Anal. Chem. **2002,** *74,* 5251−5256

Mass-Produced Ionophore-Based Fluorescent Microspheres for Trace Level Determination of Lead Ions

Martin Telting-Diaz and Eric Bakker*

Department of Chemistry, Auburn University, Auburn, Alabama 36849

The development and characterization of small, uniform, and mass-produced plasticized PVC-based sensing microspheres in view of rapid trace level analysis of lead ions is reported. Micrometer-sized particles obtained via an automated casting process were rendered selective for lead ions by doping them with highly selective components in a manner analogous to traditional optode sensing films. Single particles that contained the lipophilic ionophore *N*,*N*,*N*′,*N*′-tetradodecyl-3-6-dioxaoctane-1-thio-8-oxodiamide (ETH 5493), the chromoionophore ETH 5418 together with a lipophilized indocarbocyanine derivative as internal reference dye ($DiIC_{18}$), and lipophilic ion-exchanger sites sodium tetrakis[3,5-bistrifluoromethylphenyl]borate, yielded measurable lead responses at the low nanomolar level in pH buffered solutions. The detection limit for single particles was 3×10^{-9} M at pH 5.7. The microspheres were fabricated via a reproducible formation of polymer droplets within a flowing aqueous phase followed by collection of spherical particles of ∼13 μm in size. The particles were immobilized and assayed individually in a microflow cell via fluorescence microscopy. Selectivity patterns found were in agreement with those reported earlier for the lead-selective ligand ETH 5493, and all response functions were fully described by theory. In contrast to optode films that necessitated very long equilibration times and large sample volumes in diluted samples of analyte, particles exhibited extremely enhanced equilibrium response times. Thus, for lead sample concentrations at and above 5×10^{-8} M, response times were ∼3 min, whereas at the detection limit, complete equilibrium was recorded after just 15 min, with required sample volumes on the order of 1 mL. This new class of microspheres appears to be suitable for rapid and sensitive ion detection at trace levels in environmental and biological applications.

Lower detection limits, smaller sample volumes, faster response times, and high selectivity are among the many requirements that must be met in the trace level analysis of complex samples. Toward this end, optode films based on neutral ionophores have proven to be a highly promising technology for the analysis of heavy metal ions. Over the past decade, an increasing number of cation-exchange based systems, including those for Pb^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}, and UO_2^{2+} have been reported.[1−5]

For the analysis of lead ions, optode systems that incorporate highly selective ionophores have been used. Those that may contain sulfur coordinating functionalities, such as a dimethylthioacetamide pending group[6] or a dioxaoctanedithioamide derivative,[1] have demonstrated increased success in polymer membranes of ISEs where they have been shown to exhibit detection limits that extend even to picomolar levels with electrodes that are carefully tailored.[7,8] In optode systems, selective ionophores incorporated with a lipophilic chromoionophore and required anionic sites have been examined in anticipation of their use in environmental monitoring.[1] As for all conventional cation-exchange-based systems, lead optodes follow predicted theory as given by the associated equilibrium of transfer of the lead analyte species and H^+ ions into the plasticized PVC optode phase. In particular, the lead complexing agent 3,6-dioxaoctanedithioamide derivative, that is, ETH 5435, in conjunction with the absorption changing properties of chromoionophore ETH 5418, was found to exhibit excellent selectivity against all relevant alkaline and alkaline-earth metal ions, thus allowing measurable lead concentrations to extend to the subnanomolar range.

More recently, Antico et al.[9] reported on optode films incorporating the closely related analogue of ETH 5435, the monothio oxodiamide derivative ETH 5493 ionophore. Although the use of such an ionophore offers less good selectivity with respect to the alkaline-earth metal ions Ca^{2+} and Mg^{2+} than the dithioamide ligand, no irreversible sensing film poisoning upon exposure to Ag^+ or Hg^{2+} ions occurs.

