A BIODEGRADABLE SENSOR HOUSED IN 3D PRINTED POROUS TUBE FOR IN-SITU SOIL NITRATE DETECTION

Hongjie Jiang, Wuyang Yu, Rahim Rahimi, and Babak Ziaie
Purdue University, West Lafayette, IN, USA

ABSTRACT

In this work, we demonstrate a low-cost and biodegradable electrochemical sensor packaged in a 3D printed porous tube that can sample the nitrate ion from irrigated soil for detection while simultaneously protecting the sensor from any external interference when buried in soil; thus achieving a stable in-situ measurement of soil nitrate concentration with a controllable spatial resolution once deployed in an array format. The sensor array can also be coated/passivated by different biodegradable polymers (or thickness) having a controllable degradation rates, providing a time-delayed sensor exposure to the environment, hence allowing for a longer/tunable working period.

INTRODUCTION

Nitrate (NO_3^-) is a ubiquitous nitrogen compound existing in many different environments and used in various industries such as food [1, 2] and agriculture [3]. Nitrate presence is essential for crop growth since it is one of the key required nutrients in the soil. As one of the key targets in precision agriculture, accurate monitoring of nitrate is of extreme importance in optimizing fertilizer application rates and improving agricultural production. Nitrate also can be a source of contamination of surface and groundwater if its usage as the soil fertilizer is excessive and beyond the actual requirements. If these contaminated water is used to irrigate the plants, which are later consumed by animals and human, or directly used by animals and human, health problems can occur due to the toxicity of nitrate [3]. Therefore, for environmental and ecological sustainability, the nitrate utilities or sources need a strictly scientific regulation and management. To achieve these goals, deploying the in-field nitrate sensors is a critical step.

Based on different nitrate sensing requirements, several measurement methods capable of providing a quantitative result have been investigated [4], [5]. Optical non-contact methods, such as colorimetric or atomic absorption spectrometry, usually involve reducing nitrate to nitrite and measuring the nitrite absorbance at a specific wavelength of light. These methods need discrete soil sampling and sophisticated instruments, which are labor-intensive and costly. A more direct approach first exact the nitrate from the analyte sample and then measure its concentration through different techniques such as UV [6], fluorimetric [7], or mass spectroscopy [8]. These systems still are laboratory-based and will not allow for rapid in-situ measurement in agricultural settings. Diffuse reflectance spectroscopy has recently attracted considerable attention as a more direct method to detect the nitrate concentration in soil by measuring the level of light energy reflected by soil particles and nutrient ions. This has its unique advantages such as non-destructiveness and in-situ operation. However, this method is strongly soil dependent and require careful calibration [2], [4]. Electrochemical sensors [9] using ion selective electrode (ISE) is an attractive choice for in-field and cost-effective nitrate detection. For example, a prototype tractor-mounted field monitoring system comprised of a soil sampler and a fluidic module, to gather and transport the soil samples from 15 cm deep slot, was used to extract and analyze nitrate levels with an ISE with up to 95% accuracy [10]. In another example, an integrated agitated chamber module (IACM) was developed to contain ISEs and data acquisition system. This was attached to the commercial Veris® Mobile Sensor Platform in order

to detect the nitrate concentration in a suspension of soil and water [11]. Although these on-the-go direct nitrate measurement systems have a high accuracy, their fabrication process is still complicated and their deployment face possible mechanical or electrical problems (e.g., clogging of the extractor). The planar electrochemical ISEs that use solid state electrodes to eliminate the requirement of the inner solution of conventional electrochemical sensor are suitable for scalable manufactory through mass-printing technologies (inkjet or screen printing).

Figure 1: (a) Conceptual illustration of the printed biodegradable nitrate sensors array in 3D printed sampling tube for in-situ controllable temporal and spatial nitrate measurement; (b) Different biodegradable polymers erode at different times to expose the sensors.

In this work, we describe the development of screen printed potentiometric nitrate sensor with solid state working and reference electrodes housed in a 3D printed rectangular tube designed to have three porous and one non-porous side walls, Figure 1. The former with micro-pores is used to extract/filter the nitrate containing solution from the surrounding soil into the tube and thus create an aqueous environment for nitrate detection; the latter is used to hold the ISEs array inside the tube. Once equipped with nitrate sensors and inserted into the soil, the porous tube can be deployed at the predetermined regions or depths to achieve a desired spatial resolution of nitrate detection. In addition, a biodegradable polymer, PLGA in this context, is deposited into the sensors' sensing windows with different thicknesses. This results in different biodegradation periods, allowing the users to control the time-dependent activation of sensors. The 3D resin of tube and the substrates of sensor also can be selected to be biodegradable. Note that this conception can be extended to other electrochemical sensors for precision agricultural (e.g., pH, phosphate, and other micro and macronutrients) as a gateway to high resolution sensing platforms for precision agriculture.

