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Coupling Fiber Optics to a Permeation Liquid Membrane for Heavy Metal Sensor Development

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We present the first sensing system for metal ions based on the combination of separation/preconcentration by a permeation liquid membrane (PLM) and fluorescence detection with an optical fiber. As a model, a system for the detection of Cu(II) ions was developed. The wall of a polypropylene hollow fiber serves as support for the permeable liquid membrane. The lumen of the fiber contains the strip solution in which Cu(II) is accumulated. Calcein, a fluorochromic dye, acts as stripping agent and at the same time as metal indicator. The quenching of the calcein fluorescence upon metal accumulation in the strip phase is detected with a multimode optical fiber, which is incorporated into the lumen. Fluorescence is excited with a blue LED and detected with a photon counter. Taking advantage of the high selectivity and sensitivity of PLM preconcentration, a detection limit for Cu(II) of ~50 nM was achieved. Among five tested heavy metal ions, Pb(II) was the only major interfering species. The incorporation of small silica optical fibers into the polypropylene capillary allows for real-time monitoring of the Cu(II) accumulation process.

The speciation of trace compounds (e.g., heavy metals) in environmental systems is of great importance for explaining their role in geochemical and biological cycling.[1] A very useful method for environmental monitoring is fiber-optic sensing based on fluorescence. Up to now, several sensing schemes for heavy metals have been presented that use fluorescence measurements.

Saari and Seitz designed a fiber-optic sensor for determination of heavy metal ions.[2] They immobilized the fluorophore calcein on cellulose and fixed it in front of the distal end of a bifurcated optic fiber. However, owing to the high complexing constant of calcein for Cu(II), the metal binding was irreversible. Hieftje et al. attached rhodamine 6G electrostatically to a Nafion ion exchanger and used a fiber-optic arrangement to measure the total heavy metal concentration.[3] Metals such as Cu(II), Co(II), Cr(II), Fe(II), Fe(III), and Ni(II) were determined with a limit of detection of ~1 μM.

A more selective approach was reported by Birch et al.[4] It is based on fluorescence resonance energy transfer (FRET) between rhodamine 800 and the large absorption band of the $Cu(H_2O)_4^{2+}$ complex in the near-infrared region (λ_{max} = 810 nm). As the absorption coefficient of the complex is relatively weak, only a limit of detection of 5 mM was achieved.

Niessner et al. reported on a sophisticated fiber-optic sensor for the detection of heavy metals.[5] The sensor head consists of five compartments filled with fluorogenic dyes, each of which is selective to one metal ion. The indicator solutions are separated from the sample solution by an ion-permeable membrane. Metal diffusion across the membrane causes a change in fluorescence. The fluorescence is excited with a nitrogen laser (337 nm) and detected with a CCD camera via multimode optical fibers. The limit of detection for Cu(II) is 4.7 μM. According to the authors, a drawback of the system is the slow diffusion rate of the metal ions across the membranes. The signal reaches the equilibrium value after ~20 min. The driving force of the passive diffusion process is the concentration gradient between sample and dye solution.

Our goal was to use carrier-associated permeable liquid membranes (PLMs) to develop a sensor for Cu(II) ions. PLMs preconcentrate the analyte from the sample solution (source) into a receiver solution (strip). These two aqueous phases are separated by a hydrophobic liquid membrane that contains an analyte-selective carrier R (Figure 1).

The accumulation is driven by the gradient of the free analyte concentration between the source and the strip solution. Permeable liquid membranes allow the selective detection of a wide range of trace compounds (e.g., metal ions, carboxylic acids, and alcohols) with high sensitivity.[6] Potential advantages of PLMs are high selectivity, reduced sample handling, easy automation, real-time analysis, application to wide range of compounds, and selectivity for the free (often the bioavailable toxic) form of the analyte.[6]

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(1) Buffle, J. *Complexation reactions in aquatic systems. An analytical approach*; Ellis Horwood: Chichester U.K., 1998.

(2) Saari, L. A.; Seitz, W. R. *Anal. Chem.* **1984**, *56*, 813.

(3) Bright, F. V.; Poirier, G. E.; Hieftje, G. M. *Talanta* **1988**, *35*, 113.

(4) Birch, S. D. J.; Rolinski, O. J.; Hatrick, D. *Rev. Sci. Instrum.* **1996**, *67*, 2732.

(5) Prestel, H.; Gahr, A.; Niessner, R. *Fresenius J. Anal. Chem.* **2000**, *368*, 182.

(6) Buffle, J.; Parthasarathy, N.; Djane, N. K.; Matthiasson, L. Permeation Liquid Membranes for Field Analysis and Speciation of Trace Compounds in Waters. In *In situ monitoring of aquatic systems*; Buffle, J., Horvai, G., Eds.; IUPAC Series on Analytical and Physical Chemistry of Environmental Systems 6; Wiley: Chichester, U.K., 2000; Chapter 10.