These papers on ionophore-based optodes have shown that the selectivity and detection limits are sufficient to reach subna-

(1) Lerchi, M.; Bakker, E.; Rusterholz, B.; Simon, W. *Anal. Chem.* **1992**, *64*, 1534.
(2) Lerchi, M.; Reitter, E.; Simon, W.; Pretsch, E.; Chowdhury, D. A.; Kamata, S. *Anal. Chem.* **1994**, *66*, 1713.
(3) Lerchi, M.; Reitter, E.; Simon, W. *Fresenius' J. Anal. Chem.* **1994**, *348*, 272.
(4) Hisamoto, H.; Nakagawa, E.; Nagatsuka, K.; Abe, Y.; Sato, S.; Siswanta, D.; Suzuki, K. *Anal. Chem.* **1995**, *67*, 1315.
(5) Amiet, G. R.; Farrell, J. R.; Iles, P. J.; Sands, T. J. *Aust. J. Chem.* **2001**, *54*, 27.
(6) Malinowska, E.; Brzozka, Z.; Kasiura, K.; Egberink, R. J. M.; Reinhoudt, D. N. *Anal. Chim. Acta* **1994**, *298*, 253.
(7) Sokalski, T.; Ceresa, A.; Pretsch, E. *J. Am. Chem. Soc.* **1997**, *119*, 11347.
(8) Ceresa, A.; Bakker, E.; Hattendorf, B.; Gunther, D.; Pretsch, E. *Anal. Chem.* **2001**, *73*, 343.
(9) Antico, E.; Lerchi, M.; Rusterholz, B.; Achermann, N.; Badertscher, M.; Valiente, M.; Pretsch, E. *Anal. Chim. Acta* **1999**, *388*, 327.

10.1021/ac025596i CCC: $22.00
Published on Web 09/12/2002

nomolar detection limits. So far, the important drawback is the high sensing volume of the optode film, which requires typically on the order of 10 nmol of ions to be extracted from the sample in order to achieve the desired optical response. Consequently, massive volumes of sample (many liters) and very long response times (many hours) in a continuous flowing system have so far been required to accurately measure low levels of heavy metals in aqueous samples. A drastic miniaturization of the sensing element should be able to alleviate this important problem.

Recently, we demonstrated that optical sensing systems can be prepared as single functional micrometer-sized particles that can be spatially and spectrally characterized by fluorescence microscopy and imaging spectroscopy.[10] Particles prepared incorporating various selective components were shown to respond on the basis of bulk extraction principles behaving analogously to optode films. Lately, fully functional particles based on a sodium neutral carrier system were prepared at a rate of ~20 000 particles/s via an automated particle casting apparatus, which exhibited high reproducibility.[11] This novel approach represents a versatile sensing format for optode systems. It allows further expansion on the chemistry of microspheres that has so far been mostly limited to surface-attached reactions or polymer swelling mechanisms. In addition, with the advent of microengineered devices along with rapid developments in microfluidics, the handling and assessment of mass-produced particles for high-throughput multianalyte detection can be envisioned. Here, we report on our efforts to further expand on the technology of particle casting of optode systems by introducing the development of rapidly responsive micrometer-sized lead-sensing particles.

EXPERIMENTAL SECTION

Reagents. Poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (2-NPOE), bis(2-ethylhexyl) sebacate (DOS), 4-*tert*-butylcalix-[4]arene-tetrakis(*N*,*N*-dimethylthioacetamide) (lead ionophore IV), 11-[(-butylpentyl)oxy]-11-oxoundecyl-4-[9-(dimethylamino)5H-benzi[a]phenoxazin-5yl-idene] aminobenzoate (ETH 5418, chromoionophore VII), sodium tetrakis-[3,5-bis(trifluoromethyl)phenylborate (NaTFPB), and tetrahydrofuran (THF) were Selectophore quality from Fluka (Milwaukee, WI). The nitrate salts of heavy metal ions and the chloride salts of sodium, potassium, and magnesium acetate were all puriss p. a. from Fluka. The internal reference dye 1,1′′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($DiIC_{18}$) was from Molecular Probes (Eugene, OR), cyclohexanone (99.8%) was from Aldrich, and dichloromethane and xylenes (ACS grade) were from Fisher. The 4-(octadecylamino)azobenzene (chromoionophore ETH 5315) was synthesized according to a procedure reported elsewhere.[12] The *N*,*N*,*N*′,*N*′-tetradodecyl-3,6-dioxaoctane-1-thio-8-oxodiamide (ETH 5493) ionophore was a gift from the laboratory of Prof. E. Pretsch (ETH, Zurich). It had been synthesized as described.[9]

Optode Films. For optode film preparation, 1.14 mg of ion-exchanger NaTFPB (7.2 mmol kg^{-1}), 2.46 mg of lead ionophore (15.8 mmol mmol kg^{-1}), 0.85 mg of chromoionophore ETH 5418 (6.6 mmol kg^{-1}), 0.88 mg of internal reference dye $DiIC_{18}$, 115.66 mg of plasticizer DOS, and 58.6 mg of PVC were weighed out and dissolved in 1.5 mL of THF. After complete dissolution, a 200-μL aliquot of the sensing cocktail was deposited by spin-coating onto quartz glass plates, and any remaining solvent was left to evaporate in a hood draft for at least 30 min prior to measurements.