FABRICATION

The ion-selective membrane (ISM) consists of tetra-n-octylammonium bromide (TOA-bromide), poly (vinyl chloride) (PVC), di-n-butyl phthalate (plasticizer), and tetrahydrofuran (THF) [12]. PVC was purchased from Aldrich, and the other three components were obtained from Alfa Aesar. Potassium nitrate (KNO_3) was purchased from Alfa Aesar and used as the primary ion in all the test procedures. Potassium chloride (KCl) were bought from Mallinckrodt Chemicals and used in the fabrication of reference electrodes and the selectivity coefficient experiments. Potassium phosphate monobasic (KH_2PO_4) and sodium sulfate (Na_2SO_4) were provided by Mallinckrodt Chemicals and used in the selectivity coefficient experiments. Poly Lactic-co-Glycolic Acid (PLGA) (719897, Mw 7,000 – 17,000 g/mol) was purchased from Sigma Aldrich.

Figure 2: (a) Fabrication process of the sensor; (b) Photograph of the sensor.

Figure 3: 3D printed porous tube.

The fabrication process of sensor is shown in Figure 2. First, a duct tape (0.22 mm thick) is attached to cellulose acetate substrate and laser-machined to create two electrode patterns. Then, silver ink (CreativeMaterials) are screen-printed onto the exposed regions and subsequently dried at 70 °C for 30 min. One silver electrode is immersed into 0.1M FeCl_3 solution for 5 min to form the reference Ag/AgCl electrode, which is then covered with a mixture of UV curable epoxy and KCl powder in a 1:1 w/w ratio under 10 min UV light curing to form the solid electrolyte. Finally, a ~100 μm thick nitrate ion selective membrane (ISM) is solvent-casted on the second silver electrode to form the working electrode. Before measurement, the reference electrode is conditioned in 1M KCl solution for 30 min while the working electrode is conditioned in 0.1 M nitrate solution for 2 days.

The porous tube was 3D printed by a stereolithography (SLA)

desktop 3D printer (Formlabs. Inc) using a photo-reactive resin (mixture of methacrylic acid esters and a photoinitiator), Figure 3. The pores were designed to be 1mm diameter, allowing a fast water sampling while keeping the soil outside.

The PLGA film is made by casting the PLGA powder in acetone with 10% w/v and leaving the polymer solutions in 70 °C oven approximately 24 hours for complete drying.

EXPERIMENTS AND DISCUSSION

Potentiometric method was employed in the experiment in which the potential between the ISE and reference electrode was recorded by Agilent 34401A.

Sensor Characterization in Standard Solution

The sensitivity characterization was conducted with a set of 3 sensors tested at one time. The test solution was prepared by dissolving the KNO_3 powder in deionized water to reach four different nitrate concentrations, from 0.0001 M to 0.1 M. The sensors were then immersed into the solutions and their potentials were monitored for 5 minutes in each solution, sequentially from the lowest to the highest concentration. No ionic strength adjustment or buffer solution was used in the measurement to eliminate the effect of interference ions, especially in low nitrate concentration solution. The result showed a Nernstian sensitivity of averaged 57 mv/decade ($\text{SD}=2.7$ mv), Figure 4. The response time was short, between 5 and 10s.

Figure 4: Sensitivity characterization of nitrate sensor in four standard solutions from .0001 M to .1 M.

Next, the potentiometric selectivity coefficients were measured and calculated using the definition of the Nicolsky-Eisenman equation as follows [13]:

$$E = E_0 + \frac{RT}{(Z_A F)} \ln[a_A + \sum_B K_{A,B}^{pot} (a_B)^{Z_A/Z_B}]$$

Where E is the measured potential; E_0 is the standard potential of the electrodes; Z_A and Z_B are charge numbers of the primary ion, A, and of the interfering ion, B; a_A and a_B are the activities of the primary ion, A, and of the interfering ion, B; and $K_{A,B}^{pot}$ is the potentiometric selectivity coefficient for the primary ion A against the interfering ion, B; R is the gas constant of 8.135 J/K/mol, T is the temperature in kelvin, and F is the Faraday's constant of 9.684 x

