MICROMACHINED AMPEROMETRIC NITRATE SENSOR WITH INTEGRATED MICROFLUIDICS

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ABSTRACT

Nitrate-sensing system that consists of a micromachined sensor substrate, nitrate-permeable membrane, integrated microfluidic channels, and standard fluidic connectors has been designed, fabricated, assembled, and tested. Our microsensor was designed for *in-situ* monitoring of nitrate concentrations in ground water. A silver electrode was patterned for amperometric nitrate detection. An electrochemically oxidized silver electrode was used as a reference electrode. Microfluidic channels were fabricated as flow paths for the eluent and ground-water sample to the microelectrochemical (MEC) cell. The sensor also incorporates a nitrate-permeable membrane that is used for selective measurement of nitrate. The sensor has a linear response over concentration ranging from 1 µM to 1 mM.

I. INTRODUCTION AND MOTIVATION

NITRATE is considered to be a major contaminant in ground-water and surface-water systems, and can present significant risks to human health and the environment [1]. In addition, precise nitrate concentration management is needed for the growing field of precision farming [2]. Consequentially, federal health and environmental agencies, ecological scientists, environmental engineers, and farmers have a great need for small, low-power, reliable, and yet sensitive nitrate sensors that can be remotely operated and can measure concentrations in the range of 1 µM to 1 mM [3].

Commercially available nitrate-sensing systems based on ion chromatography, spectrometry, or electrophoresis, usually require expensive and massive instrumentation, complex measurement procedures, and are not currently amenable to remote operation or large-scale deployment. Electrochemical measurements, such as amperometry and potentiometry, are relatively simple, easily miniaturizable, low power, and yet sensitive enough for the targeted applications (i.e., 1 µM to 1 mM). Of these electrochemical techniques, amperometry usually offers simpler analyses to be performed and lower detection limits to be achieved than potentiometry [4]. Selectivity is a major concern in electrochemical measurements and many surface-modification approaches have been used, including immobilized enzymes, freshly-deposited electrode surfaces, and complexing metal ions. However, these approaches increase technical and procedural complexity, without significantly improving performance [4]. Instead, a nitrate-ion-permeable membrane provides the selectivity for our sensing system. Our ultimate research goal is to develop a remotely operable, field-deployable and miniaturized nitrate-sensing system that meets the requirements mentioned above. With the sensitive amperometric detection techniques, a nitrate-permeable membrane, and MEMS-fabrication technology, such a sensing system can be realized.

II. DESIGN AND FABRICATION

A schematic diagram of the sensing system and an illustration of its operation are shown in Figure 1. The sample reservoir is filled with a ground-water test sample and the microelectrochemical (MEC) cell is filled with an electrolytic eluent (10 mM NaOH) (Figure 1b). The sample-fluid reservoir and the MEC cell are adjacent to each other but separated by the ion-selective membrane. Hydroxide ions diffuse across the membrane due to an extreme concentration gradient, and exit the cell while nitrate ions diffuse through the membrane and enter the cell to maintain charge balance. After equilibrium is reached (~5 minutes in our design), the nitrate ions previously in the sample reservoir are transferred to the MEC cell and the amperometric measurements are performed (Figure 1c). When other anions are present in sample solution, they are also transferred across the membrane. However, nitrate diffuses much faster than the other interfering ions due to its larger diffusion coefficient in the membrane. Such a separation method is called Donnan dialysis and allows the selectivity for nitrate measurements [5, 6].
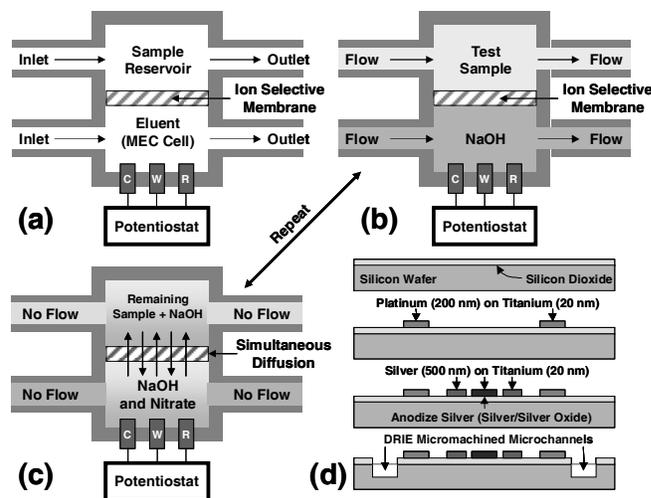

Figure 1. *Schematic diagrams of the electrochemical-sensor system, microfludics (a, b, c), and its fabrication process (d). The electrodes shown are: counter electrode (C), working electrode (W), and reference electrode (R).*
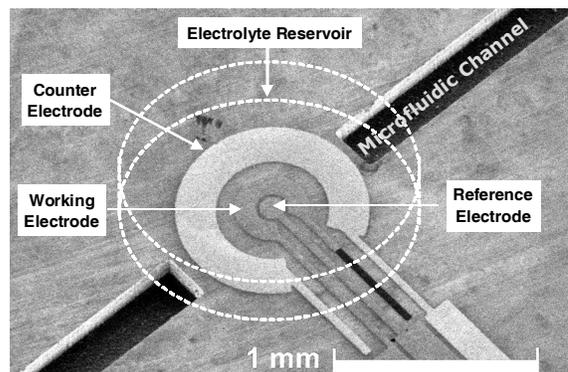

Figure 2. *SEM image of the micromachined electrodes and the 180-µm-deep microchannels in the substrate.*

