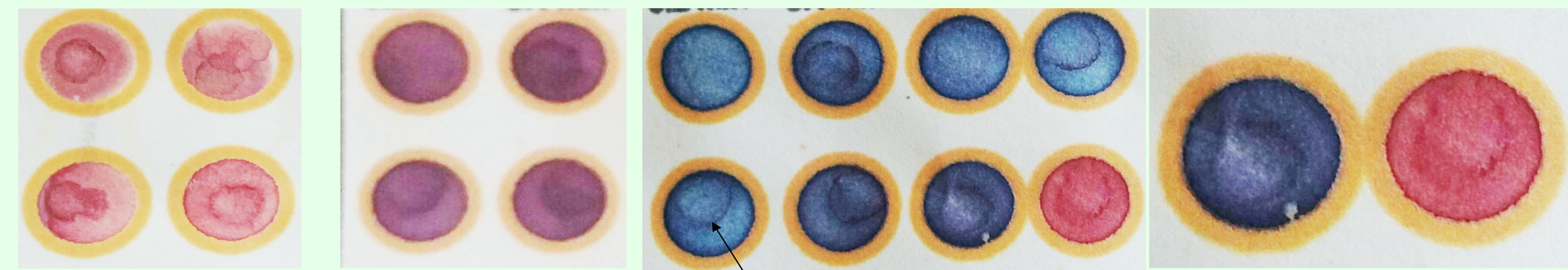


**Fig 3: Brown reaction zone is with only the Zincon solution on the µPAD**  
**Fig 4: Purple colour formed immediately on addition of Zn<sup>2+</sup> to Zincon on the µPAD.**  
**Fig 5: Complete Zincon- Zn<sup>2+</sup> reaction on µPAD**

## RESULTS AND DISCUSSION

### TIME GRAPH ZINC-ZINCON REACTIONS ON PAPER DEVICES

Table 1: Mean Colour Intensity Values of Zincon-Zn<sup>2+</sup> Reactions at Different Time with Varying Concentration (Triplicate Analysis)

| Zinc Concentration | 0mins         | 30mins        | 60mins        | 90mins        | 120mins       | 150mins      |
|--------------------|---------------|---------------|---------------|---------------|---------------|--------------|
| 0.001M             | 49.212 ± 1.95 | 65.471 ± 0.85 | 81.500 ± 4.34 | 79.257 ± 2.54 | 85.943 ± 1.86 | 86.368± 2.16 |
| 0.002M             | 50.088 ± 0.73 | 65.160 ± 0.48 | 75.310 ± 4.23 | 79.259 ± 3.29 | 85.469 ± 3.94 | 85.067± 3.73 |
| 0.004M             | 50.915 ± 2.61 | 67.409 ± 1.74 | 76.037± 2.24  | 80.044 ± 2.30 | 87.086 ± 2.76 | 86.836± 2.03 |
| 0.006M             | 52.307 ± 4.45 | 69.467 ± 5.81 | 77.994 ± 6.72 | 79.738 ± 1.96 | 86.587 ± 2.65 | 87.106± 2.77 |
| 0.008M             | 50.206 ± 1.85 | 65.931 ± 2.41 | 76.348 ±7.95  | 82.191 ± 3.20 | 89.847 ± 3.29 | 89.798±3.14  |
| 0.01M              | 50.149 ± 1.06 | 67.820 ± 1.47 | 78.190± 3.78  | 83.752± 1.84  | 89.698 ± 2.25 | 90.363± 2.28 |

Zincon-Zn<sup>2+</sup> time reactions; at different concentrations of zinc calibration standard solutions with 6uL Zincon solution immobilized with 6uL PDDA in each reaction zone. This data shows a change in colour intensities with time at different concentrations.

