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Fluorescent Ion-Selective Optode Membranes Incorporated onto a Centrifugal Microfluidics Platform

Ibrahim H. A. Badr,[‡] R. Daniel Johnson, Marc J. Madou,[†] and Leonidas G. Bachas*

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506-0055

The development of an integrated analysis system for small ions based on ion-selective optodes and centrifugal microfluidics is reported. The performance of this system was evaluated through five-point calibration plots for two types of optode membranes, one being cation-selective and the other anion-selective, which were incorporated into a microfluidics platform on which fluid motion is induced via angular rotation. Additionally, the application of the microfluidic platform to ion analysis is studied via a two-point calibration protocol used to quantify an unknown sample. Calibrant solutions are delivered from reservoirs fabricated onto the platform to a measuring area that contains the optode membrane, with a change in membrane fluorescence being monitored. This work demonstrates the first instance of a microfluidic-based analysis system with detection based on ion-selective optode membranes monitored with fluorescence transduction. Furthermore, in addition to employing a standard excitation source where a fiber-optic probe is coupled to a tungsten−halogen lamp, laser diodes such as those employed in portable CD/DVD players were studied as excitation sources to enhance the observed fluorescence signals.

Since the inception of the idea of miniaturized fully integrated analysis systems (micro total analysis systems) over a decade ago, numerous advances have been made in the areas of fluidics theory and control, microfabrication, and detection, as well as packaging and scaled-down instrumentation.[1−20] In fact, significant progress in the understanding and application of these ideas and numerous efforts to incorporate concepts from different disciplines into the microfluidics niche continues today.[21−24] Furthermore, the development of viable total analysis systems from conceptualization through field-readiness requires that research attention be focused on the fundamental areas outlined above.

Recently, a fully integrated analysis system for potassium that was based on centrifugal microfluidic propulsion and ion-selective optode detection was reported.[1] The platform was demonstrated to have advantages over systems based on electrokinetic fluid propulsion, especially with regard to the detection of small inorganic ions. The centrifugal system was based on a microfluidic architecture of channels and reservoirs microfabricated onto a rigid polymer disk substrate with fluid propulsion arising from forces created by spinning the disk. Additionally, the detection system was a standard fiber-optic-based absorbance photometer. This system was demonstrated as a simple, novel platform for calibrating optodes and measuring unknown quantities of inorganic ions in aqueous media.

* To whom correspondence should be addressed. Phone: 859-257-6350. Fax: 859-323-1069. E-mail: bachas@uky.edu.

[†] Current address: Department of Mechanical and Aerospace Engineering, University of California, Irvine, CA 92697.

[‡] On leave from the Department of Chemistry, Faculty of Science, Ain-Shams University, Cairo, Egypt.

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To take advantage of the beneficial aspects of microfluidic systems, for example, less power, space and reagent consumption, the reported centrifugal platform and associated instrumentation may require further miniaturization. It is feasible to imagine packaging the platform's motor and detection systems into a footprint similar in size to that of a portable compact disk player that can be linked to a laptop computer or personal digital assistant. This reduction in size would then make it possible to supply power to the system through a conventional battery. Furthermore, with typical modern fabrication methods, it is possible to construct a centrifugal platform that possesses up to 100 individual microfluidic architectures on a single disk, thereby reducing the per-analysis amount of reagent/sample consumed as well as waste produced. This platform could be constructed such that analyses could be performed either for a single analyte in a large number of samples or for an array of different analytes in a single sample.