Particle Preparation. The schematic setup and the general protocol for the preparation of sensing particles have been reported recently[11] and it applies here with minor modifications. Briefly, particle preparation relies on a method[13] in which two liquids coexisting as two separate phases are brought in contact by flowing from separate storage reservoirs. One of the liquids, that is, the polymer solution mixture containing the chemical sensing components (see below), is dissolved in an adequate organic solvent that acts as the core stream. This solution and a second flowing aqueous solution, the so-called sheath liquid, are directed to a chamber where the constant oscillation frequency from a piezoelectric crystal, driven by a frequency generator (BK Precision model 4011, Placentia, CA) controls the formation of polymer droplets at a high rate. As the core polymer liquid flows under precisely defined conditions into an injection tube in the chamber and emerges as a liquid jet from a ceramic orifice tip (43-μm diam), the polymer droplets form within the second moving aqueous stream, which flows around the injection tube and emerging jet. Thus, the polymer droplet solvent slowly partitions into the aqueous sheath stream, leaving behind small and highly uniform spherical particles that can be collected in a recipient solution for subsequent precipitation and separation, or as performed here, directly into small vials for immediate immobilization and analysis.

Unless otherwise indicated, a cocktail mixture was prepared by weighing out 58.5 mg of PVC, 116 mg of DOS, 1.44 mg (10.2 mmol kg^{-1}) of chromoionophore, 1.27 mg (6.9 mmol kg^{-1}) of reference dye $DiIC_{18}$, 4.05 mg (23.8 mmol kg^{-1}) of ion exchanger NaTFPB, and 14.8 mg (87.2 mmol kg^{-1}) of ionophore and dissolving it in 5 mL of cyclohexanone. The mixture was shaken in a vortex mixer for approximately 1 h and then added dropwise to 100 mL of dichloromethane under gentle stirring. After adding 1 mL of xylenes, the solution was filtered through a 0.45-μm Gelman filter and 50 mL was transferred to a gastight Hamilton syringe. The syringe containing the polymer core solution was mounted on a syringe pump (Stoelting, Wood Dale, IL) and set to flow at a rate of 0.263 mL min^{-1}. Deionized water used as the sheath liquid stream flowing at a rate of 43 mL min^{-1} was controlled via a pressure regulator. The frequency generator was operated at a setting of 12.3−12.7 kHz.

Instrumentation. A Pariss Imaging Spectrometer (Light Form, Belle Mead, NJ) combined with a Nikon Eclipse E400 microscope equipped with an epifluorescence attachment (Southern Micro Instruments, Marietta, GA) was used to optically characterize optode films and particles. The system was equipped with two CCD cameras EDC 1000L (Electrim Corp., Princeton, NJ) and a Nikon super high-pressure mercury arc lamp (Southern Micro Instruments). A filter cube with a 510−560-nm excitation filter, a 565-nm dichroic mirror, and a 590-nm long-pass emission filter was used. The system, equipped with a motorized stage

(10) Tsagkatakis, I.; Peper, S.; Bakker, E. *Anal. Chem.* **2001**, *73*, 315.
(11) Tsagkatakis, I.; Peper, S.; Retter, R.; Bell, M.; Bakker, E. *Anal. Chem.* **2001**, *73*, 6083.
(12) Bakker, E.; Lerchi, M.; Rosatzin, T.; Rusterholz, B.; Simon, W. *Anal. Chim. Acta* **1993**, *278*, 211.
(13) Fulwyler, M. J.; Perrings, J. D.; Cram, L. S. *Rev. Sci. Instrum.* **1973**, *44*, 204.

(Prior Optiscan ES9, Fulbourn, Cambs, U.K.) was operated via Pariss data acquisition software to record individual fluorescence spectra of particles and films under the field of view. The schematic representation of this optical arrangement has been reported previously.[11]

