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Fiber-optic Sensor for Sodium Ion Based on a Novel Fluorescent Dye Sodium Green™

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An optical fiber fluorescence sensor for sodium ion was developed. A dextran derivative of the fluorescent dye Sodium Green™ was covalently immobilized on a Biodyne A™ membrane disk. The disk was set at the common end of a bifurcated optical fiber and positioned in a flow cell. The leg ends of the optical fiber were coupled to the light source and detector of a fluorescence spectrophotometer via a positioner. The immobilized dye exhibited an excitation wavelength of 510 nm and an emission wavelength of 550 nm. The fluorescence signal was completely reversible and reproducible. Typical response time varied from 3 to 5 min depending on the concentration. The equilibrium sensor signal was not affected by flow rate. Calibration curves constructed for sodium ion exhibited a linear range from 5 to 40 mM ($r = 0.969$) and a sensitivity of 0.424 F.U./mM. Minimal interference was indicated for K^+, Li^+, Ca^{2+}, and Mg^{2+}. Acidic pH values were observed to inhibit sensor response.

Keywords: fiber-optic sensor; fluorescence; sodium ion; Sodium Green

INTRODUCTION

Sodium ion is a clinically significant analyte. It is the major cation in the blood and body fluids. Along with potassium, sodium functions in the maintenance of proper body water and blood pressure. It also plays an essential role in the transmission of nerve impulses. Quantitative data of sodium levels in body fluids are used in the diagnosis and treatment of aldosteronism, diabetes insipidus, Addison's disease, dehydration, and other diseases involving electrolyte imbalance [1]. The regulation of dietary salt intake likewise necessitates a reliable method for the determination of salt content of commercial food products.

The standard method for the analysis of sodium levels in serum and urine involves the use of Na^+ ion-selective electrodes (ISE). These Na^+ ISEs makes use of ionophores, such as calix(n)arenes [2], which are macrocyclic molecules that have a natural cavity that can recognize sodium ions. A potential is eventually generated at the membrane-solution interface as a consequence of the binding of sodium ions with the sodium ionophore.

The concentration-dependent binding event of the ion to the ionophore can also be evaluated through optical methods, either through the absorbance or fluorescence changes of a suitable pH indicator [3]. The pH change is effected through a cation-exchange mechanism occurring in the ionophore. A simplification of this approach was introduced with the combination of the ionophore and chromophore/fluorophore in a single molecule.

One such type of fluoroionophore is the novel dye Sodium Green™ [4]. This fluorescence dye is excitable in the visible wavelength range. It has been used for imaging intracellular free sodium ion concentrations in the range from 0.5 to 50 mM using fluorescence lifetime-based measurements [5, 6]. However, no work has been reported yet on the application of this relatively new fluorescence indicator in the immobilized phase or in a sensor configuration.

This work therefore has aimed to develop an optical fiber fluorescence sensor for sodium ion based on immobilized Sodium Green™ reagent. With its emission and excitation wavelengths occurring in the visible region, a Sodium Green™–based sensor presents the advantage of ease of fab-

ricating low-cost instrumentation systems. Such a sensor system also has the capability of being miniaturized, automated, and requiring small sample volumes. The sensor can be used for the analysis of sodium in serum, urine, and other body fluids. The sensor can also find application in the study of sodium channels and sodium channel blockers such as saxitoxin and tetrodotoxin.

EXPERIMENTAL

Materials. All reagents were used as received from commercial suppliers. Biodyne A membrane was purchased from Pall Europe Ltd. (Portsmouth, England). Glutaraldehyde, polyallylamine, and cyanuric chloride were sourced from Sigma-Aldrich, Inc. (Milwaukee, Wisconsin, USA). The fluorescent dye Sodium Green Dextran was purchased from Molecular Probes Inc. (Eugene, Oregon, USA). All other reagents used were of analytical reagent grade.