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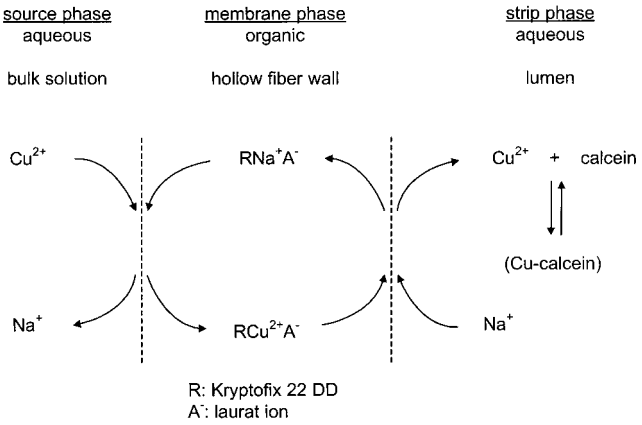

Figure 1. Principle of the PLM preconcentration of Cu(II) ions with Kryptofix 22DD as the organic carrier. The driving force is the concentration gradient of free Cu(II) ions between the source solution and the strip compartment. Charge neutrality is assured by a countertransport of sodium ions.

The wall of polypropylene hollow fibers can be used as the PLM support (HFPLM). Here, the source solution is placed outside the hollow fiber and the analyte is selectively extracted into the lumen of the fiber, which contains the strip phase. Enrichment factors of more than 1000 can be achieved due to their large source/strip volume ratio.[7] The large surface/volume ratio of the strip phase also gives high fluxes and hence reduces the transport times. Until now, the evolution of concentration in the strip solution could only be analyzed discontinuously using AAS or voltammetric methods,[7,8] which is very much time-consuming, since after each measurement the preconcentration process has to be started again. The incorporation of an optical fiber into the lumen of the PLM hollow fiber offers a solution to this problem. If the strip solution contains an indicator, then the accumulation of an analyte can be followed in real time.

Apart from the real-time measurement, combination of PLMs with fiber-optic detection would bring along other advantages. The volume of the strip compartment can be further reduced, which would result in higher preconcentration factors. A smaller strip volume will also guarantee autonomy for a larger number of analyses in case of unattended field operation.[6] Finally, optical fibers are well suited for remote sensing.

In this paper, we present the first sensing system based on the combination of a permeable liquid membrane and fiber optics. The optical detection of Cu(II) was chosen to demonstrate the feasibility of this technique because its transport system has been well developed[9] and much experience has already been gained in speciation of heavy metals with PLMs.[6−10] As the fluorophore in the strip solution, we have chosen calcein (cal; Figure 2).

Its complex formation constant with Cu(II) ($K = 10^{12.3}$) [2] is such that, for [cal] ~30 μM and pH 6.5 as used here, the degree of complexation α_{st} of Cu(II) in the strip solution is higher than in the membrane (α_{st} is the total/free concentration of Cu(II) in the strip solution). Hence, calcein can act as stripping agent and as indicator at the same time. Another reason to use calcein is that its complexing properties are complementary to that of the PLM with respect to the selectivity toward metals. Indeed, its fluorescence is hardly influenced by metals other than Cu(II) that are transported through the PLM, such as Zn(II) and Cd(II),[11] while the PLM does not preconcentrate other metals that strongly quench the calcein fluorescence such as Co(II) and Ni(II).

Calcein and its Cu(II) complex are highly soluble in water and insoluble in the organic phase of the membrane (toluene + phenylhexane = 1 + 1), which is another condition for its use as complexing agent in the strip solution. Its high fluorescence quantum yield (~0.9) and its absorption and emission maximums in the visible region (λ_{ex} = 495 nm, λ_{em} = 512 nm) make it very suitable for fiber optical sensing.

THEORY

It can be shown theoretically[6] that the total analyte concentration in the strip solution c_{st} varies with time as depicted in Figure 3. If the transport through the membrane is rate limiting, then the initial slope of the curve is proportional to the initial concentration of the free (uncomplexed) analyte in the source solution.[6]

For an analyte that is not complexed in the source solution, the initial slope is proportional to the total initial concentration of analyte in the source c_s^0. If the strip solution contains an excess of the complexing agent, then the total analyte concentration in the strip solution at equilibrium c_{st}^e is also proportional to the initial total concentration in the source. Hence, either the initial slope of the c_{st} versus time curve or the value of c_{st}^e can be used to determine the initial concentration in the source as well as the degree of complexation α_s of the analyte in the source.

For labile complexes of the analyte in the source and diffusion-based transport in the source, membrane, and strip phases, the general expression for the overall initial flux J of the analyte can be written as[6]

$$J = \frac{V}{A}\frac{dc_{st}}{dt} = \frac{c_s}{\alpha_s}\left[\frac{\delta_s}{D_s} + \frac{l}{D_m K_p} + \frac{\delta_{st}}{D_{st}\alpha_{st}}\right]^{-1} \qquad (1)$$

where V is the volume of the strip solution, A is the effective membrane surface area, c_s is the source concentration of the analyte, δ_s and δ_{st} are the thicknesses of the Nernst aqueous diffusion layers in the source and strip solutions, respectively, l is the membrane thickness, K_p is the distribution coefficient of Cu(II) between the source solution and the membrane, D_s and D_m are the diffusion coefficients in the Nernst layer and in the membrane, respectively, and α_s and α_{st} are the degrees of complexation (the ratio total/free concentrations) of the analyte in the source and in the strip solution.

