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Application of a Novel Lipophilized Fluorescent Dye in an Optical Nitrate Sensor

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A new lipophilic pH probe (1-hydroxypyrene-3,6,8-tris-octadecylsulfonamide) has been synthesized, and its spectral properties and applications in a solid state nitrate sensor are shown. The sensor is investigated with respect to sensitivity, limits of detection, and selectivity over other anions found in drinking water.

KEY WORDS: Lipophilic fluorescent dye; optical nitrate determination.

INTRODUCTION

The determination of nitrate in drinking water currently is a most important aspect of analytical chemistry. Human organism metabolizes nitrate to end up finally in the form of nitrosamines, which are carcinogenic and highly toxic to the human fetus. The maximum allowance of nitrate in drinking water can be as high as 50 ppm in many countries. Therefore, detection methods are required with a sensitivity over the 5–500 mg/L range. We present an optical sensor membrane that fulfills this requirement. It is based on fluorescence [1], which is advantageous over absorbance when monitoring turbid waters. The membrane consists of a matrix composed of plasticized PVC[3] containing both the nitrate carrier tridodecylmethylammonium chloride (TDMACl) and the lipophilic indicator dye 1-hydroxypyrene-3,6,8-tris-octadecylsulfonamide (HPTS-TOA).

The sensing mechanism is based on ion coextraction [2,3]: TDMACl carries a nitrate ion into the membrane. In order to maintain electroneutrality in the membrane, a proton is also extracted from the aqueous into the membrane phase. As a result, the pH indicator in the membrane becomes protonated and the fluorescence intensity of the base form of the indicator is reduced. Since the signal change depends on the product of the activities of nitrate *and* protons, the pH has to be adjusted or determined independently.

EXPERIMENTAL

Chemical and Reagents

Poly(vinyl chloride) (PVC; high molecular grade), bis-(2-ethylhexyl)-sebacate (DOS), tridodecylmethylammonium chloride (TDMACl), and tetrahydrofurane (THF) were obtained from Fluka AG (Buchs, Switzerland). All buffer components were of analytical grade. Triply distilled water was used throughout.

Synthesis of the Lipophilized Fluorescence Indicator

Trisodium-1-acetoxy-pyrene-3,6,8-trisulfonate **(1).** Two grams (3.7 mmol) of trisodium-1-hydroxypyrene-

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[3] *Abbreviations used:* HPTS, trisodium-1-hydroxy-pyrene-3,6,8-trisulfonate; HPTS-TO, 1-hydroxypyrene-tris-3,6,8-octadecylsulfonamide; TDMACl, tridodecylmethylammonium chloride; PVC, poly(vinyl chloride); DOS, bis-(2-ethylhexyl)-sebacate; THF, tetrahydrofurane; DMF, dimethylformamide.

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3,6,8-trisulfonate, 0.32 g (3.9 mmol) of sodium acetate, and 10 ml of acetic anhydride were stirred at 60°C for 2 h. The resulting mixture was filtered and the liquid evaporated to dryness. The crude product was recrystallized from 150 ml of methanol to give 1 g (46%) of pure **1.** The mp is 238–248°C (decomp.). ^{1}H-nmr (D_2O): 2.9 (s, 3H); 8.1–9.6 (m, 6II).

1-Acetoxy-pyrene-3,6,8-trisulfonyl chloride **(2).** One gram (1.77 mmol) of 1 and 3.4 g of phosphorus pentachloride are ground in a mortar and the mixture is heated in a round-bottom flask at 100°C for 30 min. The resulting mixture is stirred with 100 ml of toluene for 15 min and then it is filtered. The filtrate is evaporated to dryness and dried at 60°C in an oven. Yield: 0.88 g (90%) of **2** with a m.p. of 215°C. ^{1}H-nmr (D_2-DMSO): 2.62 (s, 3H); 7.12 (s, 2H); 8.18–9.33 (m, 4H).