Apparatus. An instrumentation system based on a fluorescence spectrophotometer (Hitachi F-4500) was assembled (Fig. 1a). A bifurcated optical fiber bundle (Oriel Instruments Model 77533) was coupled to the optics of the fluorometer using a fabricated positioner holder. The common end of the optical fiber is set in a flow cell connected to a flow manifold driven by a peristaltic pump (Ismatec IPC). The excitation light is directed by one leg of the bifurcated optical fiber bundle to the common end where it interacts with the reagent phase. The emission radiation is guided back to the detection optics of the fluorometer through the other leg of the optical fiber bundle. The fluorescence readings were graphically displayed and recorded on a PC-based data acquisition system that is part of the instrument.

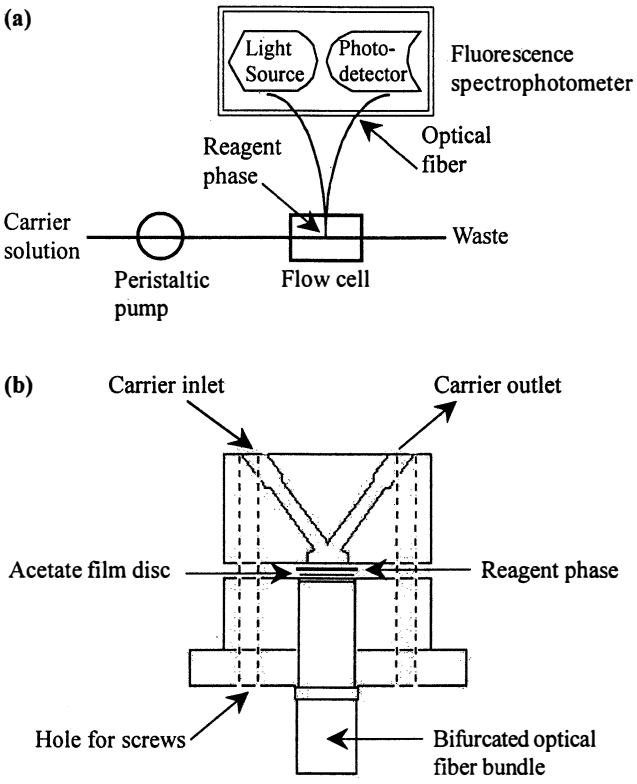

Fig. 1. (a) *Instrumentation system based on a fluorescence spectrophotometer;* (b) *Diagram of flow-cell*

A flow cell was fabricated from Perspex rods and sheets (Fig. 1b). It was designed to sandwich the reagent disc between two Perspex rod cross sections (2.85 cm dia.). The bottom piece accommodates the common end of the optical fiber while the upper piece has inlet and outlet bores for the carrier stream passing through the reagent phase. The bottom and upper pieces are fastened using screws. The internal volume of the flow cell was approximately 100 μL. A 6-mm dia. acetate film disc (100 μm thick) was used as spacer between the common end of the optical fiber bundle and the reagent disc.

Immobilization of Sodium Green™. 50 discs of Biodyne A (6.5 mm dia.) were cut out from sheets using a pointed cutter blade and a plastic template. The discs were placed in a glass vial, to which 1 mL of 0.5% glutaraldehyde solution was added. The discs were agitated in the glutaraldehyde solution by intermittent vortexing for 1 h. The glutaraldehyde solution was decanted and the discs were washed with 1 mL 0.1 M phosphate buffer pH 7.0 for 1 min. After discarding the washings, the discs were then immersed in 1 mL of (0.01534 g/mL acetone) polyallylamine solution, with intermittent vortex mixing for 30 min. The polyallylamine solution was decanted and the discs were washed twice with 1 mL portions of distilled water for 1 min. Final washing was done with two 5-mL portions of distilled water for 1 min.

The discs were soaked in 700 μL of cyanuric chloride (0.3 g/mL dry acetone) for 2.45 h. The cyanuric chloride solution was pipetted off and was disposed of accordingly. The discs were washed once in 1-mL portion of acetone and twice in 2-mL portions of acetone for 1 min each washing. The washings were pipetted off and the discs were air-dried for 45 min. A 750-μL aliquot of Sodium Green dextran solution was used to soak the discs for 4 h. The discs were washed five times in 1 mL portions of 0.1 M phosphate buffer pH 7.0. The discs were finally air-dried for two hours and were stored desiccated inside a freezer.