Under the conditions of a large excess of ligand in the strip solution ($\alpha_{st} \gg 1$) and the absence of any complexant in the sample ($\alpha_s = 1$), eq 1 simplifies to

$$J = \frac{V}{A}\frac{dc_{st}}{dt} = c_s\left[\frac{\delta_s}{D_s} + \frac{l}{D_m K_p}\right]^{-1} \qquad (2)$$

The maximal preconcentration factor F describes the performance

(7) Parthasarathy, N.; Pelletier, M.; Buffle, J. *Anal. Chim. Acta* **1997**, *350*, 183.
(8) Keller, O. C.; Buffle, J. *Anal. Chem.* **2000**, *72*, 943.
(9) Parthasarathy, N.; Buffle, J. *Anal. Chim. Acta* **1991**, *254*, 1.
(10) Parthasarathy, N.; Buffle, J.; Gassama, N.; Cuenod, F. *Chem. Anal. (Warsaw)* **1999**, *44*, 455.
(11) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes, Inc., Eugene, OR, 1996.

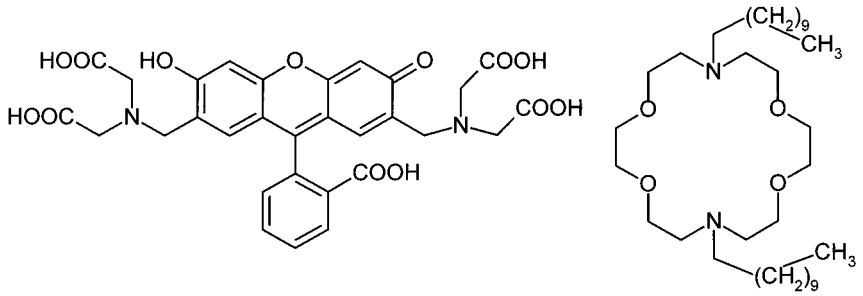

Figure 2. Structure of the fluorophore calcein and the organic carrier Kryptofix 22 DD.

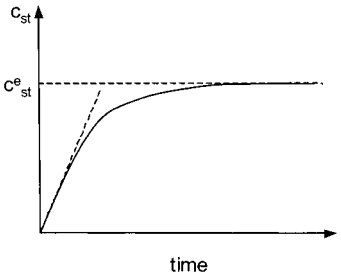

Figure 3. Principal time course of the total analyte concentration in the strip compartment, c_{st}, during a PLM preconcentration (see ref 6 for details). To determine the initial source concentration, the total strip concentraion at equilibrium c^e_{st} (in the case of excess stripping agent) or the initial slope of the c_{st} vs time curve can be used.

of a PLM. For the accumulation of the analyte in the strip solution, it is governed by the ratio of the degree of complexation in the strip α_{st} to the degree of complexation in the source compartment α_s and by the ratio of the volumes of the two compartments (V_{st} and V_s) according to the following equation:[6]

$$F = \frac{c^e_{st}}{c^0_s} = \frac{V_s\alpha_{st}}{V_{st}\alpha_{st} + V_s\alpha_s} \quad (3)$$

or

$$\frac{1}{F} = \frac{c^0_s}{c^e_{st}} = \frac{V_{st}}{V_s} + \frac{\alpha_s}{\alpha_{st}} \quad (4)$$

with c^e_{st} the total concentration of analyte in the strip solution at equilibrium and c^0_s the initial source concentration.

EXPERIMENTAL SECTION

Reagents, Materials, and Apparatus. 1,10-Didecyl-1,10-diaza-18-crown-6 (Kryptofix 22DD) was purchased from Merck AG (Darmstadt, Germany). Calcein was supplied by Molecular Probes Inc. (Eugene, OR). *trans*-1,2-Diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), lauric acid, toluene, and phenylhexane (all p.a. grade) were obtained from Fluka AG (Buchs, Switzerland). 2-(*N*-Morpholino)ethanesulfonic acid (MES) and 3-(*N*-morpholino)propanesulfonic acid (MOPS) were purchased from Sigma (Buchs, Switzerland).

The blue LED was obtained from Oshino (Tokyo, Japan), and the filters and dichroic mirror (set XF 115) were from Omega Optical Inc. (Brattleboro, VT). The optical fiber (105/125 A) was bought from CeramOptech (Bonn, Germany), and the photomultiplier H7421-50 came from Hamamatsu Photonics (Schüpfen, Switzerland). The timer/counter board CTM-05/A was purchased from Keithley Instruments S. A. (Dübendorf, Switzerland). The polypropylene hollow fiber Accurel ppq 3/2 was supplied by Membrana GmbH (Wuppertal, Germany) and the peristaltic pump by Omnilab (Mettmenstetten, Switzerland).

A scheme of the optical system is given in Figure 4.

All bulk optical elements were mounted on an aluminum plate (dimensions 42 cm × 28 cm × 1 cm). The light of the blue LED (λ_{max} = 460 nm, fwhm = 70 nm, 3.1 V) was passed through an excitation filter (λ_{max} = 475 nm, fwhm = 40 nm) and coupled into a multimode optical fiber (length 1 m, NA 0.22) via a dichroic mirror. The output power at the opposite distal end of the optical fiber at 475 nm was 15 nW. At this end, the fiber was introduced into the tubing via a 5-cm-long INOX tube (inner diameter 250 μm, corresponding to the outer diameter of the fiber-optic acrylic jacket). Calcein fluorescence was collected with the same fiber, filtered by a band-pass filter (λ_{cutoff} = 510 nm), and detected with a Peltier-cooled photomultiplier tube operated in photon counting mode. Data were retrieved with a timer/counter board and processed with a personal computer.