1-Hydroxy-pyrene-tris-3,6,8-octadecylsulfonamide **(3).** Eighty-five hundredths gram (1.53 mmol) of **2** is dissolved in 40 ml of dry dimethylformamide (DMF). This solution is slowly added to 1.24 g (4.6 mmol) of octadecylamine in 80 ml of dry DMF. The resulting mixture is stirred for 1 h and then heated to 90°C for 15 min. After cooling, the product is precipitated with distilled water, filtered, and washed with methanol. Yield: 1.58 g (85%) of **3** with a melting point of 95–97°C. Elemental analysis (calc./found) for $C_{70}H_{121}N_3O_7S_3$ (1212,9): C, 69.32/68.86; H, 10.05/10.14; N, 3.43/3.46; S, 7.93/8.45. Mass spectrum: M^+ 1212 (16%), 403 (100%). Abs. mmax. (MeOH): 413 nm (ε 13,800 M^{-1} cm^{-1}); 390 (sh); 374 (10,600).

Preparation of the Nitrate-Sensitive Membrane

A mixture of 120 mg PVC, 240 mg DOS, 4.6 mg TDMACl, and 3.7 mg HPTS-TOA **(3)** is dissolved in 1.5 ml of THF. This solution (0.1 ml) is spread onto a dust-free 175-μm polyester foil (Mylar, type GA-10, from Du Pont de Nemours & Co, Brussels) using a homemade coating device. The membrane is placed in a THF-saturated atmosphere in an exsiccator. After 1 h, the membrane is placed in ambient air for complete drying. Before measurements, the membrane, whose thickness can be calculated to be 2–4 μm, is placed in a plain buffer solution (10 m*M* NaH_2PO_4, 6.6 m*M* citric acid, and 2.5 m*M* $Na_2B_4O_7$ adjusted to pH 7.6 or 6.6 with 6 *N* H_2SO_4 and 6 *N* NaOH) for conditioning (see Fig. 4).

Apparatus

Fluorescence excitation/emission spectra and the response curves of the membranes were measured on a Aminco SPF 500 spectrofluorophotometer equipped with a 250-W tungsten halogen lamp as a light source. Response curves were recorded by placing the membranes in flow-through cells and pumping the buffer solution at a flow rate of 2 ml/min through the cell. Excitation and emission wavelengths of the response measurements were set to 510 and 550 nm, respectively.

RESULTS

Spectral Properties

The conversion of HPTS into HPTS-TOA (Fig. 1) results in a change of optical properties in that both the excitation and emission maxima are shifted to longer wavelengths. The absorption spectrum of **3** in methanol shows a longwave maximum at 413 nm, which, on addition of 1 *M* NaOH, shifts to 472 nm. This is in accordance with the spectra of HPTS itself. The molar absorbance is moderate and higher for the anion. Spectra and pH effects could not be studied in aqueous solution because of the insufficient solubility of HPTS-TOA in water. Data are summarized in Table I, and spectra are shown in Fig. 2.

Similar to HPTS, the fluorescence of HPTS-TO under 470-nm excitation decreases on decreasing the pH. Embedding HPTS-TO into a lipophilic matrix and addition of TDMACl as the anion carrier results in a pH-dependent sensor membrane. This membrane, on exposure to buffer solutions of varying pH, shows a sigmoidal pH plot (Fig. 3). The apparent pK_a depends on both the fraction of the plasticizer [4,5] and the amount of cationic or anionic carrier added [6].

Sensing Scheme

The anion carrier TDMACl reversibly extracts nitrate into a lipophilic polymer matrix. In order to maintain electroneutrality, the equivalent amount of protons diffuses into the polymer. This coextraction process results in the protonation of a pH indicator and, consequently, an optical signal change which can be correlated with the amount of nitrate in the analyte solution as can be seen in Fig. 4.