Measurement procedure. With the reagent disk set in the flow cell, baseline is established with the blank, which could either be distilled water or buffer solution. The sodium solution is then passed through the flow cell and the signal is followed until a steady state fluorescence emission value is attained. The baseline is then reestablished with the blank. The fluorescence intensity signals were corrected against a distilled water blank.

RESULTS AND DISCUSSION

The reagent phase. The sodium fluorescence indicator used is Sodium Green™, a visible light-excitable probe developed at Molecular Probes, Inc. USA. In solution, it exhibits a λ_{ex} of 488 nm and λ_{em} of 533 nm. Sodium Green™ consists of a fluorescein analog linked to each of the nitrogens of a crown ether with a cavity size that confers selectivity for the sodium ion (Fig. 2) [4]. Upon binding to sodium, Sodium Green™ exhibits an increase in fluorescence emission intensity with little shift in wavelength. For immobilization purposes, the dextran conjugate of Sodium Green™ indicator was used. The dextran provides hydroxyl groups which could be conjugated to the amine groups of Biodyne A.

The immobilized Sodium Green™ indicator showed a λ_{ex} of 510 nm and λ_{em} of 550 nm. These represent a shift to higher wavelength values compared with those in the indicator solution phase. The reagent phase was subjected to flow conditions continuously for 96 h, setting the flow rate of the distilled water blank at 3.0 mL/min. The baseline fluorescence signal was degraded to 45% of the original signal after the 96-hour observation period. The fluorescence signal for 0.05 M NaCl solution (pH 7.0) was reduced to 50% of the initial value after 44 h. It was reduced further to 30% of the initial response after 96 h.

Sensor response. The sensor equilibrium response is attained within 3 to 10 min depending on the sodium ion concentration. Attainment of both the equilibrium signal and baseline is highly reproducible (<5%) indicating excellent reversibility of sodium ion binding.

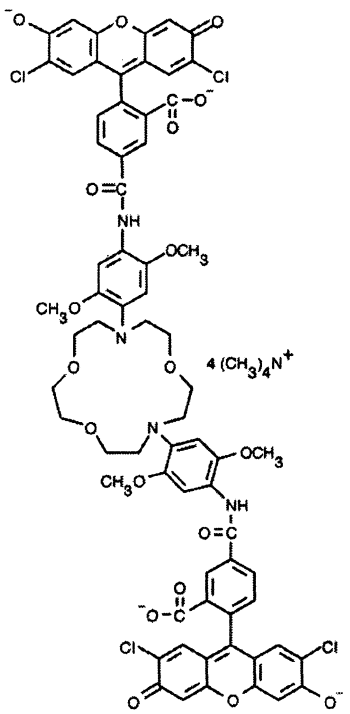

Fig. 2. Tetra(tetramethylammonium) salt of Sodium Green™.

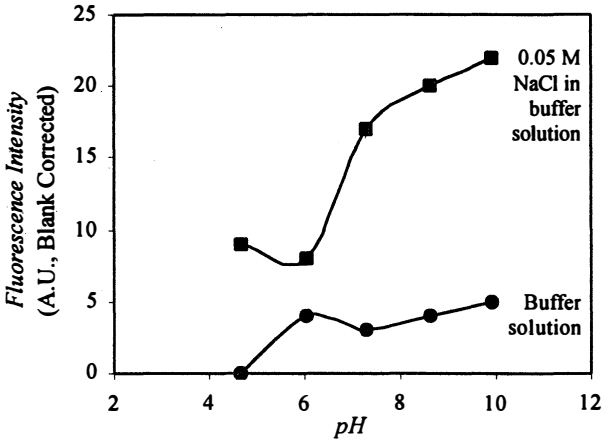

Fig. 3. Effect of pH on fluorescence signal (Britton-Robinson Universal Buffer: 0.04 M HAc, 0.04 M $H3PO_4$, 0.04 M H_3BO_3).

Flow rate was found to have no significant effect on the signal. The same value for the equilibrium signal can be reached both under flow and stopped-flow conditions. The temperature of the solution likewise did not have a significant effect on the sensor response. However, ambient air temperature was observed to affect the baseline signal. This could be attributed to the temperature effects on the electronics of the fluorescence spectrophotometer.

