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Imaging fiber microarray fluorescent ion sensors based on bulk optode microspheres

Katarzyna Wygladacz, Eric Bakker*

Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849, USA

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Abstract

Optical imaging fibers with micrometer-sized wells were used as a sensing platform for the development of microarray optical ion sensors based on selective bulk extraction principles established earlier for optodes. Uniform 10 μm sized microspheres based on plasticized poly(vinyl chloride) containing various combinations of ionophores, fluoroionophores and lipophilic ion-exchangers were prepared for the detection of sodium, potassium, calcium and chloride, and deposited onto the wells of etched fiber bundles. Specifically, sodium sensing particles were based on *tert*-butylcalix[4]arene tetraacetic acid tetraethylester, potassium particles on 2-dodecyl-2-methyl-1,3-propanediyl bis[*N*-[5′-nitro(benzo-15-crown-5)-4′-yl]carbamate] (BME-44), calcium particles on an acrylic derivative of ETH 129 (AU-1) covalently attached to a methacrylic polymer, and chloride particles based on the anticrown ionophore [9]mercuracarborand-3 (MC-3). The fluorescence emission characteristics of individual microspheres were observed from the backside of the fibers and were found to selectively and rapidly change as a function of the sample composition. The optical characteristics of the particles were found to be comparable to that of corresponding thin optode films and particles deposited onto microscope glass slides. The measuring ranges (logarithmic molar concentrations) at pH 7.0 were found as −3 to 0 for sodium, −3.5 to −0.5 for potassium, −7 to −2 for calcium, and −5 to 0.5 for chloride. Selectivities were determined over other common electrolytes and found to be sufficient for physiological applications. The simultaneous deposition of sodium and chloride sensing particles was successfully performed, demonstrating that such microarray sensors are capable of simultaneously sensing multiple analytes. This technology is compatible with other microsphere-based fluorescent sensing principles, forming a promising total analysis platform for a variety of applications.

Keywords: Optode microspheres; Ionophore; Human saliva; Imaging fiber bundles; Total analysis system

A single, compact probe containing a manifold of chemical and biological recognition principles that could all be simultaneously and independently interrogated forms an excellent foundation for the development of clinical and environmental total analysis systems. Imaging fibers consisting of a fused fiber bundle that can each be optically addressed independently are very useful for optical imaging applications. As the group of Walt has convincingly shown, they can also be used for the development of sensing arrays via selective etching of each fiber core to form high density microwell arrays that are capable of accepting size-matched microsphere sensing elements [1–5].

A myriad of recognition and sensing principles have already been integrated into microsphere formats that are compatible with such fiber bundle microarrays. For example, the direct modification of the etched wells of such fibers by immobilization of mouse fibroblasts [6], yeast (*Saccharomyces cerevisiae*) [7], and bacteria (*Escherichia coli*) [7] was performed. The imaging and sensing characteristics of living cells by using fibers modified with polymers with a grafted dye have also been reported [8]. Fiber-based optical sensors were applied for crevice, pitting and galvanic corrosion monitoring [9,10]. An optical biosensor for acetylcholine was fabricated by coating the etched surface of the fiber with a polymer that contained both a fluorescence active dye and the enzyme acetylcholine esterase [11]. Using a discrete sensing approach, optical sensors for pH and penicillin [12], and for

* Corresponding author. Tel.: +1 334 8446972; fax: +1 334 8446958.
E-mail address: eric.bakker@auburn.edu (E. Bakker).

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oxygen and glucose were designed [13]. An imaging fiber that contained three different sensing regions was proposed for simultaneous measurements of oxygen, pH and CO_2 [14]. Optical fibers were applied as a sensing platform for oligonucleotide (DNA) detection [1,3,5] and used for the design of odor sensors [15] and artificial noses [2].

So far, the large palette of ionophore-based chemistry available to the development of electrochemical and optical sensors [16] has not yet been implemented into optical fiber bundle sensing systems. Indeed, the selective determination of a variety of ionic analytes is possible by use of polymeric films doped with lipophilic complexing agents, as the widespread use of ion-selective electrodes in clinical analyzers attest. Fiber optic probes for the detection of a number of ions have been developed via fluorescence measurements [17–19] and subsequently expanded to include intracellular submicron-sized probes [20]. Our group has recently introduced highly uniform fluorescent microspheres based on this extraction and complexation chemistry originally described for traditional thin film optodes [21–25]. These microspheres were found to possess excellent selectivity, generally mirroring that of the corresponding ion-selective electrodes, that can be tuned by the choice of the active sensing ingredients in the polymer [22,26]. These systems have been characterized on microscope slides [22,24,26] and in analytical flow cytometry [27], and recent efforts have focused on covalent attachment approaches [28,29] and the design of plasticizer-free polymers containing no leachable components [25,30]. We report here on the first study to implement this sensing technology to the design of optical fiber microarrays.