The detection system presented in our previous publication is limited by a high detection limit (submillimolar) and a narrow linear range, which are typical characteristics of absorption mode measurements.[1] In this paper, we present the next step in the evolution of the centrifugal platform-based analysis system. Detection in the system is provided through the change in the fluorescence properties of optode membranes that is associated with varying concentration of analyte ions. The impetus for employing fluorescence detection lies in the improved measuring capabilities associated with this technique, as opposed to reflectance or absorbance measurements, particularly for small volume analyses. For instance, it is well-known that fluorescence-based detection schemes are more sensitive methods; therefore, the optically interrogated dimensions can be much smaller than in absorbance measurements without sacrificing signal-to-noise ratio. In addition to the large gain in sensitivity anticipated from fluorescence-based measurement, fluorescence is expected to provide a wider linear dynamic range over absorption methods and lower detection limits. A further advantage of fluorescence is its compatibility with laser excitation sources that not only furnish an intense, highly monochromatic light but also can be focused efficiently onto a very small area, such as the optical membrane integrated onto the CD platform. Moreover, using a laser source is expected to increase the sensitivity by increasing the fluorescence intensity, thus enhancing the signal-to-noise ratio.

We also demonstrate, herein, the use of diode laser sources, as opposed to providing excitation light via a lamp and monochromator, since fluorescence can be enhanced by employing the more intense laser excitation. Additionally, laser diodes are amenable to miniaturized instrumentation and are relatively inexpensive light sources. Finally, to demonstrate the flexibility of optode detection and to preview the capability of the platform for array or highly parallel analyses, we now present analysis systems based on both cation- (K^+) and anion-selective (NO_2^-) sensing membranes.

EXPERIMENTAL SECTION

Reagents. 9-(Diethylamino)-5-(2-octadecylamino)benzo[*a*]phenoxazine (proton chromoionophore III), 2-dodecyl-2-methyl-1,3-propanediylbis[*N*-[5′-nitro(benzo-15-crown-5)-4′-yl]carbamate] (BME 44, potassium ionophore III), 4′,5′-dibromofluorescein octadecyl ester (proton chromoionophore VI), cyanoaqua-cobyrinic acid heptakis(2-phenylethyl ester) (nitrite ionophore I), potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate (KTFPB), high-molecular-weight poly(vinyl chloride) (PVC), bis(2-ethylhexyl)-sebacate (DOS), and Selectophore-grade tetrahydrofuran (THF) were obtained from Fluka (Milwaukee, WI). *N*-[2-Hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid] (HEPES, molecular biology grade) was obtained from Sigma (St. Louis, MO). Tris(hydroxymethyl)aminomethane, molecular biology grade was obtained from Research Organics, Cleveland, OH. Sodium nitrite was purchased from J. T. Baker (Phillipsburg, NJ). Potassium chloride was obtained from Fisher Scientific (Cincinnati, OH). Transparent polypropylene adhesive tape, available commercially as Scotch mailing tape, was obtained from 3M (St. Paul, MN). All aqueous solutions were prepared with 14 MΩ deionized distilled water produced by a Milli-Q water purification system (Millipore, Bedford, MA). Potassium standards were prepared in 0.0500 Tris/HCl buffer, pH 6.9, and nitrite standards were prepared in 0.0100 M HEPES/NaOH buffer, pH 7.4.

Preparation of Optode Membranes and Centrifugal Platform. Membrane cocktails were prepared with the following composition: 45 mg of PVC, 100 mg of DOS, 2.0 mg of nitrite ionophore I, and 1.0 mg of chromoionophore VI dissolved in 1.0 mL of THF or 80 mg PVC, 160 mg DOS, 7.3 mg of BME 44, 2.1 mg of chromoionophore III, and 3.4 mg KTFPB dissolved in 1.5 mL of THF. A 5-cm^2 piece of adhesive tape was attached, avoiding the trapping of air bubbles between the tape and glass, to the surface of a clean, dust-free glass plate of the same dimensions via the tape's adhesive layer. The tape-covered glass plate was secured in a spin-on device under a THF-saturated atmosphere (Model KW-4A, Chemat Technology, Northridge, CA) by applying vacuum. The plate was rotated at 2000 rpm, and 200 μL of the membrane cocktail was injected onto the rotating plate, thereby forming a membrane on the nonadhesive side of the tape. After a spinning time of ~9 s, the plate/membrane was removed from the spin coater and allowed to dry in air for 10−15 s. The resulting membranes had an average thickness of 2−4 μm. When not in use, the membranes were stored in a desiccator and in the dark.