10^4 C/mol. In this work, the selectivity coefficients of nitrate sensors were measured in two separate solutions with the same ion activity ($a_A = a_B$), one contained only the primary ion, NO_3^- and the other contained only the interfering ion, Cl^- , or H_2PO_4^- , or SO_4^{2-} . The interfering ions were chosen as they are the most universal ions in the soil. The measurement protocol was the same as the sensitivity characterization, i.e., the sensor was continuously measured in each solution for 5 minutes. The result is shown in Table 1. The sensor has a high selectivity to the nitrate since all the measured selectivity coefficients were smaller than 1. The chloride ion has a little higher interference as compared to other interfering ions; however, as the normal nitrate concentration in soil is higher than 0.0001 M [2], this however will not compromise the nitrate sensor functionality, especially in nitrate pollution regulation since interfering of chloride ion decreases as nitrate concentration increases.

Table 1: Selectivity coefficients of the fabricated nitrate sensor

	Cl^-	H_2PO_4^-	SO_4^{2-}
0.0001M	5.02 E-01	1.82 E-01	3.10 E-04
0.001M	2.82 E-01	7.97 E-02	6.44 E-05
0.01M	1.39 E-01	1.63 E-02	6.41 E-06
0.1M	1.27 E-01	6.23 E-03	3.39 E-06

Finally, the long-term drift of the sensor was characterized by immersing the sensors in 0.1 M nitrate solution and continuously measuring the potential change at an interval of 50 s for one day. The results showed a ~ 0.8 mV/hour linear positive drift (19.2 mV in 24 hours, or 0.1 M drift) due to the change of inner potential induced by the formation of a thin aqueous layer [14], Figure 5. The long-term drift can be reduced or totally avoided by the biodegradation passivation on the sensing region as the sensor would only be activated and work at a programmed window.

Figure 5: Drift of nitrate sensor immersed in 0.1M nitrate solution for one day.

Sensor Characterization in Soil Samples

The sensor capability to detect nitrate in real soil samples (silt loam) was also evaluated. First, the soil was mixed with DI water in a ratio of 3:5 w/w and nitrate levels were measured using the fabricated sensor and a commercial sensor (Cole-Parmer). High accuracy ($\sim 95\%$) was achieved, showing the nitrate concentration of soil as $\sim 0.00017\text{M}$, Figure 6(a). Later, the soil was mixed with standard nitrate solution in 3:5 w/w ratio and measured only by the fabricated sensor. The result was then compared to in nitrate solution without adding soil, where both the measurements were almost the

same in the range of 0.001 to 0.1M while the 60% difference in the mixture of 0.0001M nitrate solution and soil was mostly ascribed to the intrinsic nitrate of soil ($\sim 0.00017\text{M}$), thus further confirming the high accuracy, Figure 6(b).

Figure 6: (a-b) Accuracy characterization of sensor with soil samples.

The PLGA degradation characteristics was measured by immersing a 100 μm thick PLGA film in the soil sampling solution and measuring its weight by gravimetric analysis. At the same time, a nitrate sensor with the sensing area coated with a layer of 100 μm thick PLGA was also immersed in the same solution with its potential monitored at a shorter interval. The results showed that the PLGA film lost an average of 15% weight within 10 days, enough to expose the sensor and initiate the measurement, Figure 7.

Figure 7: Biodegradation characterization of PLGA.

Finally, we tested in-situ performance of the sensor housed in the porous tube and inserted into soil. This was done by pouring DI water into the soil until the water reached the sensor through the tube pores, Figure 8(a). After an initial sharp increase from zero to a high value due to the surrounding medium changes from air to water, the measured potential dropped quickly to a stable value within few

minutes after the completion of nitrate ion sampling from soil to the water inside tube. Figure 8(b) shows the outputs, the sensor using tube sampling can detect a ~30 mv difference from two kinds of soils (compared to a ~20 mv difference measured by a commercial nitrate sensor directly inserted into the soil). This shows that the porous tube can be used to house the ISE potentiometric sensor and provide for an accurate, stable, and fast in-situ detection of nitrate concentration in the soil.

Figure 8: Nitrate measurement using in-situ sampling tube. (a) Experiment setup, (b) comparison of test results for between with (left) and without sampling tube (right).

CONCLUSIONS

In this work, we developed a screen-printed nitrate sensor using solid state working and reference electrodes. The fabricated sensor was able to achieve a 57 mv/dec linear sensitivity within the nitrate concentration range of 0.0001 M to 0.1 M, covering the normal range of nitrate in soil. The sensor had an average of 0.8 mv/hr drift, which could be mitigated by covering the sensing regions by a biodegradable polymer layer of varying thickness or chemical composition. A 3D printed porous tube was used to house the sensor and act as a sampling and protective enclosure. The sensor and its housing can be used for in-situ measurement of nitrate in farming soil at various spatio-temporal scale for precision agriculture.