The microfabrication process was used to produce the microelectrode substrates with integrated microfluidic channels (Figure 1d). Electrode materials widely used for amperometry, such as Pt, Au, and glassy carbon, are not electrochemically active for nitrate detection. We chose to use silver as the working-electrode material after through electrochemical studies in a 10-mM NaOH supporting electrolyte, since silver has an excellent activity for reducing nitrate to nitrite or ammonia [7, 8]. Therefore, the MEC cell has a silver working electrode patterned on a silicon-dioxide substrate. To simplify fabrication, the reference electrode

was made of silver that is electrochemically anodized in 10-mM NaOH to form silver oxide. The counter electrode was made of platinum. The metal electrodes are deposited using a CHA e-beam evaporator. Titanium was used as an adhesion later instead of chromium since it is not electrochemically etched during measurements. A pair of 180-µm-deep microchannels are dry etched with the BOSCH-DRIE process. A SEM image of the concentric electrodes and the microchannels are shown in Figure 2. The microsensor chip is then assembled into a sensor unit with the ion-permeable membrane and EDPM (ethylene-propylene-diene-methylene) rubber plates that define the reservoir and fluidic ports sized to accept standard connectors (Figure 3). The completed and assembled sensor unit is shown in Figure 4.
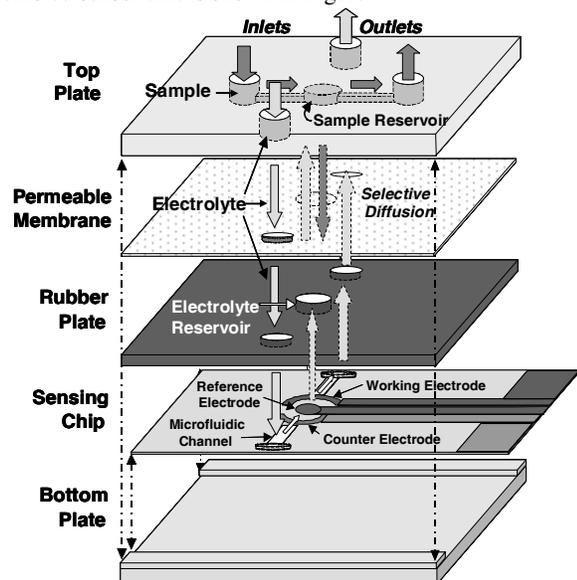

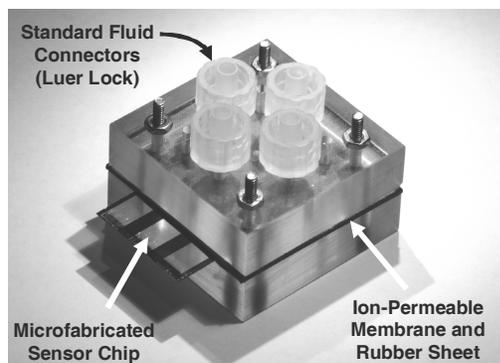**Figure 3**. *Exploded schematic diagram of the complete sensor unit.*

Figure 4. *Assembled sensor unit with standard connectors.*

III. EXPERIMENTAL RESULTS

Reagents were prepared by standard methods. Measurements of the electrochemical process were done using a laboratory potentiostat and data-acquisition system.

In order to obtain repeatable and sensitive measurements, the working electrode needs to be activated before analysis [8]. This is done by sweeping potential from -1.2 V to 0.8 V vs. Ag/AgCl a total of 20 times at a sweep rate of 1 V/s. Calibration curves for nitrate were obtained using chronocoulometry (Figure 5), in which the working electrode is biased at -0.85 V vs. Ag/AgCl and the nitrate reduction current is integrated for 0.5 s. With standard addition methods (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 µM nitrate), the integrated current (charge) is plotted with respect to concentration and slopes are calculated with linear regression. The calibrations represent the greatest and smallest slopes of the sensing chips tested. The data also illustrates the linearity of our amperometric method. The detection limit of the microsensors is ~1 µM of nitrate.

In order to examine the selectiveness of our microsensor, its response to 100-µM nitrate in a mixture of typical interfering ions in ground water (i.e., 100 µM each of PO_4^{-2}, SO_4^{-3}, F^-, Cl^-) was measured. The sensor output is only 13.9% higher than the average response for 100-µM nitrate without interfering ions.

IV. CONCLUSIONS

By incorporating amperometric detection techniques, a nitrate-permeable membrane, and MEMS-fabrication techniques, we successfully demonstrated the feasibility of using a microsensor system to monitor nitrate concentration in ground water. The performance of the nitrate microsensor is sufficient for this application (i.e., detection range from 1 µM to 1 mM with good selectivity). Long-term qualification tests are presently underway. Once integrated with a chip-scale potentiostat, miniature pumps, check valves, and a wireless sensor node, we anticipate that a revolutionary instrument for nitrate monitoring in water systems could be realized.
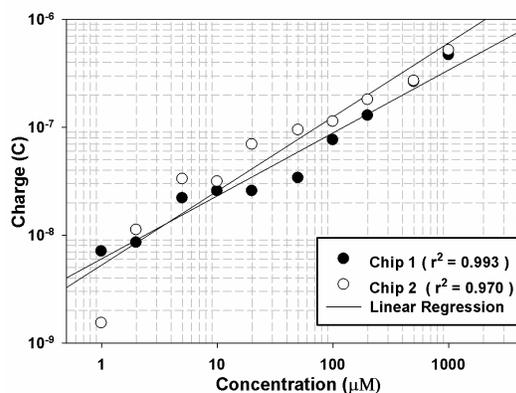
Figure 5. Calibration curves for the nitrate-sensor system with a minimum detection level of ~1 µM.

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