The polypropylene hollow fiber (length 61 mm, inner diameter 600 μm, outer diameter 1 mm) was fixed on both ends to Teflon tubes (6 cm long, inner diameter 1.0 mm) with a 1-cm-long heat-shrinkable polypropylene tube. A peristaltic pump was used to fill and rinse the hollow fiber with the appropriate solutions. A four-port valve allowed to pass the solutions through the polypropylene hollow fiber or to bypass it (Figure 5).

Membrane Preparation and Measurements. The PLM was prepared following a standard procedure.[7] A 1-mL aliquot of a 200 mM solution of Kryptofix 22DD in phenylhexane and 1 mL of a 200 mM solution of lauric acid in toluene were mixed. Then the wall of the polypropylene hollow fiber (wall thickness 200 μm) was impregnated with this carrier solution from the outside by slowly flowing the solution with a Pasteur pipet. After the impregnation, the hollow fiber was placed in Milli-Q water and the lumen was rinsed with Milli-Q water for 10 min to remove the excess of carrier solution. Then the strip solution was passed through the lumen for 10 min.

The strip solution had been prepared by dissolving 0.5 mg of calcein in 25 mL of a 10 mM buffer solution (MES or MOPS) and adjusting the pH with ultrapure HNO_3 or NaOH. Prior to use, the

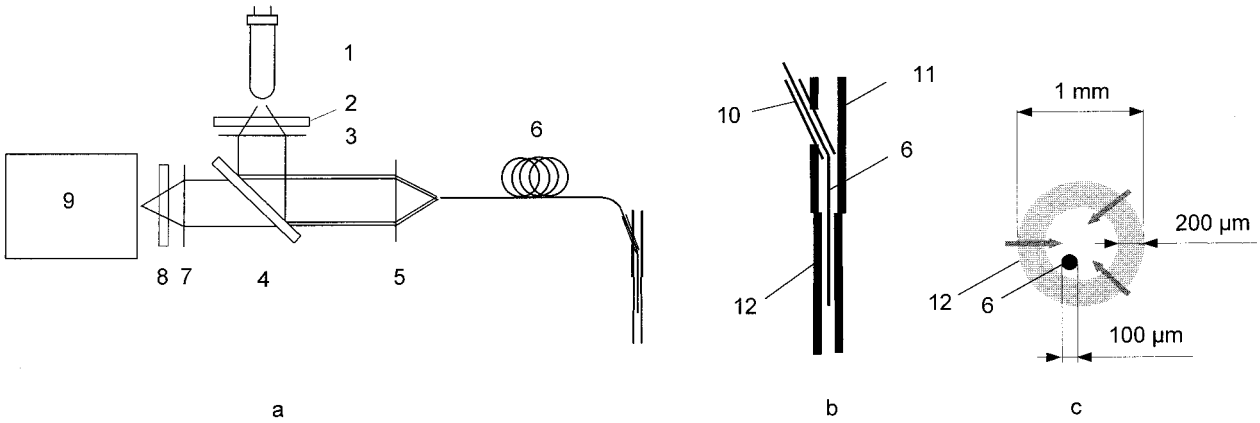

Figure 4. (a) Scheme of the optical system: 1, blue LED; 2, 8, filters; 3, 5, 7, lenses; 4, dichroic mirror; 6, sensing fiber (diameter 105 μm); 9, photon counter. (b) Zoom into the sensing region: 10, INOX tube; 11, PTFE tube; 12, polypropylene capillary. (c) Cross section of the polypropylene capillary with the optical fiber incorporated into its lumen, drawn to scale. The wall of the polypropylene fiber (thickness 200 μm) serves as support for the permeation liquid membrane. The arrows indicate the radial diffusion of the analyte into the lumen.

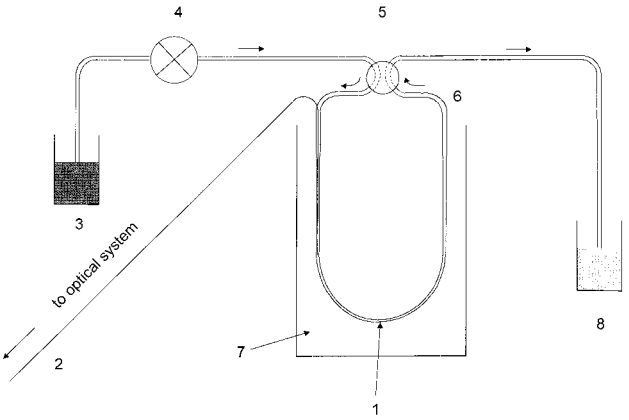

Figure 5. Preconcentration system: 1, polypropylene hollow fiber; 2, sensing fiber (105 μm); 3, calcein solution; 4, peristaltic pump; 5, four-way valve; 6, PTFE tubing; 7, sample (= source) solution; 8, waste container.