Measurements. Particles were collected in 20-mL small glass vials directly from the emerging jet of the casting apparatus. A 100-μL aliquot of liquid containing resuspended particles was deposited on a 22-mm-wide Fisherbrand microscope cover glass immediately after collection and was allowed to settle in the dark. The glass substrate containing the physically immobilized particles was mounted into a flow cell, fitted on the motorized stage of the microscope, and connected to a small peristaltic pump (Dakota Instruments). Fluorescence spectra were taken under static conditions and after equilibrating the flow cell with given test concentrations. For response time measurements, a borosilicate glass microcapillary cell of 1.0-mm i.d. and 0.15-mm wall thickness was used in order to reduce the dead volume. A 50-μL aliquot of collected particles was pipetted inside the capillary cell, and the particles were then left to settle for a couple of hours. The capillary was positioned over a glass fixture, attached at each end with polyethylene tubing, and connected to a peristaltic pump operated at a rate of 0.1 mL min^{-1}. Measurements were taken every 30−60 s for 5 min and every minute or longer thereafter. In all cases, the image of a particle was obtained using a 40× microscope objective to capture a specific slice under the field of view, typically using transmission mode to avoid photobleaching. The spectral image of this slice was then taken in the fluorescence mode. For all measurements, neutral density filters 4 and 8 were used to decrease the light intensity from the source. Exposure time for spectral acquisition of particles was 500 ms and for films, was 300 ms.

Standard 5×10^{-3} M stock solutions were prepared by dissolving the metal salt in a 1 mM magnesium acetate buffer of desired pH. Test solutions were then prepared by stepwise gravimetric dilution with the same buffer. For concentrations below 10^{-7} M, aliquots of the 10^{-6} M solution were diluted with increasing volumes of buffer to give the final concentration. All calibrating solutions were prepared in polyethylene beakers that had been pretreated with 0.01 M HNO_3.

For drinking water measurements, 500 mL of a commercially bottled water sample (Aquafina) acidified with 10^{-4} M nitric acid (final concentration) was stirred to remove residual carbon dioxide and subsequently buffered with magnesium acetate to a pH of 5.7. An aliquot of 100 μL of the above-mentioned lead stock solution was added to 100 mL of the buffered sample. This solution was serially diluted 10-fold with buffered water sample to obtain spiked samples with known concentrations of lead in the range of 5×10^{-6} to 5×10^{-9} M.

RESULTS AND DISCUSSION

Micrometer-sized optical particles reported in this work operate according to cation-exchange principles that have been described in detail for optode films by several authors.[14,15] Here, a cocktail mixture containing a lipophilic ionophore selective for lead ions, a chromoionophore selective for H^+ ions and required anionic sites incorporated in a PVC matrix plasticized with DOS were used. For such a composition, the Pb^{2+} ion activity (a_{Pb}) is dependent on the pH of the sample. The value α, which corresponds to the relative fraction of unprotonated chromoionophore, is introduced as an experimentally accessible parameter that relates to the metal ion activity (a_I) in the sample. As shown in eq 1, the value α also depends on several film parameters that include the pH and the total concentrations of the ionophore L_T, the chromoionophore C_T and the lipophilic cation-exchanger sites R_T,

$$\alpha_I = K_{exch}^{-1}\left(\frac{\alpha a_H}{1-\alpha}\right)\frac{R_T - (1-\alpha)C_T}{z\left(L_T - \frac{n}{z}\{R_T - (1-\alpha)C_T\}\right)^n} \quad (1)$$

where z is the charge of the analyte I^{z+}, and n is the ion−ionophore complex stoichiometry. Most typically, the degree of protonation of the chromoionophore $(1-\alpha)$ is used to represent the response function of optode systems in absorbance mode. Given the need to spatially and spectrally characterize microspheres via fluorescence microscopy, a judicious choice of the chromoionophore is required. Indeed, the chomoionophore must display appreciable fluorescent quantum efficiency, and it should also exhibit a pK_a value that permits effectively shifting the dynamic range and detection limit of Pb^{2+} to best-suited levels. Furthermore, it must ideally lend itself to ratiometric measurement in order to minimize any possible photobleaching effects and variations in positioning, size, and light intensity. Efforts to resort to the azoderivative ETH 5315, of which the pK_a value of 5.5 determined in optode membranes[12] appeared suitable for heavy metal detection in acidic media, proved unsuccessful, as demonstrated by its very poor fluorescence emission when incorporated in optode films. Alternately, the more basic chromoionophore ETH 5418 (pK_a 8.8) which has been used in absorption-based measurements for lead and other heavy metal ions was chosen. However, the use of ETH 5418 for fluorescent-based measurements requires the development of an inner filter approach,[16] since its basic form is not light-emitting. Thus, the fluorescence spectral characteristics of the lipophilic indocarbocyanine dye $DiIC_{18}$, which was shown earlier to be modulated by the absorbance changing properties of a different chromoionophore, ETH 2439,[17] was used in this work. The basic form of the chromoionophore ETH 5418 absorbs between 450 and 600 nm, whereas the protonated form absorbs in the region of 600−700 nm.[1] Therefore, only the absorbance band of the latter form overlaps significantly with the emission band of the reference dye. An absorbance increase of the protonated form should result in an emission decrease of the reference dye. Figure 1 shows the spectral fluorescence response of an optode film prepared containing the chromoionophore ETH 5418, the modulator dye $DiIC_{18}$, the lead ionophore Pb-IV, and anionic sites $TFPB^-$ as a function of various Pb^{2+} concentrations in $Mg(OAc)_2$-buffered sample at pH 4.7. As seen from Figure 1, the fluorescence peaks of the ETH 5418-$DiIC_{18}$ pair are nicely resolved. Because deprotonation of ETH 5418 occurs in response to lead activity in the sample, the fluorescence of the protonated form at 717 nm decreases, and the emission intensity of the $DiIC_{18}$