Response Behavior

The forward response time (t_{90}) of the anion-sensitive membrane is 3–5 min; the reverse response time is 6–10 min. The response times generally decrease on lowering the fraction of plasticizer. Relative signal changes are as high as 95%. (Fig. 4). The relative signal

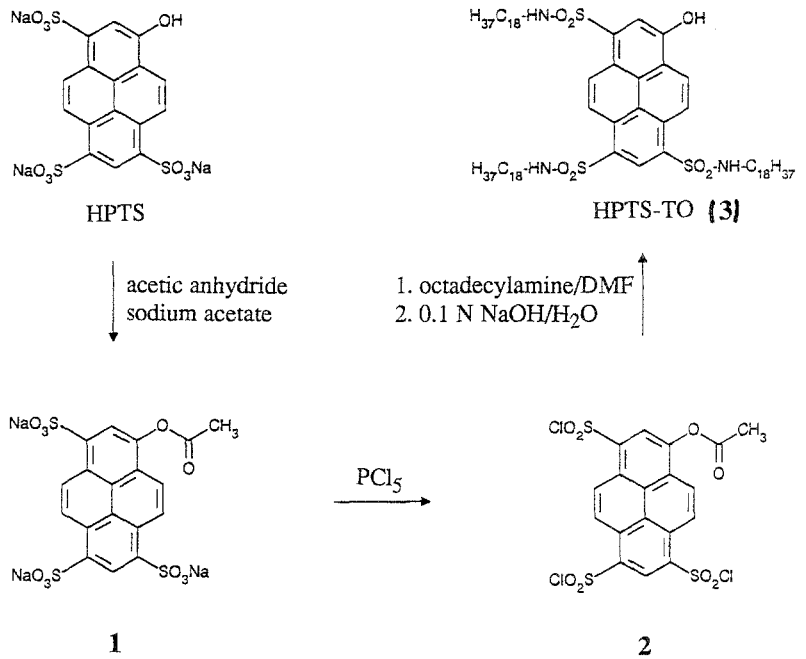

Fig. 1. Synthetic pathway for 1-hydroxy-3,6,8-tris-octadecylsulfonamide (HPTS-TO).

Table 1. Excitation and Emission Maxima of HPTS and HPTS-TO

λ_{max} (nm)	$exc._{acid\ form}$	$em._{acid\ form}$	$exc._{base\ form}$	$em._{base\ form}$
HPTS[a]	403	512[b]	455	512
HPTS-TOA[c]	422	455	521	548

[a]In water.
[b]Anion fluorescence due to photodissociation.
[c]In PVC/DOS.

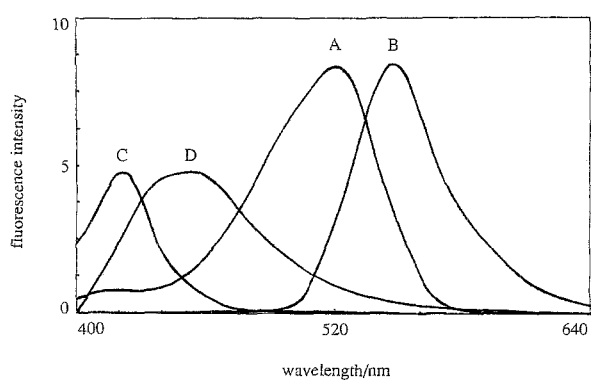

Fig. 2. Excitation and emission spectra of the anion-sensitive membrane. A, Excitation of base form; B, emission of base form; C, excitation of acid form; D, emission of acid form.

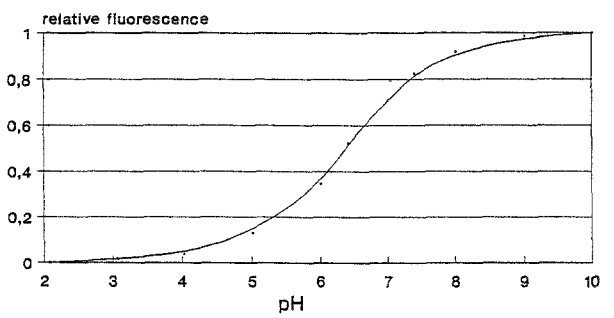

Fig. 3. Fluorescence of HPTS-TOA in PVC/DOS plus TDMACl, exposed to buffer solutions of various pH, composed of 10 m*M* NaH_2PO_4, 6.6 m*M* citric acid, and 2.5 m*M* $Na_2B_4O_7$, and adjusted to the appropriate pH with 6 *N* H_2SO_4 or 6 *N* NaOH.

changes on exposure to concentrations typical for drinking water are −4% for 50 μ*M* (3.1 ppm) nitrate, −18% for 0.5 m*M* (31 ppm) nitrate, and −50% for 5 m*M* (310 ppm) nitrate.

