THINK BIG WE DO℠

A Case Study of Nitrate Reduction in Paper-Based Devices

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RHODE ISLAND CONSORTIUM FOR Coastal Ecology Assessment Innovation & Modeling

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Overview

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2. Nitrate Detection
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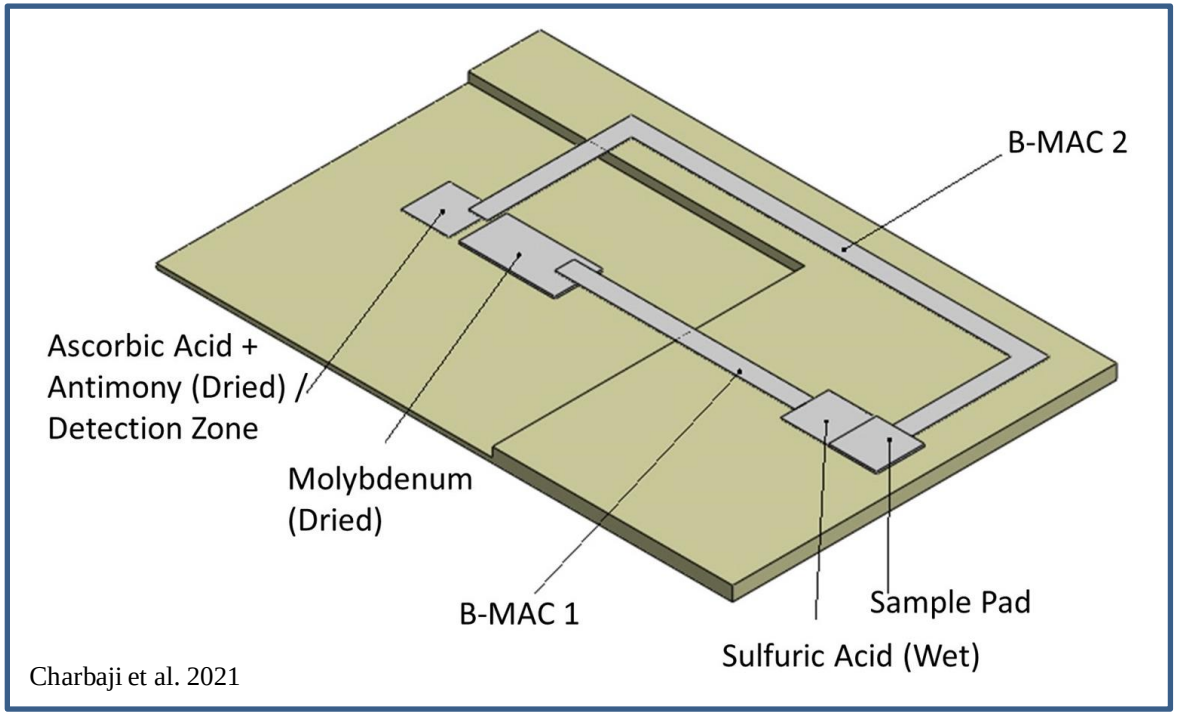
Paper-based Microfluidic Technology

1. Paper-based microfluidic technology has been gaining a lot of attention over the past several years for the many advantages it provides.

2. Paper-based microfluidic technology allows the development of low cost, portable and easy to use devices and sensors that can be easily disposed of. These devices can also provide qualitative or quantitative results and data at the point of care without the need of specialized equipment or power sources.



Charbaji et al. 2021

Charbaji A, Heidari-Bafroui H, Kumar A, et al (2021) Characterization and Modeling of Paper-based Bi-Material Actuator Cantilever; Application in Phosphate Detection. In: Innovations in Microfluidics. Boston

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Paper-based Microfluidic Technology

1. Paper-based devices are generally made up of several different sections that serve difference purposes.

2. The simpler paper-based devices generally include a sample port, transport channels, reactions zones and a detection zone at which a qualitative or quantitative signal can develop and be measured.

3. Properties of the material used in paper-based devices influence assay performance and have a substantial impact on the development of paper-based sensors. (Fernandes et al. 2017)

4. Proper material selection and optimization is critical to enhance the performance of assays in paper-based devices. (Kasetsirikul et al. 2020)

5. This is usually an iterative and an ongoing process to learn and adapt different advancements in the field of paper-based technology to check for the possibility of improving the output and performance of paper-based sensors.