(14) Seiler, K.; Simon, W. *Anal. Chim. Acta* **1992**, *266*, 73.
(15) Bakker, E.; Buehlmann, P.; Pretsch, E. *Chem. Rev.* **1997**, *97*, 3083.
(16) Werner, T.; Klimant, I.; Wolfbeis, O. S. *J. Fluoresc.* **1994**, *4*, 41.
(17) Shortreed, M.; Bakker, E.; Kopelman, R. *Anal. Chem.* **1996**, *68*, 2656.

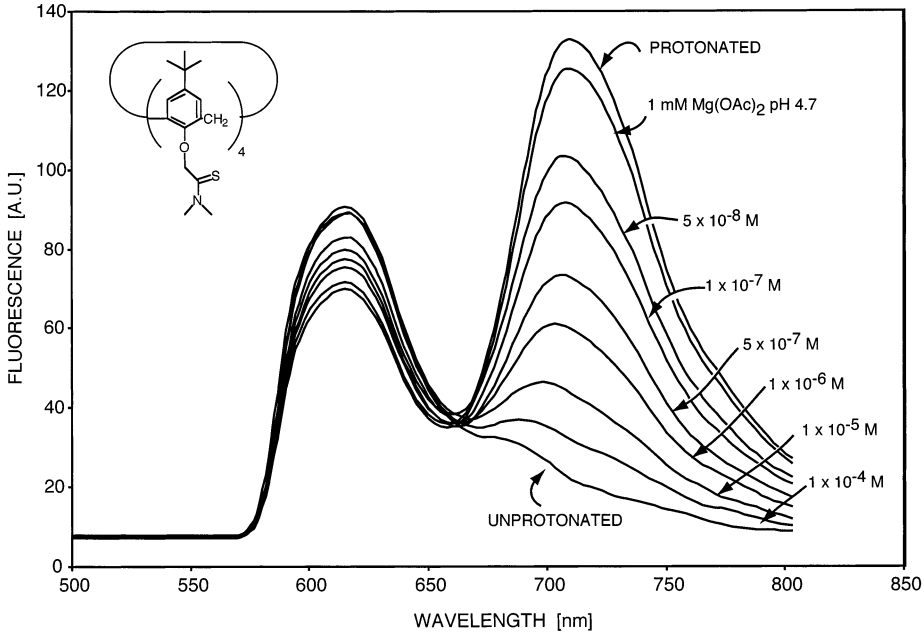

Figure 1. Fluorescence spectra of a Pb^{2+}-selective optode film after equilibration with different lead concentrations in $Mg(OAc)_2$ buffer at pH 4.7. The optode film contains the ETH 5418 chromoionophore (basic form, 717 nm) modulating the fluorescence emission of the lipophilic $DiIC_{18}$ dye (617 nm). Other components, lead ionophore Pb-IV, NaTFPB, DOS, and PVC.

increases as a result of the reduced inner filter effect. Consequently, the degree of protonation of the fluorescent chromoionophore is described as a function of the observed fluorescence ratios as follows:[18]

$$1 - \alpha = \frac{[\mathrm{IndH}^+]}{\mathrm{Ind_T}} = 1 - \left(1 + \frac{R_{\max} - R}{R - R_{\max}}\right)^{-1} \qquad (2)$$

where R, $R_{\min}$, and $R_{\max}$ are the fluorescence intensity ratios for a given equilibrium and at minimum and maximum protonation of the chromoionophore.