Selectivity and Sensitivity

The response to anions other than nitrate follows the Hofmeister series [7] and has been investigated previously by Simon et al. [2]. Anions that can occur at higher concentrations in drinking water include chloride,

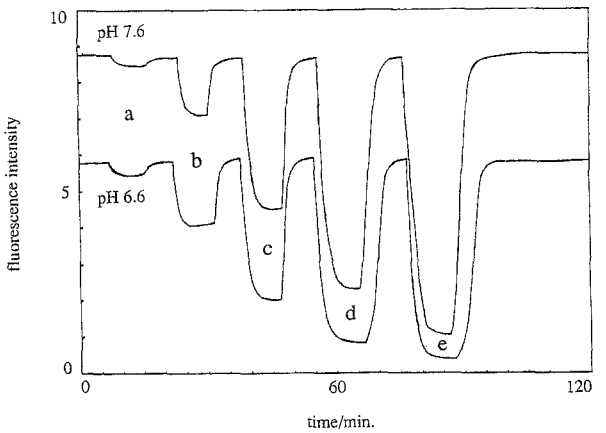

Fig. 4. Signal change, response curve, and reproducibility of the sensor membrane when exposed to various concentrations of nitrate at pH 7.6 and 6.6. a, 50 μ*M*; b, 0.5 m*M*; c, 5 m*M*; d, 50 m*M*; e, 500 m*M* nitrate.

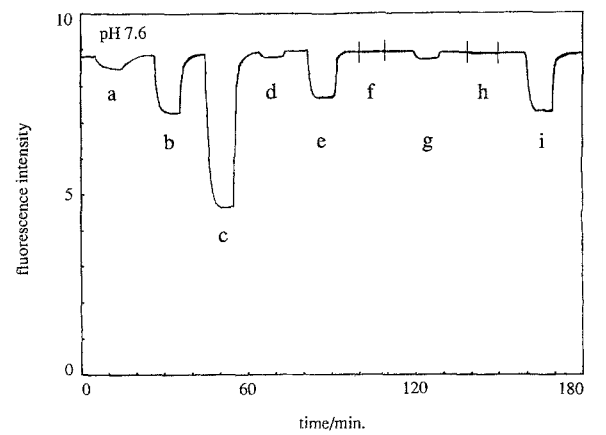

Fig. 5. Response of the membrane toward nitrate (a, 50 μ*M*; b, 0.5 m*M*; c, 5 m*M*), chloride (d, 5 m*M*; e, 50 m*M*), sulfate (f, 5 m*M*; g, 50 m*M*), hydrogen carbonate (h, 50 m*M*), and nitrite (i, 0.5 m*M*) at pH 7.6.

sulfate, and hydrogen carbonate. Figure 5 shows the relative signal change caused by different concentrations of chloride, sulfate, hydrogen carbonate, and nitrite in comparison to various concentrations of nitrate. The relative signal changes are −2% for 5 m*M* and −15% for 50 m*M* chloride, −0% for 5 m*M* and −2% for 50 m*M* sulfate, −0% for 50 m*M* hydrogen carbonate, and −18% for 0.5 m*M* nitrite at pH 7.6. Because the coextraction process is coupled to a proton transfer, pH is the most serious interferent (Fig. 3).

The sensor is capable of detecting 1 ppm nitrate at pH 7.6. The dynamic range is as wide as from 1 to 10,000 ppm, with the best resolution obtained between 30 and 3000 ppm, which is the range of interest when sensing nitrate in drinking water.

CONCLUSION

The sensor material presented here is capable of monitoring nitrate over the 3–1000 mg/L concentration range. Response times are of the order of minutes, and the sensor exhibits sufficient selectivity toward anions commonly encountered in ground and drinking water. However, the sensor is not applicable for monitoring seawater due to limited selectivity toward chloride (~100). Because it is highly pH dependent, the sample pH must be carefully adjusted, or results must be corrected for pH effects.

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