The design and construction of the centrifugal platform (Figure 1) and incorporation of optode membranes onto the disk have been described elsewhere.[1,25] Briefly, the platform consists of five reservoirs from which fluids flow sequentially into a measuring area where the optode is positioned. After integration with the centrifugal platform and prior to use, membranes were conditioned for 1 h with Tris buffer (for potassium-selective optodes) or with HEPES buffer (for nitrite optodes) by introducing the buffers into the measuring reservoir via a pipet.

Measurement Setup. Once the optode membrane was conditioned, the channels and reservoirs were sealed with a piece of adhesive tape. To fill the reservoirs, a syringe tip was used to create a hole in the sealing layer above the center of each reservoir. Solutions were then introduced into the reservoir through the hole. Finally, a second layer of adhesive tape was placed over the entire fluidics system to ensure an airtight seal to avoid fluid leakage. The fluid-loaded disk was placed on the hub of the centrifugal platform's motor.

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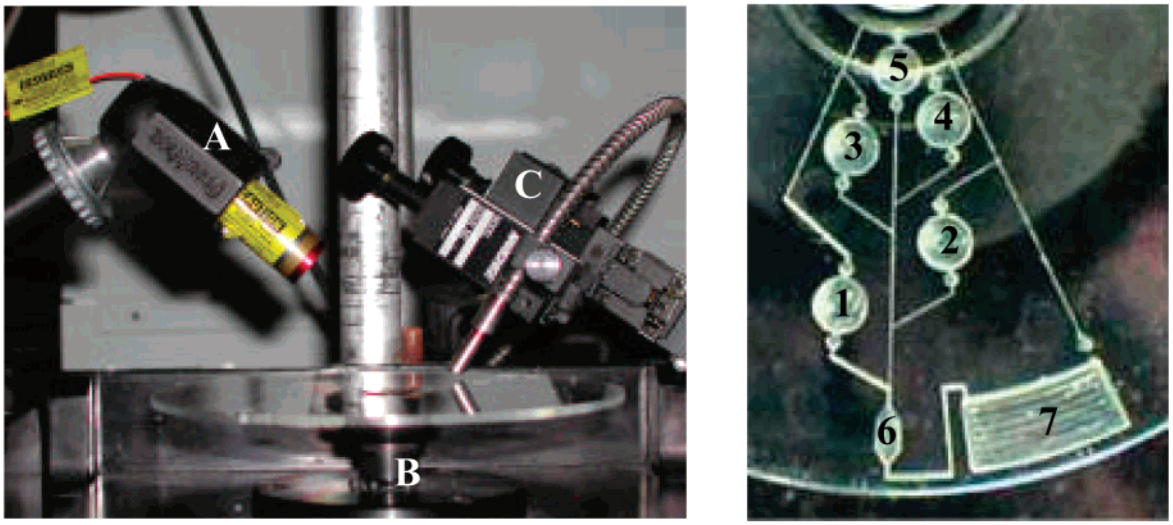

Figure 1. Experimental setup for laser-diode-based measurements. Left photograph shows the laser diode (A), CD motor stage (B) and micromanipulator holding the fiber-optic bundle (C). Right photograph shows the microfluidic architecture employed throughout this study with five solution reservoirs (numbered 1−5), a detection chamber (6), and a waste reservoir (7).

A standard instrumental system that was composed of two main sections, a system for motor control/positioning and a fiber-optic-based photometer has been described in a previous work.[1] For the studies described herein, the instrument is the same except for modifications to allow detection of membrane fluorescence. For the nitrite-selective membrane measurements described, the monochromator wavelength of excitation was set to 470 nm, and a 590-nm (emission wavelength) or 510-nm (wavelength at isosbestic point) band-pass filter was employed between the centrifugal platform and PMT. Measuring fluorescence at a second landmark wavelength was performed so that ratiometric data could be studied. On the other hand, with measurements involving the potassium-selective optode, the excitation wavelength was set to 601 nm, and a 670-nm emission filter was employed.