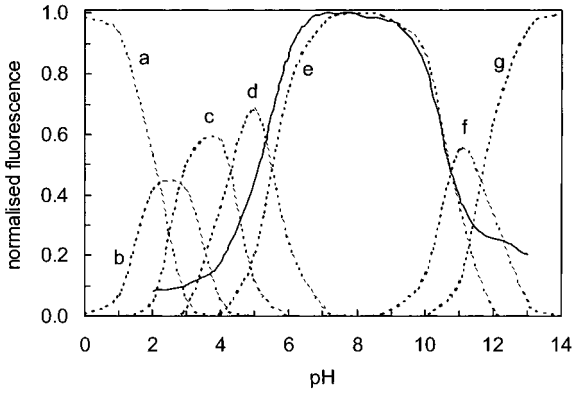

Figure 6. Dependence of the normalized fluorescence intensity on the pH in an unbuffered 30 μM calcein solution measured with the fiber-optic setup (solid line). The experimental curve consists of 85 single experimental points. The distribution of the different protonated forms of calcein (dashed lines: a, H_6cal; b, H_5cal^{-}; c, H_4cal^{2-}; d, H_3cal^{3-}; e, H_2cal^{4-}; f, $Hcal^{5-}$; g, cal^{6-}) was calculated with the software COMICS12 using the pK_a values published in ref 13 (pK_a values: 2.1, 2.9, 4.1, 5.4, 10.1, 12.0).

strip solution was degassed with nitrogen for ~30 min to prevent oxidation.

After the impregnated hollow fiber was rinsed with the strip solution, the peristaltic pump was switched off and the valve was closed. Then the hollow fiber, filled with the enclosed strip solution (volume 17 μL), was placed in a solution of 10 mM MES (pH 6.0). Now aliquots of the stock solution ($Cu(NO_3)_2$ concentration 1 mM) were added and the fluorescence was recorded as a function of time. For the selectivity tests, aliquots of stock solutions of the nitrate salts of Pb(II), Co(II), Ni(II), Cd(II), and Zn(II) (all at 1 mM concentrations) were added to the source solution. The metal concentrations in the source solution stayed constant throughout all experiments. This was verified with AAS measurements.

After each measurement, the hollow fiber was rinsed with a solution of 1 mM CDTA and Milli-Q water (10 min each).

RESULTS AND DISCUSSION

Optimization of the Calcein Concentration and the pH of the Fluorescent Strip Solution. The sensing system was first optimized with respect to the pH of the strip solution by measuring the pH dependence of the calcein fluorescence. The optical fiber was dipped into 10 mL of a stirred, unbuffered calcein solution (concentration 30 μM). The pH was varied in the range pH 2−13 by adding small-volume aliquots of HNO_3 or NaOH solutions and the pH of the resultant mixture was measured with a pH meter. The final increase in volume was ~1%. The maximum fluorescence signal at pH 8, corresponding to 550 000 counts/s, was set to unity. (When the optical fiber was dipped into Milli-Q water, the count rate was 3200 counts/s.).

Calcein fluorescence was found to be constant in the range of pH 6.5−9.5 (Figure 6). The pK_a values for calcein have been determined by several research groups.[13−15] In Figure 6, the calculated protonated forms of calcein based on the pK_a values published by Iritani and Miyahara (determined by titration in 0.1 N KOH, room temperature)[13] are presented in addition to the measured fluorescence. Comparison of the experimental curve with the calculated curves shows that the fluorescence is mainly due to the 4 times-deprotonated calcein (H_2cal^{4-}) at the excitation

(12) Perrin, D. D.; Sayce, I. G. *Talanta* **1967**, *14*, 833.
(13) Iritani, N.; Miyahara, T. *Jpn. Anal.* **1973**, *22*, 174.
(14) Markuszweski, R. Ph.D. Thesis, Iowa State University, Ames, IA, 1976.
(15) Wallach, D. F. H.; Surgenor, D. M.; Soderberg, J.; Delano, E. *Anal. Chem.* **1959**, *31*, 456.

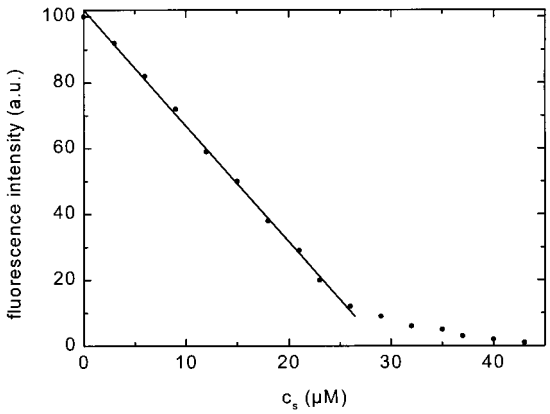

Figure 7. Calibration curve for Cu(II) in 30 μM calcein and 10 mM MES (pH 6.5).

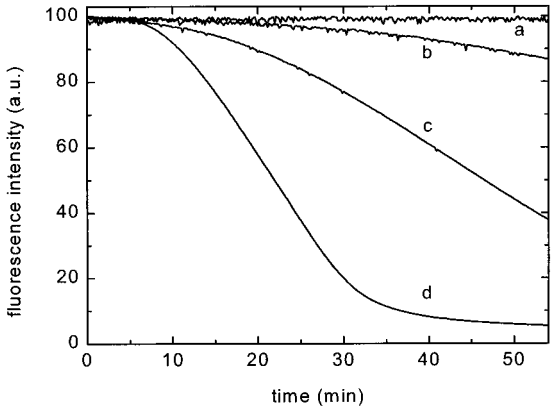

Figure 8. Fluorescene intensity vs time for different Cu(II) concentrations in the source solution. The fluorescence−time curves were followed on-line with the fiber optic setup. Strip solution: 30 μM calcein in 10 mM MES buffer, pH 7.9. The sample solutions were prepared by adding different volumes of a Cu(II) stock solution to 250 mL of 10 mM MES buffer (pH 6.0; sample concentrations: a, no Cu; b, 0.12 μM Cu; c, 0.40 μM Cu; d, 1.00 μM Cu).