Effect of pH. The response of the sensor was found to be affected by the pH of the sodium ion solution (Fig. 3). The fluorescence intensity increased with the pH of the solution. This can be attributed to the ionization of the phenolic hydroxyl and the carboxyl groups in Sodium Green™. Ionization of these functional groups extends electronic conjugation and enhances the fluorescence of the indicator. The same effect was also observed in the reagent phase in the absence of sodium ion.

The difference in fluorescence signal between the plain buffer solution and 0.05 M NaCl in buffer solution increased from pH 7.0 to 10.0. However, since serum and urine samples commonly exhibit near-neutral pH values, the pH value that was chosen for the sodium standard solutions was 7.0. The type of buffer used and the buffer concentration were observed to have no significant effect on the fluorescence signal. For the sodium standard solutions, 0.10 M HEPES buffer was nominally chosen as solvent because it is more suited for biological samples.

Effect of sodium ion concentration. The sensor response varied with sodium concentration (Fig. 4). A linear relation was observed (r = 0.969) within the range from 5 to 50 mM Na^+. A sensitivity value of 0.424 F.U. per mM was observed.

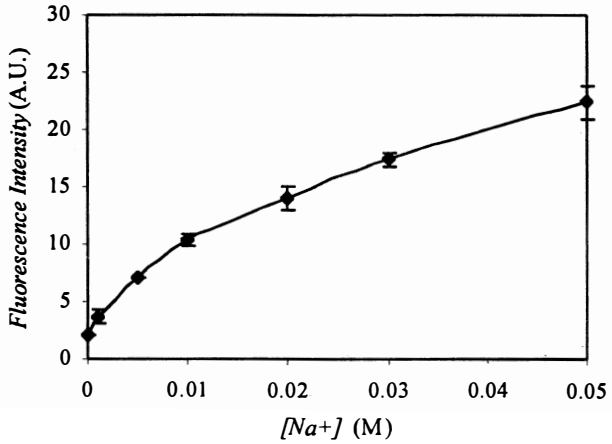

Fig. 4. Calibration curve for sodium ion (n = 3).

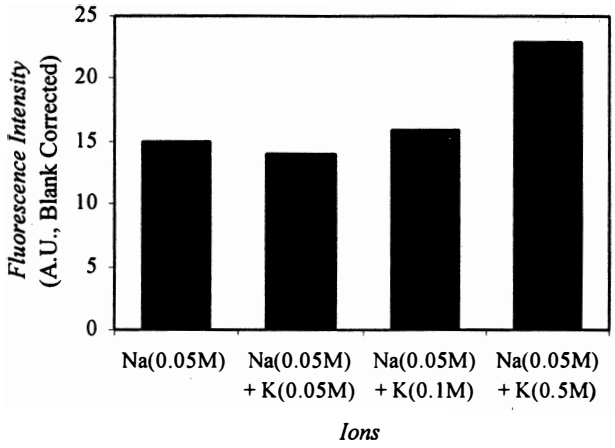

Fig. 5. Interference from potassium ion.

Interference. The effect of other ions on the sensor response was investigated. Minimal interference was indicated for K^+, Li^+, Ca^{2+}, and Mg^{2+} at equimolar concentration as that of sodium ion. Interference from potassium ion was further studied because K^+ is the other important ion found in body fluids (Fig. 5). Potassium exhibited significant interference at a higher concentration than sodium. Sodium Green™ is claimed to exhibit a 41-fold selectivity for Na^+ over K^+ [4]. Interference from potassium ion and other metal ions can theoretically be eliminated through the use of the standard addition technique.

CONCLUSION

An optical fiber fluorescence sensor for sodium ion based on a new reagent was developed. Sodium Green™ was covalently immobilized on a Biodyne A membrane disc. The fluorescence signal was completely reversible and reproducible. The sensor exhibited an acceptable response time and a linear range and sensitivity that is good enough for the analysis of sodium ion in serum, urine and other body fluids. Proper dilution of the sample should be done in order to ensure that the analysis is performed within the linear range of the calibration curve.

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