Aside from the standard photometer setup, laser diodes were also used as excitation sources in some potassium-selective optode experiments instead of a conventional lamp (see Figure 1). In these cases, the diode laser was mounted via a table-mounted optical holder such that laser light intercepted the measuring reservoir/optode at a low angle relative to the plane of the centrifugal platform. Detection was carried out using the fiber-optic/filter/PMT module. Either a red (635 nm) or green (530 nm) laser diode (Coherent, Auburn, CA) was employed in these studies, and a 670 nm filter was interposed between the optode membrane and PMT during experiments with each laser diode.

Capillary burst valves were fabricated into the microfluidic architecture so that fluids from each of the five reservoirs flowed to the optode membrane at separate times. Burst valves operate on the idea that two pressures, one associated with the induced centrifugal force and the other due to fluid surface tension, act on liquids from varying reservoirs when these flowing fluids encounter an abrupt enlargement in the channel structure.[26] When the platform is rotated at low angular frequencies, the flow-restricting surface-tension-based pressure is predominant, so fluid flow is effectively stopped. However, when the angular frequency of rotation is increased such that the centrifugal pressure equals or is greater that the surface tension pressure, the fluid will begin to flow once again. The angular frequency, ω, at which this condition is met is described by the following equation:

$$\omega = \left(\frac{a(4\gamma/d_H) + b}{\rho \bar{r} \Delta r} \right)^{1/2}$$

where γ is the surface tension of the fluid, d_H is the hydrodynamic diameter of the channel, ρ is fluid density, $\bar{r}$ is the average distance of fluid from the center of rotation, Δr is the radial extent of the fluid, and a and b are parameters that depend on the geometry of the microfluidic architecture and fluid/platform material interactions, respectively.[26]

In all experiments, the angular frequency of the disk was gradually increased until release of fluid from the first reservoir occurred. Upon this fluid filling the measuring reservoir, the disk was decelerated to a stop, and the intensity of emitted light was measured at both the emission wavelength and, in cases where discussed, at the isosbestic point after a 1-min equilibration period. This process was repeated for the remaining filled reservoirs. The disk was washed with ethanol and deionized water before reuse.

RESULTS AND DISCUSSION

Ion-selective optode membranes have been known and widely studied for a little over 10 years,[27,28] yet until recently, these sensors were studied primarily in either a flow cell arrangement or coated onto the tip of a fiber-optic bundle. With the spread of bioanalytical chemistry and interest in microfabricated systems, new sensing constructs based on optodes have begun to emerge. Recent reports have demonstrated platforms based on micro- to nanoscale optodes at the tips of single optical fibers or as free-

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standing microspheres.[29−33] Only within the past year or two, however, have optode membranes been integrated with microfluidics to create useful analytical devices for assaying ions.[1] Furthermore, prior to the study presented herein, fluorescence-based optodes had not been explored for detection on microfluidic platforms.

Five-Point Calibrations of Optode Membranes. Potassium-selective optode membranes were constructed from the bis-crown ether ionophore BME44 and the H^+-sensitive Nile Blue derivative, chromoionophore III. Chromoionophore III is a basic dye (pK_a = 12.0) and exists in the membrane in the fully protonated state. When potassium from the sample solution is extracted into the optode membrane, these cations exchange with H^+ from the chromoionophore as a result of the electroneutrality requirement within the bulk membrane. As a result of the change in protonation state of the chromoionophore, fluorescence of the optode membrane should decrease in intensity at the wavelength corresponding to the protonated form of the chromoionophore and should increase at the wavelength associated with the deprotonated form. In the experiments described herein, the studied wavelength was 670 nm, which is the wavelength of maximal fluorescence (λ_{em}) for the protonated chromoionophore when excitation occurs at 601 nm.