The fabrication of particles with the same lead-based ionophore system whose fluorescent film response is depicted in Figure 1 was attempted with the casting apparatus outlined in the Experimental Section. Thus, a batch of PVC-DOS plasticized particles incorporating the Pb-IV ionophore, ETH 5418, DiIC18 reference dye, and anionic sites was prepared. Although single particles were obtained whose shape, size, and acid−base response were as expected, exposure to dilute buffer solutions resulted in complete or partial deprotonation of the chromoionophore, thus yielding irreproducible results. The very high selectivity coefficients of Pb−IV ionophore reported for polymeric membranes of ISEs[8] supported the fact that interference by buffer ions was unlikely. Instead, the loss of ionophore during the casting process was the most likely explanation. Efforts to greatly increase the concentration of Pb−IV were hindered by the limited solubility of Pb−IV in DOS plasticizer. Indeed, the ionophore was shown to crystallize in the membrane over time at concentrations of 20 mmol kg^{-1}. Recent reports on the use of Pb-IV in membranes of ISEs plasticized with *o*-NPOE demonstrating lead ion response in the nanomolar concentration level[19] prompted us to formulate batches with this plasticizer. In this case, such efforts were met with the inability to deprotonate the chromoionophore, which may be indicative of near-complete leaching of the more polar plasticizer (the produced particles were significantly smaller than with DOS under the same conditions).

Table 1. Selectivity of Lead-Selective Sensing Particles[a]

ion J^{z+}	z	n[b]	log K_{exch}	log $K_{\mathrm{I,J}}^{\mathrm{opt}}$
Pb^{2+}	2	2	−2.3	0
Na^+	1	1	−3.6	−5.2
Ca^{2+}	2	2	−7.0	−10.9
Cd^{2+}	2	2	−1.2	1.0
K^+	1	1	−3.5	−5.1

[a] Procedure according to the references.[1,20] [b] n is the assumed complex stoichiometry,[9] and K_{exch}, the experimentally determined ion-exchange constant for the listed ion (see eq 1).

As a result of these initial studies, we opted for the use of the ligand ETH 5493 recently reported by Antico et al.[9] Because solubility of this ionophore in various plasticizers does not seem to be a problem at high concentrations, we prepared particle batches of typical compositions described in the Experimental Section. Optical ion-exchange constants (eq 1) and selectivity coefficients for particles prepared by incorporating this ionophore were determined for some relevant ions and presented in Table 1 (complex stoichiometries are from ref 9). As shown, the particles display excellent selectivity characteristics, which is in line with earlier described data.[9]

Figure 2 depicts a typical spatially resolved fluorescence spectrum obtained from a single microsphere with the chro-

(18) Kurihara, K.; Ohtsu, M.; Yoshida, T.; Abe, T.; Hisamoto, H.; Suzuki, K. *Anal. Chem.* **1999**, *71*, 3558.

(19) Telting-Diaz, M.; Bakker, E. *Anal. Chem.* **2001**, *73*, 5582.

(20) Bakker, E.; Simon, W. *Anal. Chem.* **1992**, *64*, 1805.

(21) Bakker, E.; Willer, M.; Pretsch, E. *Anal. Chim. Acta* **1993**, *282*, 265.

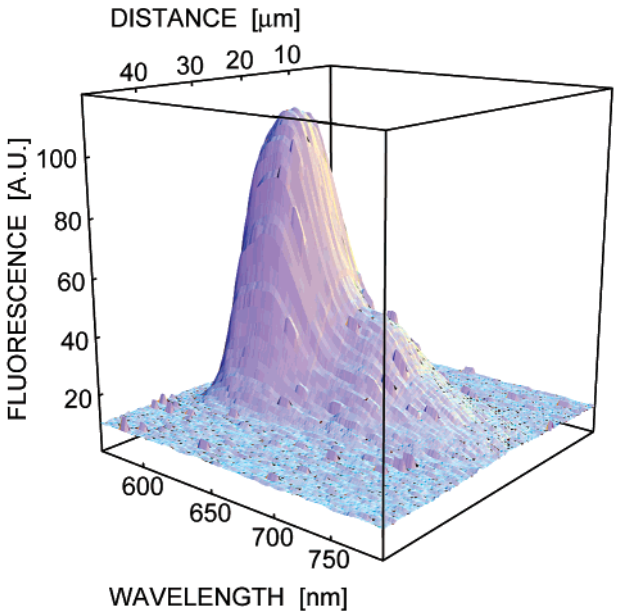

Figure 2. Spatially resolved fluorescence spectrum of a single sensing lead particle in its deprotonated form, as observed with the fluorescence spectrophotometric microscope. The spatial position of highest fluorescence intensity marks the middle of the microsphere. Particle composition: 10.2 mmol kg^{-1} chromoionophore ETH 5418, 6.9 mmol kg^{-1} modulator dye $DiIC_{18}$, 87.9 mmol kg^{-1} lead ionophore ETH 5493, and 23.8 mmol kg^{-1} anionic sites NaTFPB.