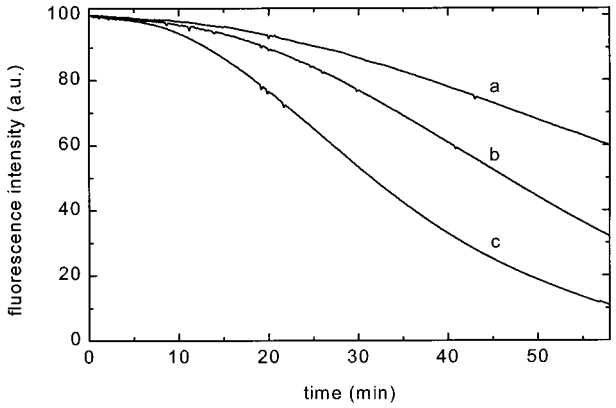

Figure 9. Fluorescene intensity vs time for different strip solution pH values (a, pH 8.5; b, pH 7.9; c, pH 6.5). The Cu(II) concentration in the source solutions were in all cases 0.40 μM Cu(II) in 10 mM MES buffer (pH 6.0).

and the emission wavelengths used here. To achieve maximal sensitivity, the pH of the strip solution should thus be fixed between 6.5 and 9.5.

In this work, we used a PLM to separate and preconcentrate Cu(II) ions from aqueous samples. The preconcentration factor F of a PLM system is determined by the degrees of complexation in the strip phase and in the source compartment (α_{st} and α_s) and the volumes of the two compartments (eq 4). If $\alpha_{st} \gg \alpha_s$, then the preconcentration factor at equilibrium depends on the volume ratio V_{st}/V_s. As the lumen of the hollow fiber served as strip compartment, a high volume ratio V_{st}/V_s and hence a high preconcentration factor can be obtained. To take advantage of the capabilities of the preconcentration system, a large α_{st} (i.e., in our case, a high calcein concentration) is necessary. However, at calcein concentrations greater than 30 μM, a reddish precipitate was formed on the inner side of the polypropylene hollow fiber. The precipitate could possibly result from photooxidation with air oxygen. In particular, it is known that fluorescein and its derivatives can undergo photobleaching.[16] A more likely explanation for the formation of this precipitate, however, is the formation of copper hydroxide or mixed calcein−copper hydroxide complexes. The fact that the precipitation was more pronounced at higher pH values of the strip solution supports this hypothesis. On the basis of the above-mentioned observation, a calcein concentration of 30 μM was chosen as the optimal concentration for our experiments.

To calibrate a buffered 30 μM calcein solution, small volumes of a Cu(II) stock solution were added to 10 mL of a stirred calcein solution and the fluorescence intensity was recorded with the fiber optical setup. A linear decrease in fluorescence intensity was observed up to Cu(II) concentrations of 23 μM (Figure 7).

Effect of the Strip Solution pH on the Response Time. In the first assays, we used a strip solution containing 30 μM calcein dissolved in 10 mM MOPS buffer at pH 7.9. The sensing region consisted of an impregnated, 61-mm-long HFPLM filled with the strip solution. An optical fiber (105-μm core diameter) was incorporated into the hollow fiber to follow the fluorescence evolution in real time. Measurements were carried out by dipping the sensing region into 10 mM MES (pH 6.0). Then aliquots of Cu(II) stock solutions were added to the source solution to obtain the desired Cu(II) concentrations.

The typical time lag due to the establishement of steady-state diffusion through a HFPLM is experimentally[7−10,17] and theoretically[18] observed to be ∼2 min. In our case, however, a much longer starting period was observed in which the fluorescence intensity remained unchanged (Figure 8). The intensity started to decrease only after about 10−25 min, depending on the Cu(II) sample concentration.

Experiments with strip solutions at different pH values but with equal Cu(II) concentrations in the source solution (c_s = 400 nM) show that faster responses are obtained at lower strip solution pH values (Figure 9).

The faster decrease of fluorescence intensity at lower strip pH values (Figure 9) and at higher Cu(II) concentrations in the source solution (Figure 8) could be due the quick formation of a copper(II) hydroxide complex or a mixed calcein−copper hydroxide complex at the interface PLM/strip solution followed by its slow dissociation into the more stable nonfluorescent complex

(16) Kasche, V.; Lindqvist, L. *J. Phys. Chem.* **1964**, *4*, 817.

(17) Parthasarathy, N.; Pelletier, M.; Tercier-Waeber. M.-L.; Buffle, J. *Electroanalysis* **2001**, *13*, 1305.

(18) Keller, O.; Poitry, S.; Buffle, J. *J. Electroanal. Chem.* **1994**, *378*, 165.