The potassium-selective optode membrane was calibrated with five standard solutions ranging in concentration from 10^{-5} to 10^{-1} M. Solutions were delivered to the area on the platform where the optode was positioned in a sequential manner from the five reservoirs by increasing the angular frequency of rotation of the platform. Control of the release of each reservoir was achieved by incorporating passive capillary burst valves that operate on the basis of surface tension and fluid-substrate interactions. The release frequencies of the reservoirs were separated by 80−150 rpm. Approximate rpm values for the release of each reservoir, as well as the separation in rpm stated was "built" into the architecture by accounting for the distance of each valve from the center of platform rotation and by varying the hydrodynamic diameter of the valve channel. The calibrant solutions were delivered in order of increasing concentration (beginning with 10^{-5} M and ending with 10^{-1} M).

The result from the five-point calibration of the potassium-selective optode under fluorescence detection is shown in Figure 2. Fluorescence of the optode membrane decreases with increasing potassium concentration as expected when measuring fluorescence of the protonated chromoionophore. Although the response profile shown is expected to become more sigmoidal in shape if fitted to a theoretical response function, the plot in Figure 1, which is constructed from raw fluorescence signals, is linear over a wide range of potassium concentrations. The measuring range of the optode studied correlates well with a potassium optode containing similar components and designed for intracellular use by dip-coating the membrane onto an optical fiber.[29] This previously studied optode possessed a linear range of nearly four decades of concentration, as in the case described herein. The limiting concentrations used to calibrate the optode on the centrifugal platform are actually the points at which the linearity begins to be lost to the sigmoidal shape of optode response. The similarity in response profile between the optode studied here and the previously studied example indicates that the platform is capable of delivering calibrant solutions to the optode area in a resolved manner with little carry-over or mixing of solutions occurring. It is worth mentioning that a large enhancement in both detection limit and linearity of the response function were obtained using the fluorescence mode of measurement in comparison to the absorbance mode. At least a one-decade improvement in the detection limit and two-decade enhancement in the linearity of the response function were obtained using the fluorescence mode (compare data in Figure 4 with that in Figure 2 of ref 1).

Aside from the potassium-selective optodes that had been discussed to date, a number of ionophore-based optical sensors exist for a host of analytes, including cations, anions, and neutral species.[30] To demonstrate the flexibility of the platform toward incorporation of other optodes, a nitrite-selective optode was combined with the centrifugal platform. The optically active

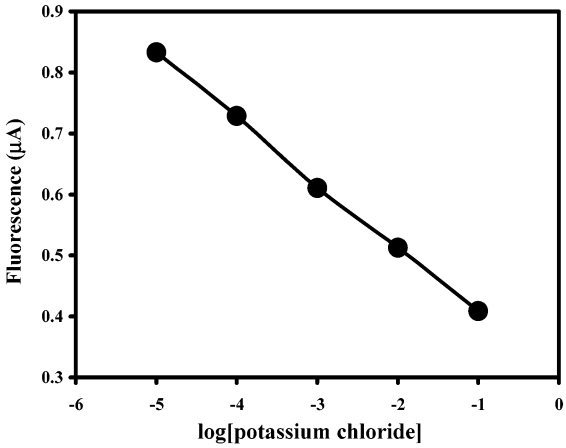

Figure 2. Five K^+-standard calibration of a BME 44-based optode membrane on a centrifugal microfluidics platform. Circles represent experimental raw fluorescence data.

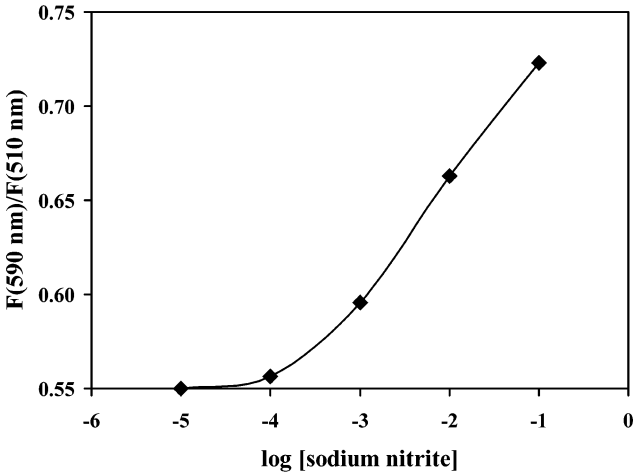

Figure 3. Typical plot of the ratio of fluorescence signals versus log[nitrite] at the emission and isosbestic wavelengths of a NO_2^--selective optode on the centrifugal platform.