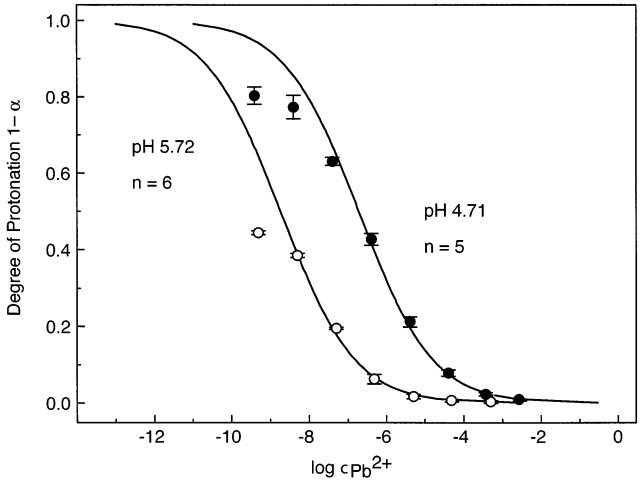

Figure 3. Response function of single sensing particles to $Pb(NO_3)_2$ solutions in $Mg(OAc)_2$ buffer at pH 4.7 (solid circles) and pH 5.7 (open circles) and calculated according to eq 1. Dashed lines indicate the detection limit at each pH calculated according to ref 21. Both curves have been generated with log K_{exch} of −2.4 (see eq 1).

moionophore in its deprotonated form. The particles were found to be uniform in size and spherical, and their surface appeared smooth under the microscope. Typical responses of particles obtained for lead concentrations spanning the 5×10^{-10} to 5×10^{-3} M level range are illustrated in Figure 3 at pH 4.7 and pH 5.7. As shown, the particles respond with a high degree of sensitivity and in full agreement with the expected theoretical values calculated on the basis of eq 1. Furthermore, the results emphasize the excellent particle to particle reproducibility that is obtained via this casting method, because the RSD of the

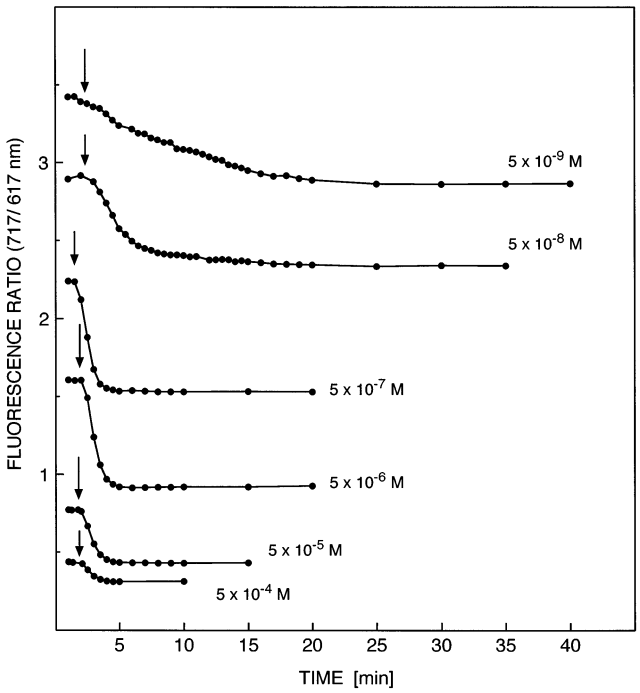

Figure 4. Response time behavior for a single sensing particle immobilized in a capillary flow-cell at different lead concentrations at pH 4.7. Arrows indicate the moment of injection as lead solutions of smaller concentrations flow past the particles until equilibration is achieved.

measurements is within a few percent ($n = 5-6$; see error bars in Figure 3).