1. Experimental

1.1. Reagents

Poly(vinyl chloride) (PVC), bis(2-ethylhexyl) sebacate (DOS), *tert*-butylcalix[4]arene tetraacetic acid tetraethylester (sodium ionophore X), 2-dodecyl-2-methyl-1,3-propanediyl bis[*N*-[5′-nitro(benzo-15-crown-5)-4′-yl]carbamate] (potassium ionophore III, BME-44), 9-(diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine (chromoionophore I, ETH 5294), 9-(diethylamino)-5-[4-(15-butyl-1,13-dioxo-2,14-dioxanodecyl) phenyl-imino]benzo[a]phenox-azine (chromoionophore VII, ETH 5418), *N*,*N*-dicyclohexyl-*N*′,*N*′-dioctadecyl-3-oxapentane amide (calcium ionophore IV, ETH 5234) were purchased from Fluka (Milwaukee, WI). The internal reference dye 1,1′′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiIC18) was from Molecular Probes (Eugene, OR), Sodium tetrakis-[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) was purchased from Dojindo Laboratories (Gaithersburg, MD). The calcium ionophore acrylic derivative of ETH 129 (AU-1) covalently attached onto a methyl methacrylate and dodecyl methacrylate polymer matrix as well as the chloride ionophore [9]mercuracarborand-3 (MC-3) were synthesized in our laboratory as described [28,31]. Dichloromethane (DCM), ethylacetate (Fisher), xylenes (EM Sciences), cyclohexanone (99.8%) (Aldrich Baker), tetrahydrofuran (THF) (Fluka), poly(ethylene glycol) (PEG) (Polysciences, Inc.) were ACS grade and were obtained from the indicated suppliers. Tris(hydroxymethyl) aminomethane (Tris) was reagent grade from Sigma. 2-Morpholinoethanesulfonic acid (MES), 3-morpholinopropanesulfonic acid (MOPS) were obtained from Fluka. All solutions were prepared with freshly deionized water (18 MΩ cm) using a Nanopure Millipore water purification system. Salts, acids and bases of the highest available quality were used. Standard 6 μm microspheres were purchased from Polysciences, Inc. (Warrington).

1.2. Spectroscopic and microscopic characterizations

A PARISS Imaging Spectrometer (Light Form, Belle Mead, NJ) combined with a Nikon Eclipse E400 microscope was used to characterize thin optode films, particles and fibers covered with microspheres or before particle deposition. This system possesses two EDC 1000L CCD cameras (Electrim Corp., Princeton, NJ) and a Nikon mercury lamp (Southern Micro Instruments). It is equipped with a motorized stage (Prior Optiscan ES9, Fulbourn, Cambridge, UK) operating via PARISS data acquisition software, which records either individual fluorescence or absorbance spectra under the field of view [22–24,26].

Scanning electron microscopy (SEM) images of the etched optical fiber bundle were obtained using a Zeiss DSM 940 Scanning Electron Microscope at 5 kV. Before measurement the fiber was mounted on aluminum stub and sputter-coated for 60 s using 30 A with 10–20 nm of Au/Pd on a Pelco SC-7 Auto Sputter-Coater.

Atomic force microscopy (AFM) was applied to estimate the depth of the etched wells of the fiber and performed at ambient condition using contact-mode (Autoprobe CP Atomic Force Microscope, Park Scientific Instruments). The imaging was done using the following set-up: force set point −2.5 nN, cantilever spring constant −0.6 N/m and scan rate 0.6 Hz. During measurement the fiber was mounted on a steal stub.

1.3. Microwell preparation

A 3200 μm diameter imaging fiber containing ca. 7000 individual optical fibers (Edmund Industrial Optics) was polished using 12, 9, 3, 1 and 0.02 m lapping films. The polished fiber was cleaned with tape and distilled water followed by sonication in distilled water and allowing it to dry. The etching of the wells of the optical fiber bundle was performed as described [32]. Briefly, the distal face of the fiber was immersed into a mixture containing ammonium fluoride (0.2 g), hydrofluoric acid (100 μL) and deionized water (600 μL) for 30 min followed by immediate immersion in deionized water to stop the etching process. The fiber was then rinsed