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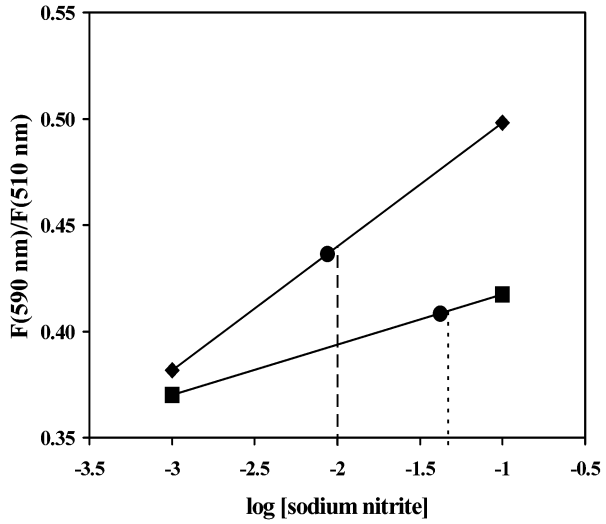

Figure 4. Two-point calibration-based determination of test samples, employing two different optode membranes. Diamonds and squares are calibration points, and circles denote the position of the unknown [NO_2^-] as determined. Vertical dashed lines display the position of actual logarithm concentrations of nitrite in the test sample, which were −2 and −1.33.

component in the membrane is a fluorescein derivative, chromoionophore VI that exists in a deprotonated form at a pH of 7.4, which was the measuring pH in all nitrite optode studies. Contrary to the ion-exchange mechanism of the potassium optode, the extraction of negatively charged nitrite into the membrane is accompanied by the co-extraction of H^+ into the membrane, which subsequently associates with the chromoionophore. It is expected, then, that in this case, an enhancement of fluorescence will be observed if the protonated form of the chromoionophore is monitored.

Figure 3 demonstrates the typical response of the nitrite optode toward five standard nitrite solutions at consecutively increasing concentrations. The observed enhancement in fluorescence of the optode at 590 nm was expected, since this wavelength corresponds to λ_{em} for the chromoionophore's protonated form, which increases in concentration as NO_2^- and H^+ are coextracted into the membrane. Additionally, the shape of the response profile for the five-point calibration is that of the lower half of the sigmoidal type that is generally expected for optode response. For the optode composition and measuring conditions employed in these experiments, the optode responds over a range of 10^{-5} to 10^{-1} M, with a linear response region from $\sim 2 \times 10^{-4}$ to 10^{-1} M, which is almost identical to what has been reported for the same optode in flow-cell-based measurements.[31] Indeed, these results further indicate that the centrifugal platform can be successfully employed with optodes that are selective toward various analytes and that the optode response characteristics are similar to those obtained via other formats (e.g., flow cells, fiber-optic-mounted, etc.).

It also should be noted that the fluorescence parameter plotted in Figure 3 is not the absolute fluorescence at 590 nm, but rather, the ratio of the optical signals at 590 and 510 nm. This ratioing approach is common practice for many fluorescence-based methods and has been employed for optodes in the past.[29] In the work herein, there are several advantages provided by ratiometric measurements, including compensating for source fluctuations and mitigating differences in response that may arise over time as a result of changes in membrane composition. More importantly, though, ratiometric measurements account for changes in positioning of the centrifugal platform on the motor hub or relative to the fiber-optic probe, for example, because of mechanical vibration. Although it is possible to perform analysis and calibration without such a ratiometric method, as demonstrated with the potassium optode-based system, the ability to perform this type of procedure is another inherent advantage of employing fluorescence detection in microfluidic applications.