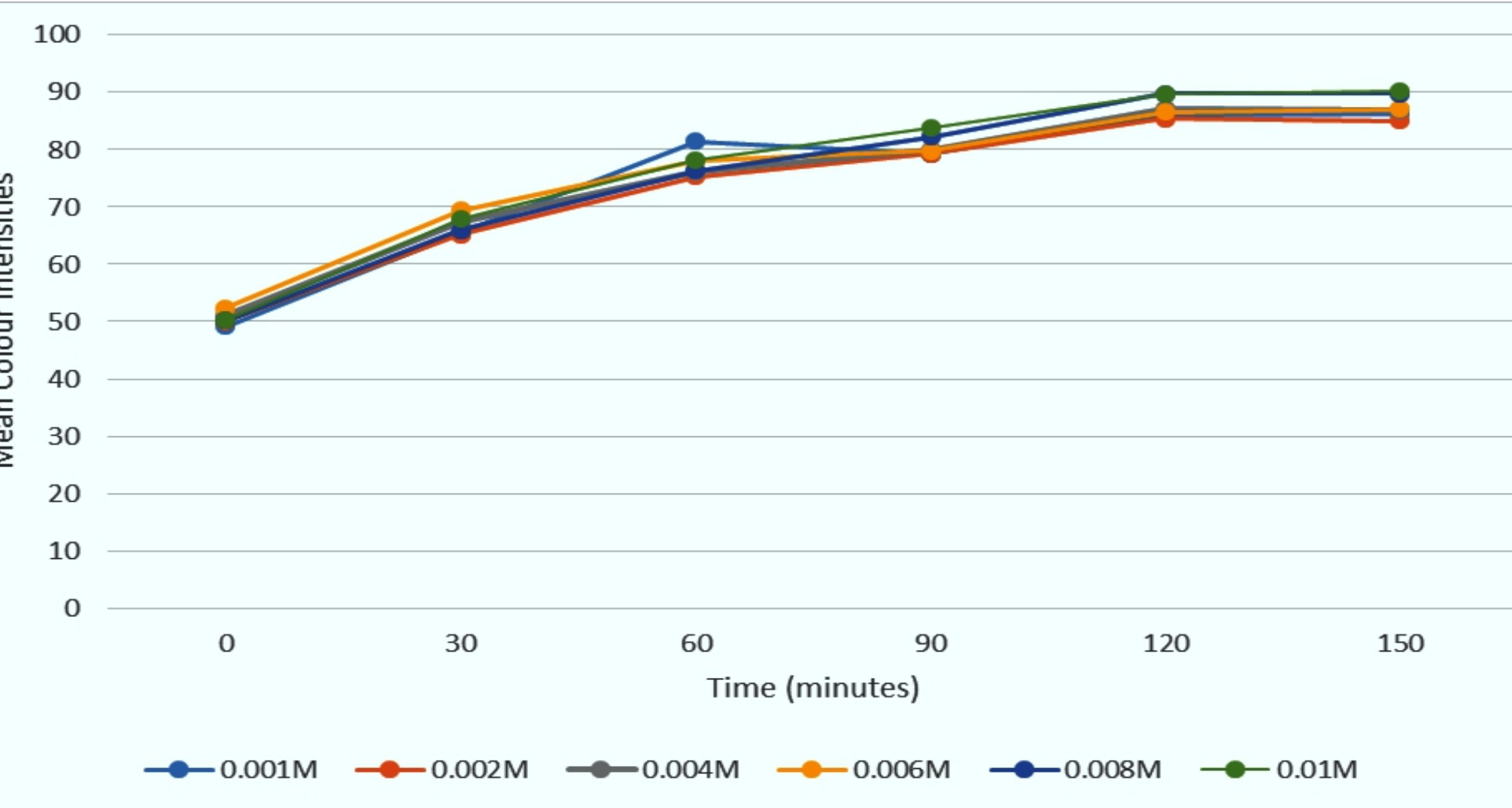


**Fig 6: A plot of mean colour intensity of zincon-zinc reaction against time using 0.001, 0.002, 0.004, 0.006, 0.008 and 0.01M Zinc calibration standard solutions respectively.**

- The time graph colour intensity data were obtained from image analysis of the triplicate determinations of Zincon- Zn<sup>2+</sup> reactions. In each reaction zone, 6uL zincon and 6uL PDDA were added (at 30minutes interval after drying). 30uL of the Zn<sup>2+</sup> calibration standard solution was added to the reaction zones.
- The paper devices were captured using a camera phone every 30mins; from 0-150mins and downloaded into a computer for image analysis with the Image J software using the RGB colour mode without conversion.