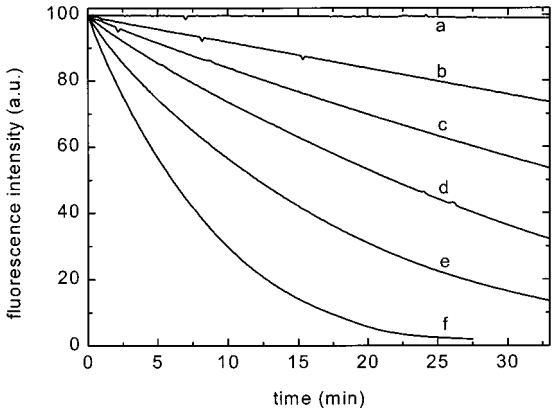

Figure 10. Fluorescene intensity vs time for different source Cu(II) concentrations (source, 10 mM MES buffer (pH 6.0) with the following variables: a, no Cu; b, 0.02 μM Cu; c, 0.05 μM Cu; d, 0.15 μM Cu; e, 0.32 μM Cu; f, 0.64 μM Cu). Strip solution: 30 μM calcein in 10 mM MES buffer (pH 6.5).

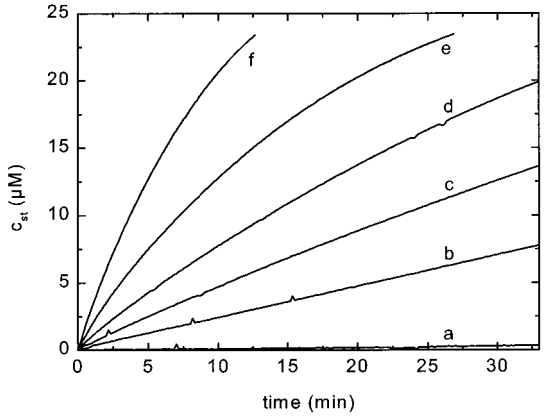

Figure 11. Total strip concentration of Cu(II) c_{st} vs time for different Cu(II) source concentrations. The strip Cu(II) concentrations were determined from fluorescence intensity vs time curves (Figure 10) and the calibration of 30 μM calcein in 10 mM MES (pH 6.5) with Cu(II) (Figure 7).

Cu(cal). Hering and Morel[19] and Miyahara[20] reported on the formation of mixed ligand−Cu(II)−calcein complexes with different stoichometry and hydroxo−copper complexes are known to form at pH >6.5 and [Cu(II)] >10^{-6} M.[21] A more detailed study of this interferring process was not performed, but subsequently, measurements were carried out under the following conditions: for each Cu(II) concentration in the source, the HFPLM was dipped for a 25-min preconditioning time into the source solution that already contained the Cu(II). The strip solution (pH 6.5) was renewed after the 25 min by means of the peristaltic pump. Then the pump was stopped, and the fluorescence intensity was measured. According to this procedure, even the time lag corresponding to the establishement of steady-state gradients by diffusion in the membrane is eliminated, as can be seen in Figure 10.

Sensitivity and Calibration. The sensitivity of our sensing system was investigated by measuring the change in fluorescence intensity at various Cu(II) concentrations in the source solution following the experimental procedure described above.

(19) Hering, J. G.; Morel, F. M. M. *Environ. Sci. Technol.* **1990**, *24*, 242.
(20) Miyahara, T. *Jpn. Anal.* **1977**, *26*, 615.
(21) Martell, A. E. *Stability Constants of Metal-Ion Complexes: Supplement No. 1*; Alden Press: Oxford, U.K., 1971.

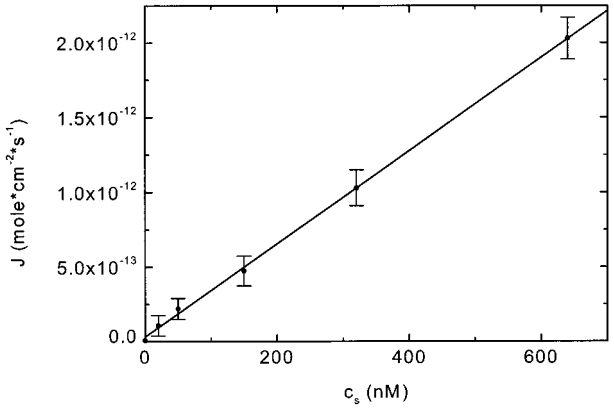

Figure 12. Dependence of the overall flux J on the Cu(II) source concentration. J was determined with eq 2. Errors are standard deviations.

Table 1. Comparism of Fluxes J Measured at $t = 0$ with Calculated Fluxes J_{calc}[a]

c_s (nM)	$\Delta c_{st}/\Delta t$ (10^{-11} mol·cm^{-3}·s^{-1})	J (10^{-12} mol·cm^{-2}·s^{-1})	J_{calc} (10^{-12} mol·cm^{-2}·s^{-1})
0	0.02	0.01 ± 0.01	0.00
20	0.29	0.11 ± 0.05	0.06 ± 0.01
50	0.60	0.22 ± 0.07	0.16 ± 0.01
150	1.30	0.48 ± 0.10	0.47 ± 0.01
320	2.83	1.03 ± 0.12	1.01 ± 0.02
640	5.58	2.03 ± 0.14	2.01 ± 0.04

[a] J were determined according to eq 2 (left-hand term). $\Delta c_{st}/\Delta t$ was measured using the fiber-optic sensor (Figure 11). The values published in ref 22 were used for calculations (δ_s = 15 μm, D_s = 7.8 × 10^{-6} cm^2·s^{-1}, l = 200 μm, D_m = 2.55 × 10^{-7} cm^2·s^{-1}, and K_p = 622). The errors in J_{calc} were calculated from the errors in the membrane parameters.[22]

At higher Cu source concentrations, the fluorescence is quenched faster than for lower Cu(II) concentrations (Figure 10). This is due to the quicker saturation of the calcein with Cu(II) ions in the strip solution. After calcein saturation, the concentration of free Cu(II) ions in the strip solution equals the Cu(II) source concentration and the preconcentration process stops.