Because of the small-diameter scales associated with microfluidic platforms, Reynolds numbers for fluid flow in microchannels are so small that flow is almost solely laminar. In many applications in which mixing of fluids is desired, laminar flow is often a problematic issue, since turbulent flow must be induced to achieve mixing not based solely on diffusion.[8,34] In the case described herein, however, laminar flow is actually advantageous, because it circumvents problems of the cross-contamination of calibration solutions induced by mixing. In the measuring reservoir, as one calibrant is displaced by the next, little mixing occurs between the two solutions except through diffusion at the solution-solution interface. Each fluid reservoir on the platform is constructed to have a volume of ∼ 3-fold the optode compartment volume; hence, the area of diffusional contact mentioned is pushed through the measuring area and into the waste before cross-contamination of calibrants can occur. Indeed, previous studies have shown that in such a case as described here, incorporating a buffer wash between calibrants yields no benefits to the measurement procedure, since little mixing occurs between the solutions.[1]

Two-Point Calibration-Based Nitrite Measurements. The generation of five-point calibration plots demonstrated that optodes on the centrifugal platform perform in a manner similar to what is observed in other formats. To further study the applicability of the system, studies to quantify test solutions using a two-point calibration protocol were undertaken. In most situations, using two standard points to define the linear region of response provides substantial improvements in the accuracy of analysis over the one-point calibrations used in many hand-held analyzers. Two-point calibration also offers the advantages of reduced standard consumption or waste production and reduced analysis times as compared to calibrations using more standards. For practical applications of ion-selective membranes and for the sake of simplicity, often calibration and measurements are performed in the most linear region of the optode response. The rationale behind this assertion was detailed recently.[1] Indeed, it was determined that the linear two-point calibration provides an acceptable means by which integrated optode analysis can be performed.[1]

For centrifugal analysis platforms based on fluorescence optodes, the types of calibrations described above can also be employed for quantification of a test sample of "unknown" concentration. In such a protocol, the reservoirs (refer to Figure 1) contain fluids as follows, beginning from disk edge and moving to the center: (1) first standard nitrite solution, (2) empty, (3) second standard nitrite solution, (4) empty, and (5) test solution.

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The flexibility associated with the five-reservoir architecture, though, allows a number of different potential calibration protocols as desired, including those with more calibrants or incorporating washing steps. Two examples of the described two-point calibrations and analyses, with different optode membranes, are demonstrated in Figure 4. It was found that the concentration of a test sample could be quantified with an error of <4% employing a fluorescence optode with ratioing and two-point calibration protocols. For instance, in the two examples demonstrated in Figure 4, the error in quantification of the "test" solution was found to be 3.0% for the diamond-represented calibrations and 3.6% for the square-represented calibration. Furthermore, the error in the determination of an unknown is similar, regardless of the position of the test sample's concentration within the linear region, corroborating that with a judicious choice of the two-point calibration interval, a linear fit can be employed for such analyses.

Laser Diodes as Excitation Sources. Laser-induced fluorescence is a widely employed technique when the dimensions of the sample volume are reduced or for other instances in which high sensitivity is required in detection. The improvement in sensitivity is due to the capability of more intense laser light to populate molecular excited states to a greater extent, thereby increasing the resultant fluorescence signal. In fact, there have been recent reports of micro- and nanospherical optode constructs that were interrogated with a laser.[32,33] Additionally, laser diodes are present in many devices that read CDs, including CD-ROM drives and DVD players, so many packaging and circuitry concerns associated with employing laser diodes in instrumental design have been addressed previously. Figure 1 depicts the optical geometry employed in laser diode measurements on the centrifugal platform.

Two laser diodes with wavelengths at 535 and 635 nm were studied in concert with a 670-nm emission filter to determine which laser provided maximum sensitivity of a two-point calibration of the potassium-selective optode. These lasers were chosen not only because of their wide commercial availability but also because both are capable of exciting fluorescence of chromoionophore III. Fluorimetry studies reveal that 635-nm light excites predominantly the protonated form of the dye, whereas 535-nm light will induce fluorescence from primarily the deprotonated form of chromoionophore III. It is expected, then, that the slopes of calibrations employing the two laser diodes should be opposite in sign.