Figure 4 displays typical response times of a single sensing particle immobilized in a glass capillary cell and assessed in the flowing stream of lead test samples. The arrows in Figure 4 represent the apparent moment of injection in which the sensing particle first transitions from lower to higher concentrations of lead. As seen in Figure 4, the equilibrium response time for a 13-μm particle at lead ion concentrations of 5×10^{-8} M and higher, is reached essentially in 2.5 min. For concentrations at detection limit levels, that is, $\sim 5 \times 10^{-9}$ M, the time needed for complete response is ~15 min. Equilibrium response values on the order of minutes represent a considerable enhancement over the response time behavior of optode films, which have been found to exhibit extremely long response times in highly diluted samples. Indeed, optode membranes proposed earlier for the analysis of Pb^{2+} at the nanomolar level necessitated hours of equilibration for stable signals to be obtained.[1] More recently, optode systems developed for other environmentally relevant ions e.g., Ag^+,[2] have also been shown to require response times in excess of a few hours at detection limit levels and of tens of minutes for higher concentrations. Because the rate-limiting step in optode films in contact with dilute solutions is thought to depend on the convective mass transport to the membrane, the saturation of the bulk equilibrium is expected to be massively shortened for micrometer-size particles of the type described here. Such behavior clearly arises from the drastically reduced amount of active components found within the particle. Indeed, a 10-μm particle requires only on the order of 2×10^{-11} mol lead ions to reach an accurate optical response (at $\alpha = 0.5$), which is a full 4 orders of magnitude smaller than with traditional thin films. Herein

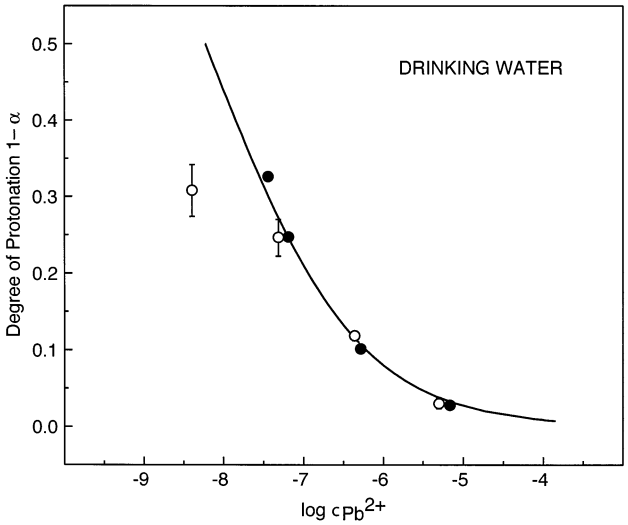

Figure 5. Degree of protonation of lead sensing microspheres for a commercial drinking water sample spiked with indicated $Pb(NO_3)_2$ concentrations and buffered to pH 5.6 (open circles, $n = 5$). Solid circles represent measurements of total $Pb(NO_3)_2$ concentrations in pure water buffered to pH 5.6 with $Mg(OAc)_2$. The solid curve represents the theoretical values calculated according to eq 1.

lies the true benefit of miniaturization of this sensing principle. Figure 4 also illustrates the high response stability observed for the various concentration changes, thus indicating minimum or negligible drift following multiple exposures to the light source.

To demonstrate applicability of the lead sensing microspheres prepared here, commercial samples of drinking water from natural springs were obtained and spiked with known amounts of $Pb(NO_3)_2$. Prior to measurements, the samples were acidified with HNO_3 to eliminate carbonate content and buffered at pH 5.6 with $Mg(OAc)_2$. Figure 5 shows a typical response curve obtained with added lead concentrations demonstrating excellent agreement with analogous measurements performed in 1×10^{-4} M $Mg(OAc)_2$ buffered solutions at the same pH. The results obtained revealed good selectivity of the spheres over other relevant ions present, and the interference from the drinking water background signal was apparent only for lead level concentrations below 4.7×10^{-8} M. Thus, the microspheres presented here were useful in rapidly ascertaining the absence of lead ions in the samples chosen at their near detection limit levels.

CONCLUSIONS

Fluorescent plasticized PVC microspheres incorporating a selective ionophore for lead metal ions were prepared via a casting apparatus that allows for the mass production of highly reproducible spherical particles. Immobilized particles assayed in a flowing stream of analyte were imaged via fluorescence spectroscopy and shown to follow predicted theory, allowing lead ion determinations at the low nanomolar level. Responses were characterized by high stability and reproducibility with a detection limit comparable to those found for optode films. However, in contrast to optode thin films that require typical equilibration times of hours following exposure to nanomolar level concentrations, the particles prepared here were shown to respond rapidly in just a few minutes and required drastically reduced sample volumes on the order of 1 mL. Miniaturized ionophore-based sensing microspheres are therefore a very promising platform for the assessment of trace level concentrations in a variety of samples.

ACKNOWLEDGMENT

The authors gratefully acknowledge financial support by the National Institutes of Health (GM 59716) for this research.

Received for review February 22, 2002. Accepted August 19, 2002.

AC025596l