6. An example is the selection of a suitable reducing agent to be used in a paper-based device meant for detecting nitrate in water.

Fernandes SC, Walz JA, Wilson DJ, et al (2017) Beyond Wicking: Expanding the Role of Patterned Paper as the Foundation for an Analytical Platform. Anal. Chem. 89:5654–5664
Kasetsirikul S, Shiddiky MJA, Nguyen NT (2020) Wicking in paper strips under consideration of liquid absorption capacity. Chemosensors 8:1–13.

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Nitrate Detection

1. Nitrate is part of the nitrogen cycle and is an essential nutrient needed for plant growth. However, it plays a significant role in water nutrient pollution when present in excessive amounts.

2. Nitrate is the most stable form of nitrogen in oxygenated systems and all other forms of nitrogen containing compounds can become a source for it. (Silva et al. 2018 & Gupta et al. 2010)

3. Ingesting nitrate has been linked to colorectal cancer, thyroid disease, and central nervous system birth defects. (Ward et al. 2018)

4. Therefore, it is important to measure nitrate levels in water for environmental monitoring purposes and for ensuring its safety for consumption.

Fish kill reported in Canada Pond in 2019, Providence, Rhode Island (WPRI).

Silva CG, Pereira MFR, Órfão JJM, et al (2018) Catalytic and Photocatalytic Nitrate Reduction Over Pd-Cu Loaded Over Hybrid Materials of Multi-Walled Carbon Nanotubes and TiO2. Front Chem 6:632.
Gupta S, Gupta RC, Gupta AB, et al (2010) Pathophysiology of Nitrate Toxicity in Human and its Mitigation Measures. Bull Reg Assess React Nitrogen 20:1–78
Ward MH, Jones RR, Brender JD, et al (2018) Drinking water nitrate and human health: An updated review. Int. J. Environ. Res. Public Health 15

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Nitrate Detection

1. Several paper-based sensors have been developed for the rapid and inexpensive detection of nitrate in water, food and human saliva samples.

2. All of the paper-based devices developed thus far for measuring nitrate levels have used the Griess assay for detection since it is the most commonly used spectrophotometric method for quantifying concentrations of nitrate and nitrite. (Wang et al. 2017 & Mahmud et al. 2020)

3. The Griess reaction involves 2 steps. First, a diazotization reaction of nitrite occurs. Then a coupling reaction follows to allow the production of the colored azo dye.

$$\text{Sulfanilamide} + \text{NO}_2^- \xrightarrow{acid} \text{Diazonium salt} \quad (1)$$

$$\text{Diazonium salt} + \text{N}-(1\text{naphthyl})\text{ethylenediamine} \xrightarrow{acid} \text{Pinkish}-\text{red azo dye} \quad (2)$$

4. The Griess assay is specific to nitrite. Therefore, nitrate molecules have to be first reduced to nitrite before detection.

Wang QH, Yu LJ, Liu Y, et al (2017) Methods for the detection and determination of nitrite and nitrate: A review. Talanta 165:709–720
Mahmud MAP, Ejeian F, Azadi S, et al (2020) Recent progress in sensing nitrate, nitrite, phosphate, and ammonium in aquatic environment. Chemosphere 259:127492

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Nitrate Reducing Agents

1. There are several different reducing agents that can reduce nitrate to nitrite such as cadmium, copperized cadmium, zinc, nitrate reductase, irradiation by ultraviolet light, hydrazine sulfate, titanium (III) chloride, vanadium (III), hydroxylamine, tin chloride or ascorbic acid.

2. Some of these reducing agents are not suitable for use in paper-based devices while others have been tested and used in this type of sensors.

3. Nitrate reductase, irradiation by ultraviolet and hydrazine require lengthy reduction times (Ellis et al. 2011). Therefore, they may not be suitable for use in paper-based sensors due to concerns of sample evaporation.

4. Titanium (III) chloride is violet in color and absorbs light in the same range as the azo dye product of the Griess assay. (Ellis et al. 2011).

5. Ferreira et al. (2020) tested tin chloride, hydroxylamine, ascorbic acid and zinc microparticles. They used zinc microparticles in their paper-based nitrate sensor since the other agents tested did not extensively reduce nitrate to nitrite.