The evolution of the total Cu(II) concentration in the strip solution c_{st} with time (Figure 11) was determined from the fluorescence versus time curve (Figure 10) and the calibration curve (Figure 7). As the calcein had to be used at a relatively low concentration (30 μM), it becomes completely complexed in a relative short time. Under such conditions, the Cu(II) concentration in the strip solution at equilibrium depends only on the total calcein concentration and strip pH and not on the Cu(II) concentration in the source solution. Hence, only the initial flux given by the linear part of the fluorescence versus time curve can be used to determine the free Cu(II) concentration in the source solution (Figure 2).

The slope of the initial linear domain $\Delta c/\Delta t$ provides the overall flux J across the permeable liquid membrane (left-hand side of eq 2). Figure 12 indeed shows that J is proportional to the Cu(II) source concentration. This relation can be used for the calibration of the sensing system.

Our setup has a detection limit of ~50 nM Cu(II) in the source solution. The fluxes J determined experimentally correspond well to the values, J_{calc}, that can be calculated by means of membrane-specific parameters and eq 2 (Table 1).

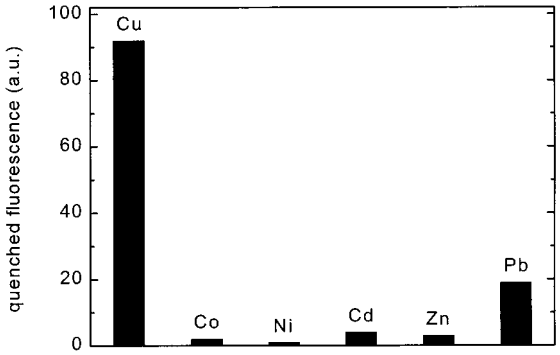

Figure 13. Quenched calcein fluorescence for six different heavy metals after 60-min preconcentration time (strip solution, 30 μM calcein in 10 mM MOPS, pH 7.9). Sample metal concentrations were 600 nM in 10 mM MES buffer, pH 6.0.

Experimentally, radial diffusion occurs in the HFPLM[7,23] whereas eq 2 holds for planar diffusion. Equation 2, however, is valid for the purpose of comparison in Table 1, since only initial fluxes are computed and the ratio $l/\delta_s = 13$ is significantly larger than 1.

Selectivity. To investigate the selectivity of our system, the sensing head was exposed to 600 nM solutions of Cu(II), Co(II), Ni(II), Cd(II), Zn(II), and Pb(II) in 10 mM MES (pH 6.0) and the resulting fluorescence intensity−time curves were recorded. Apart from Cu(II), only Pb(II) caused a significant decrease in fluorescence intensity after 60 min (Figure 13).

The high selectivity of our sensing system results from the combined selectivities of calcein and the transport through the PLM: while the calcein fluorescence in solution is effectively quenched by Cu(II), Ni(II), and Co(II),[11] the latter two ions do not pass significantly through the PLM system. On the other hand, Cd(II) and Zn(II) are preconcentrated but they only act as weak quenchers for calcein. Pb(II) is the only metal that passes the PLM and at the same time decreases the calcein fluorescence to some extent. It should be noticed that Pb(II) concentrations in nature are about 1000 times lower than these of Cu(II), suggesting that the cross-reactivity to Pb(II) is probably be a minor problem. Since a PLM usually is sensitive to free metal ions, the degrees of complexation of Cu(II) and Pb(II) in the test water may effect the selectivity. It should be noted, however, that Cu(II) and Pb(II) often have similar degrees of complexation for the same ligands. In such cases, the selectivity should be similar to that observed in our experiments.

CONCLUSION

In this work, we present the first sensing system based on a permeable liquid membrane coupled to fiber optics. It combines the potential advantages of fiber optics such as remote sensing suitability, inertness to electromagnetic radiations, and the potential of miniaturization with the selectivity and sensitivity inherent to PLM preconcentration using polypropylene capillaries as the membrane support. The incorporation of small silica optical fibers allows for real on-line monitoring of the accumulation time course. As a model system for a PLM-based fiber optical sensing system, we developed a sensor for Cu(II) ions. We chose the metallofluorochromic dye calcein as indicator to detect Cu(II) via fluorescence quenching. The high quantum yield of calcein and fluorescence detection with a photon counter allow the use of a LED as the light source. We were able to detect concentrations of Cu(II) with a limit of detection of ~50 nM in aqueous solution with high selectivity.

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(22) Guyon, F.; Parthasarathy, N.; Buffle, J. *Anal. Chem.* **1999**, *71*, 819.
(23) Danesi, P. R.; Rickert, P. G. *Solvent Extr. Ion Exch.* **1986**, *4*, 149.