Employment of a 535-nm laser diode resulted in calibrations with slope of 4.00×10^{-8} A/decade change in K^+ concentration, which is 200-fold smaller than the sensitivity obtained with a 635-nm laser diode (-1.98×10^{-6} A/decade). The sensitivities of the calibration with 535-nm laser diode excitation are positive in slope, resulting from an increase in fluorescence signal, as opposed to the negative slope obtained with the tungsten−halogen lamp at 601 nm and the 635-nm laser diode. This result is consistent with monitoring the deprotonated state of the chromoionophore at an excitation wavelength of 535 nm, since this form increases in concentration as larger amounts of potassium replace H^+ in the optode membrane.

It is worth mentioning that the sensitivity obtained with 635-nm laser diode excitation is at least 30-fold larger than that obtained using the conventional tungsten−halogen lamp excitation. This is can be seen in Figure 5, which displays a 5-standard calibration of the same optode over the same concentration range with both tungsten−halogen lamp and 635-nm laser-diode excitation. This highlights the relevance of laser excitation in the enhancement of the signal sensitivity. Furthermore, laser diodes and fluorescence detection are more compatible with the CD platform, since laser diodes are amenable to miniaturization and do not require additional optics to provide monochromatic radiation. In contrast to the conventional excitation sources, laser diodes do not require a high voltage power supply, which makes them more suitable for the design of a portable system based on a CD platform.

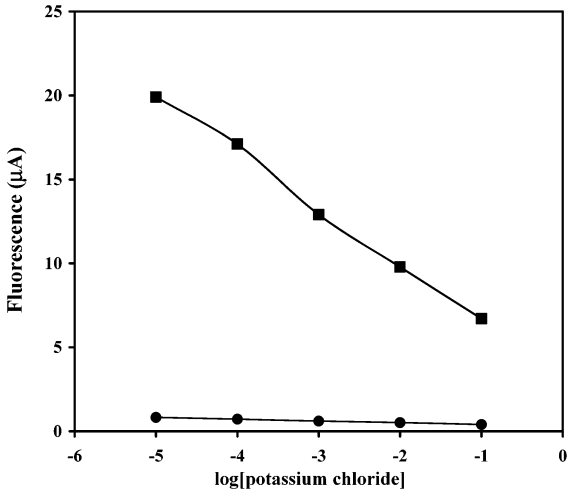

Figure 5. Comparison of 2 five-point calibrations of the same potassium optode using either a tungsten halogen lamp (experimental points are circles) or a 635-nm laser diode (experimental points are squares) as an excitation source.

CONCLUSIONS

This paper presents the first evaluation of ion-selective optodes interrogated via fluorescence on a microfluidic platform. Fluorescence detection offers much lower detection limits, as compared to absorbance-based measurements, as well as a wider linear response range. A further enhancement of the fluorescence mode of detection could be achieved using laser diodes that results in 30-fold higher sensitivity, as compared to a conventional excitation source. Thus, coupling the fluorescence mode to the microfluidic platform offers improved characteristics that enable miniaturization. With a centrifugal microfluidic platform, solutions containing known or unknown concentrations of ions can be delivered in a sequential manner as governed by capillary burst valves to an optode membrane integrated into the fluidic structure. Optodes calibrated with five calibrant solutions delivered in order of increasing concentration demonstrate responses, particularly linear ranges and detection limits, similar to optode membranes studied via fluorescence in other formats, such as flow cells, fiber-optic probes, and micro-/nanospheres. Since optode constructs exist for a number of different ions, it is feasible to create a platform where multiple ions or samples could be studied in a parallel fashion. Finally, smaller architectures on the centrifugal platform (with significantly reduced detection areas) could be

coupled with miniaturized instrumentation, similar in footprint to that of a portable CD player, to develop a fully integrated μ-TAS. Work in this direction is currently underway in our laboratory.

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