Ellis PS, Shabani AMH, Gentle BS, McKelvie ID (2011) Field measurement of nitrate in marine and estuarine waters with a flow analysis system utilizing on-line zinc reduction. Talanta 84:98–103.
Ferreira FTSM, Mesquita RBR, Rangel AOSS (2020) Novel microfluidic paper-based analytical devices (μPADs) for the determination of nitrate and nitrite in human saliva.

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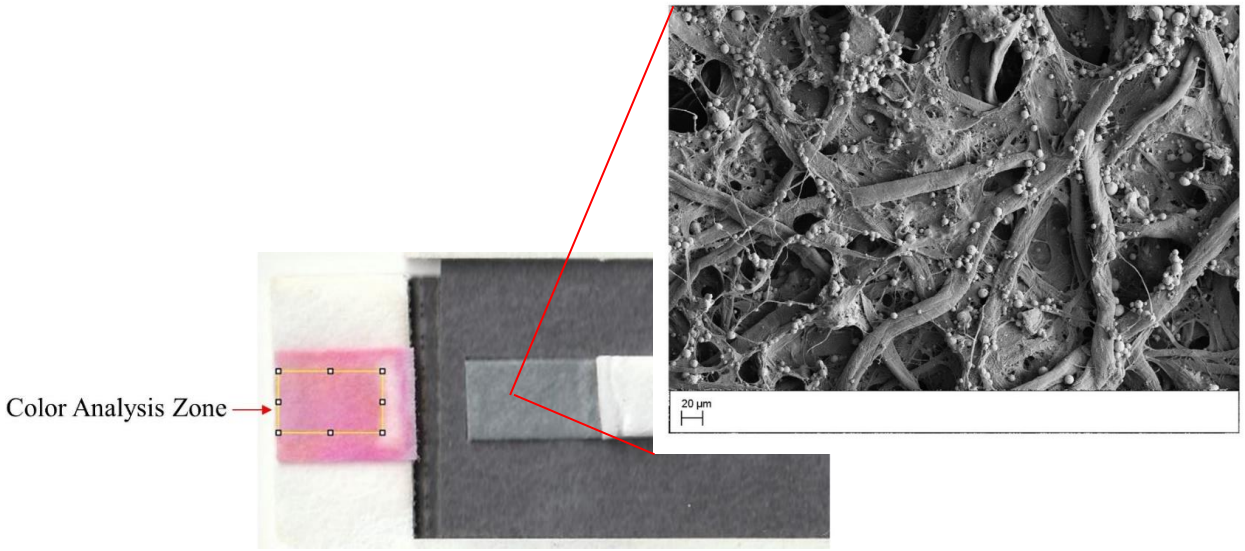
Nitrate Reducing Agents

1. Experimental results by Jayawardane et al. (2014) showed that cadmium and zinc microparticles produced similar results for nitrate reduction in their paper-based device. They opted for zinc microparticles due to the higher toxicity of cadmium.

2. Thongkam et al. (2020) developed a very simple paper-based device for measuring nitrate and nitrite concentrations in food samples and they used vanadium (III) chloride to reduce nitrate before detection.

3. We had previously developed a sensitive paper-based nitrate sensor that incorporated a new composite material made-up of zinc microparticles and cellulose fibers to enhance nitrate reduction.



Jayawardane BM, Wongwilai W, Grudpan K, et al (2014) Evaluation and Application of a Paper-Based Device for the Determination of Reactive Phosphate in Soil Solution. J Environ Qual 43:1081–1085.

Thongkam T, Hemavibool K (2020) An environmentally friendly microfluidic paper-based analytical device for simultaneous colorimetric detection of nitrite and nitrate in food products. Microchem J 159.

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Paper-Based Devices for Nitrate Detection

Reference	Media	LOD (ppm)	LOQ (ppm)
Charbaji et al. 2021	Water	0.533	1.765
Jayawardane et al. 2014	Water	1.178	2.976
Teepoo et al. 2019	Food Sample	3.6	12
Ratnarathorn et al. 2020	Food Sample	0.4	NA
Thongkam et al. 2020	Food Sample	0.4	1.4
Ferreira et al. 2020	Human Saliva	4.96	16.74

1. The results obtained by Thongkam et al. (2020) for nitrate detection in food samples by using vanadium (III) chloride as a reducing agent are very promising.