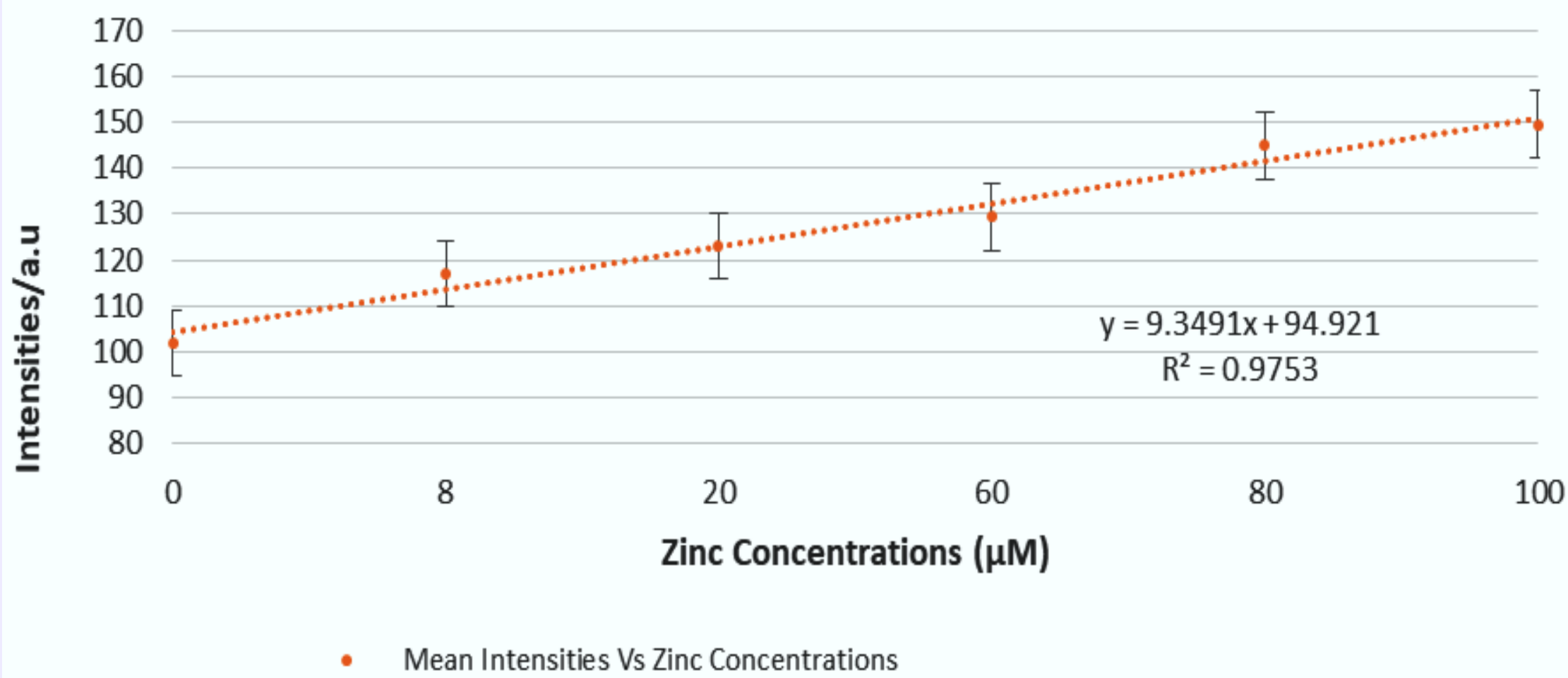


**Fig 7: A plot of mean colour intensities against Zn<sup>2+</sup> concentrations.**

· The data was obtained from image analysis of the triplicate determinations of Zincon-Zn<sup>2+</sup> reactions using the cafetiere style system.

· In each reaction zone of the paper devices, 0.5uL zincon (0.01M) and 6uL PDDA (0.1M). 100mL of five Zn<sup>2+</sup> calibration standard solutions were added to the cafetiere respectively.

· The linear calibration graph under optimum conditions was found between the blue colour intensity (Y-axis) and the Zn<sup>2+</sup> concentrations (X-axis in mol/L). The limit of detection using the calibration curve was found to be at 0.00003M while the limit of quantification was at 0.00099M

**Table 2: Mean Colour Intensities from the Triplicate Determinations of Zinc-Zincon Reactions of Paper Microfluidic Devices using the Cafetiere Style (Push down system).**

| Zinc Concentrations(µM) | Mean Intensities |
|-------------------------|------------------|
| 0                       | 102              |
| 8                       | 117.12           |
| 20                      | 122.98           |
| 60                      | 129.37           |
| 80                      | 144.88           |
| 100                     | 149.51           |

❖ Mean Intensity of the Tab water was at 102.73 a.u

## FINDINGS AND CONCLUSION

- Calibration curves showing a linear correlation from the triplicate determinations of Zincon- Zn<sup>2+</sup> reactions at an optimized sample/reagent volume and concentration in the course of this experimental study were applicable with zincon immobilized with PDDA for paper-based analytical devices.
- The data obtained from the experimental trial of the cafetiere style system was successful both at high and low concentrations of the zinc standard solutions; at microliter volumes of Zincon solutions, colour reaction was also observed.
- A linear calibration curve from the standard solutions used in this study was also obtained using the cafetiere style system. Real water samples (water from the tap at the laboratory) was analyzed using the cafetiere style system following the procedures aforementioned. The intensities also fit into the calibration curve at 102.73 a.u thus the concentration could also be determined.
- However, this analytical method can be used for the determination of unknown samples due to its effectiveness and also its cheap, fast and the paper devices are biodegradable.

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