ACKNOWLEDGEMENTS

The authors thank the staff at Purdue University Birck Nanotechnology Center and the Ziaie Biomedical Microdevices Laboratory members for their assistance in fabrication and experiments.

REFERENCES

- [1] W. Clements, S.-R. Lee, and R. Bloomer, "Nitrate Ingestion: A Review of the Health and Physical Performance Effects", *Nutrients*, vol. 6, no. 11, 2014.
- [2] H.-J. Kim, K. a Sudduth, and J. W. Hummel, "Soil Macronutrient Sensing for Precision Agriculture", *J. Environ. Monit.*, vol. 11, no. 10, pp. 1810–24, 2009.
- [3] N. S. Bryan, D. D. Alexander, J. R. Coughlin, A. L. Milkowski, and P. Boffetta, "Ingested Nitrate and Nitrite and Stomach Cancer Risk: an Updated Review", *Food Chem. Toxicol.*, vol. 50, no. 10, pp. 3646–3665, 2012.

- [4] M. Moorcroft, "Detection and Determination of Nitrate and Nitrite: A Review", *Talanta*, vol. 54, no. 5, pp. 785–803, 2001.
- [5] A. Azmi, A. A. Azman, S. Ibrahim, and M. A. Yunus, "Techniques in Advancing the Capabilities of Various Nitrate Detection Methods: a Review", *International Journal On Smart Sensing and Intelligent Systems*, vol. 10, no. 2, pp. 223–261, 2017.
- [6] D. C. Schroeder, "The Analysis of Nitrate in Environmental Samples by Reversed-Phase HPLC", *J. Chromatogr. Sci.*, vol. 25, no. 9, pp. 405–408, 1987.
- [7] S. H. Lee and L. R. Field, "Postcolumn Fluorescence Detection of Nitrite, Nitrate, Thiosulfate, and Iodide Anions in High-Performance Liquid Chromatography", *Anal. Chem.*, vol. 56, no. 14, pp. 2647–2653, 1984.
- [8] D. Tsikas, I. Fuchs, F. M. Gutzki, and J. C. Frölich, "Measurement of Nitrite and Nitrate in Plasma, Serum and Urine of Humans by High-Performance Liquid Chromatography, The Griess Assay, Chemiluminescence and Gas Chromatography-Mass Spectrometry: Interferences by Biogenic Amines And N(G) -Nitro-L-Arginine An", *J. Chromatogr. B Biomed. Appl.*, vol. 715, no. 2, pp. 441–448, 1998.
- [9] E. Cells, "Electrochemical Sensors", *Anal. Chem.*, pp. 161–180, 2013.
- [10] K. J. Sibley, J. F. Adsett, and P. C. Struik, "an on-the-go Soil Sampler for an Automated Soil Nitrate Mapping System", *Transactions of the ASABE*, vol. 51, no. 3, pp. 1895–1904, 2008.
- [11] B. Sethuramasamyraja, V. I. Adamchuk, A. Dobermann, D. B. Marx, D. D. Jones, and G. E. Meyer, "Agitated Soil Measurement Method for Integrated on-the-go Mapping of Soil pH, Potassium and Nitrate Contents", *Comput. Electron. Agric.*, vol. 60, no. 2, pp. 212–225, 2008.
- [12] R. Pérez-Olmos, A. Rios, J. R. Fernández, R. A. S. Lapa, and J. L. F. C. Lima, "Construction and Evaluation of Ion Selective Electrodes for Nitrate with a Summing Operational Amplifier. Application to Tobacco Analysis", *Talanta*, vol. 53, pp. 741–748, 2001.
- [13] Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, and S. Amemiya, "Potentiometric Selectivity Coefficients of Ion-Selective Electrodes. Part I. Inorganic Cations (Technical Report)", *Pure Appl. Chem.*, vol. 72, no. 10, pp. 1851–2082, 2000.
- [14] R. De Marco et al., "Evidence of a Water Layer in Solid-Contact Polymeric Ion Sensors", *Phys. Chem. Chem. Phys.*, vol. 10, no. 1, pp. 73–76, 2008.

CONTACT

H. Jiang; jiang289@purdue.edu
 *B. Ziaie; bziaie@purdue.edu