2. Vanadium (III) chloride will be tested for reducing nitrate in water samples.

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Color Analysis Methodology

1. We developed a dip strip using vanadium (III) chloride for reducing nitrate before detection.

2. We followed the procedure outlined by Thongkam et al. (2020) in preparing the detection chemistry for nitrate and nitrite and used the optimum values they had previously found.

3. Concentrations of nitrite and nitrate used were 0.5, 1, 2.5, 5, 10, 20 and 40 ppm added to ASTM Type 1 deionized water.

4. Matlab was used to fit the data to an exponential decay function of the form y = a . exp (-x/b) + c

5. The symbolic toolbox in Matlab was used to calculate the limit of detection and quantification for nitrite and nitrate using the following equations:

   - Limit of detection (LOD): $y_{LOD} = \bar{y}_B - 3\,\sigma_B$
   - Limit of quantification (LOQ): $y_{LOQ} = \bar{y}_B - 10\,\sigma_B$

Where $\bar{y}_B$ corresponds to the mean color intensity of the blank solution (0 ppm) and σ_B is its standard deviation.

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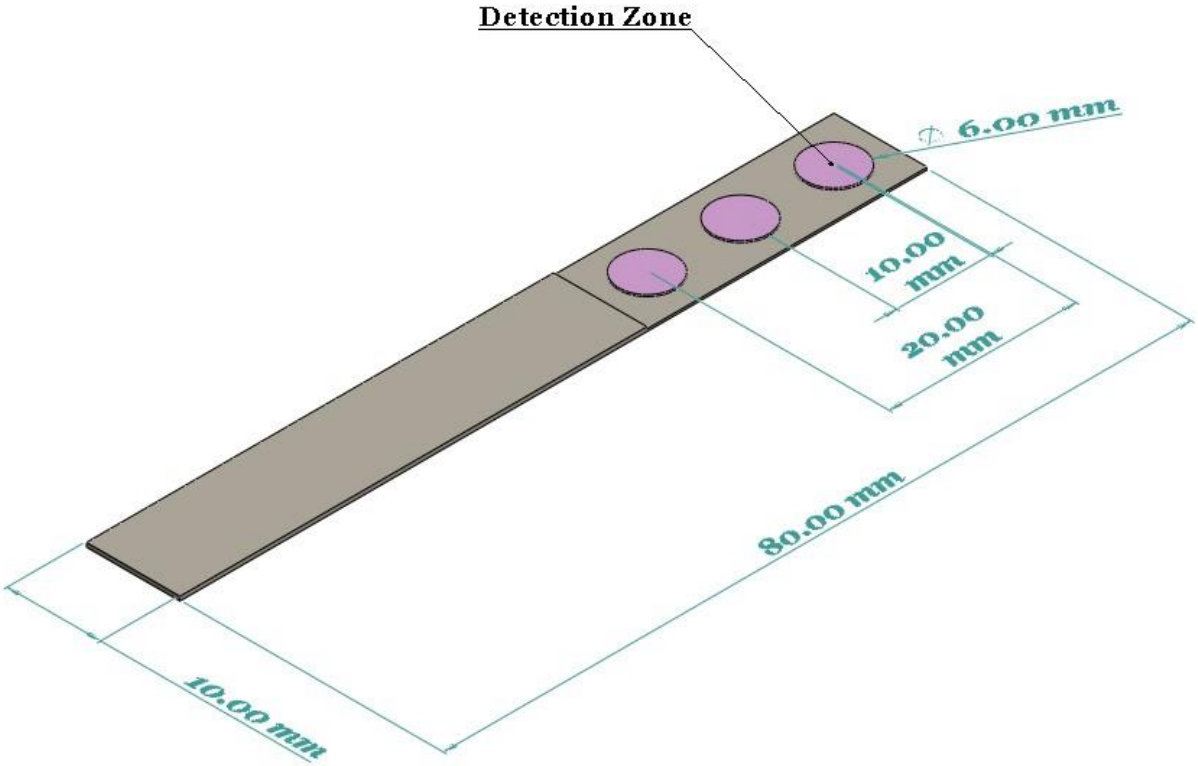
Dip Strip Developed



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Results

Nitrate after
10 minutes

0 ppm 0.5 ppm 1 ppm 2.5 ppm 5 ppm 10 ppm 20 ppm 40 ppm

Nitrite after
5 minutes

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Results

Nitrate after 1 hour

0 ppm 0.5 ppm 1 ppm 2.5 ppm 5 ppm 10 ppm 20 ppm 40 ppm

Nitrite after 1 hour

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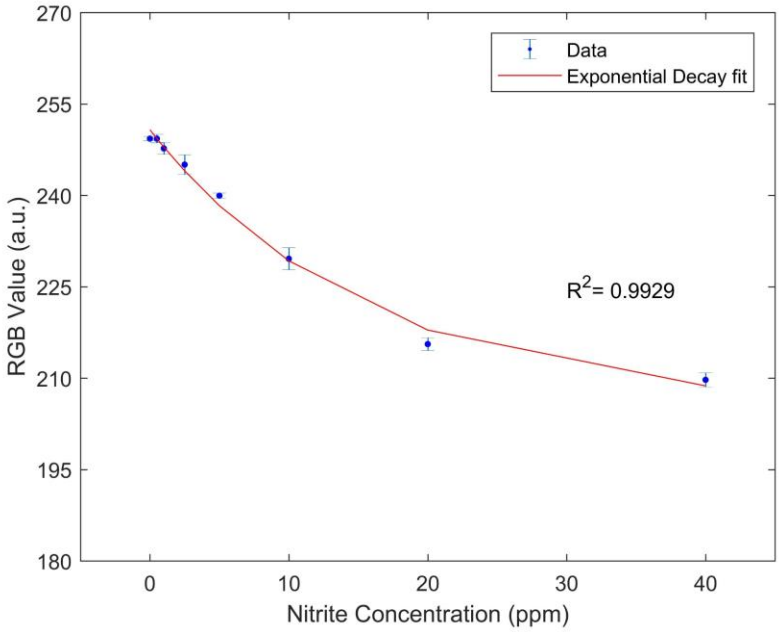
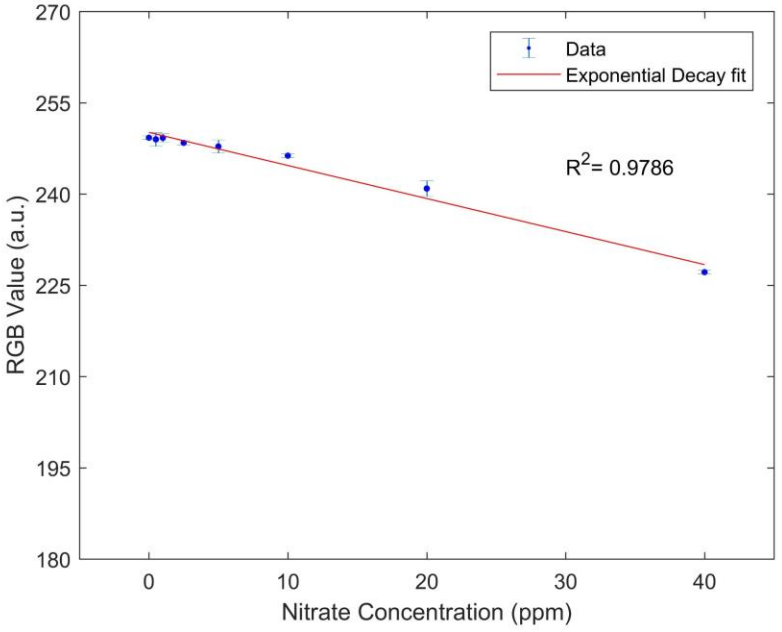
Results





1. The limits of detection and quantification for nitrite after 1 hour are 0.889 ppm and 1.823 ppm.

2. The limits of detection and quantification for nitrate after 1 hour are 3.352 ppm and 7.437 ppm.

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Conclusions

1. Zinculose is a composite material that can be incorporated in any paper-based device. The zinc microparticles in Zinculose are held in place by the matrix which allows the passage of more sample through the material and the reduction of more molecules as they pass through it. This allows for signal amplification as more molecules become available to be captured and detected.

2. Vandium (III) chloride allows for the development of simple dip strips since the reducing reagent can be mixed with the detection chemistry and easily deposited in the detection zone. However, the limits of detection and quantification achieved by dip strips utilizing vanadium (III) chloride are not as good as those obtained in more intricate designs using zinc microparticles.

3. Each of the two reducing agents, zinc microparticles and vanadium (III) chloride, has its own set of advantages and should be used in specific applications with an appropriate device design.

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