Anal. Chem. **1999,** *71,* 1534−1539

Plasticizer-Free Optode Membranes for Dissolved Amines Based on Copolymers from Alkyl Methacrylates and the Fluoro Reactand ETH^T 4014

Gerhard J. Mohr,*,[†] Nicola Tirelli,[‡] and Ursula E. Spichiger-Keller[†]

Centre for Chemical Sensors, ETHZ Technopark, Technopark Strasse 1, CH-8005 Zurich, Switzerland, and Institute for Polymers, ETH Zürich, Universitätstrasse 6, CH-8092 Zurich Switzerland

A new class of fluorogenic copolymers has been synthesized that reversibly interacts with dissolved aliphatic amines resulting in a change in fluorescence. The copolymers are composed of different methacrylate monomeric units, such as hexyl methacrylate and the dye monomer 4-[*N*,*N*-bis(11-methacroyloxyundecyl)amino]-4′-trifluoroacetylstilbene (ETH^T 4014). Upon exposure to aqueous amine solutions, thin layers of the copolymers show a decrease in fluorescence at a wavelength around 520 nm and an increase in fluorescence at around 420 nm. The change in fluorescence is based on the nucleophilic addition of amines to the trifluoroacetyl group of the reactand, thus causing a change in the degree of electron delocalization. Since the sensor layers are composed of copolymerized components, they exhibit superior operational stability to sensors based on plasticized PVC, and their lack of plasticizers enhances the shelf lifetime.

We have recently introduced a new type of optical sensor based on chromogenic and fluorogenic reactands for the determination of analytes, such as primary amines, alcohols, and humidity.[1−6] Unlike the “ligands” used in ion-selective sensors, which interact by complexing the analyte reversibly, the interaction between the analyte and the reactive compound includes a reversible chemical reaction. Since the reactive host compound is incorporated into a polymer film, the determination of the analyte is feasible in a reagent-free system. Therefore, the reactive host compound is named “reactand” in analogy to “ligands” for complexing agents. Optical sensors have traditionally been prepared with thin layers of plasticized PVC as the polymeric matrix in which the lipophilic reactands were dissolved. However, the inherent disadvantages of plasticizers (such as evaporation, leaching, and phase separation) prompted us to develop a new approach to optical sensor layers in which no plastification at all is needed.

In the past decade, there have been several attempts to use copolymerized compounds in optical sensing. Munkholm et al. made use of acryloylfluorescein copolymerized with acrylamide−methylenebis(acrylamide) for optical sensing of pH.[7] Hisamoto et al. introduced a method to prepare poly(hydroxyethyl methacrylates) (polyHEMA) with copolymerized solvatochromic pH indicator dyes for sensing of pH and the polarity of the sample solution.[8] They copolymerized the components directly on the glass support by exposure to UV light. Ambrose and Meyerhoff developed an optical polyion probe by using photo-cross-linked decyl methacrylate.[9] However, so far there have been no attempts to use copolymers for selective sensing of neutral species such as alcohols or amines.

Chromogenic and fluorogenic molecules with structures comparable to the compounds presented in this paper have been used in recent years due to their nonlinear optical (NLO) properties. Mostly, they were produced by copolymerization of methacrylic dyes with methacrylates.[10−15] The results obtained using NLO copolymers have motivated us to develop optical sensors based on structurally related copolymers. Methacrylates were chosen as the basic monomer materials since they are commercially available and easily synthesized and polymerized. Furthermore, we synthesized a methacrylate derivative of a reactand that is sensitive to nucleophilic species such as amines. However, in contrast to the NLO-based polymers, which require high T_g values for a stable orientation of the dyes, polymers for optical sensors require high elasticity and, therefore, low T_g values. Thus, we have prepared copolymers composed of butyl, hexyl, and dodecyl

---

* Corresponding author: (e-mail) gerhard@chemsens.pharma.ethz.ch, http://www.chemsens.ethz.ch.

[†] Centre for Chemical Sensors.

[‡] Institute for Polymers.

(1) Mohr, G. J.; Demuth, C.; Spichiger, U. E. *Anal. Chem.* **1998**, *70*, 3868.
(2) Mohr, G. J.; Lehmann, F.; Grummt, U.-W.; Spichiger, U. E. *Anal. Chim. Acta* **1997**, *344*, 215.
(3) Mohr, G. J.; Spichiger, U. E. *Anal. Chim. Acta* **1997**, *351*, 189.
(4) Mohr, G. J.; Citterio, D.; Spichiger, U. E. *Sens. Actuators, B* **1998**, *49*, 226.
(5) Mohr, G. J.; Spichiger, U. E. *Mikrochim. Acta* **1998**, *130*, 29.
(6) Spichiger-Keller, U. E. *Chemical Sensors and Biosensors for Medical and Biological Applications*; Wiley VCH: Weinheim, Germany, 1998.
(7) Munkholm, C.; Walt, D.; Milanovich, F. P.; Klainer, S. M. *Anal. Chem.* **1986**, *58*, 1427.
(8) Hisamoto, H.; Manabe, Y.; Yanai, H.; Tohma, H.; Yamada, T.; Suzuki, K. *Anal. Chem.* **1998**, *70*, 1255.
(9) Ambrose, T. M.; Meyerhoff, M. E. *Anal. Chem.* **1997**, *69*, 4092.
(10) S'Heeren, G.; Persoons, A.; Rondou, P.; VanBeylen, M.; Samyn, C. *Eur. Polym. J.* **1993**, *29*, 975.
(11) Altomare, A.; Ciardelli, F.; Ghiloni, M. S.; Solaro, R.; Tirelli, N. *Macromol. Chem. Phys.* **1997**, *198*, 1739.
(12) Dworczak, R.; Fabian, W. M. F.; Kieslinger, D.; Junek, H. *Dyes Pigments* **1997**, *34*, 13.
(13) Brown, D.; Natansohn, A.; Rochon, P. *Macromolecules* **1995**, *28*, 6116.
(14) Zhao, M.; Bautista, M. O.; Ford, W. T. *Macromolecules* **1991**, *24*, 844.
(15) Tirelli, N.; Suter, U. W.; Altomare, A.; Solaro, R.; Ciardelli, F.; Follonier, S.; Bosshard, Ch.; Günter, P. *Macromolecules* **1998**, *31*, 2152.

10.1021/ac9812983 CCC: $18.00

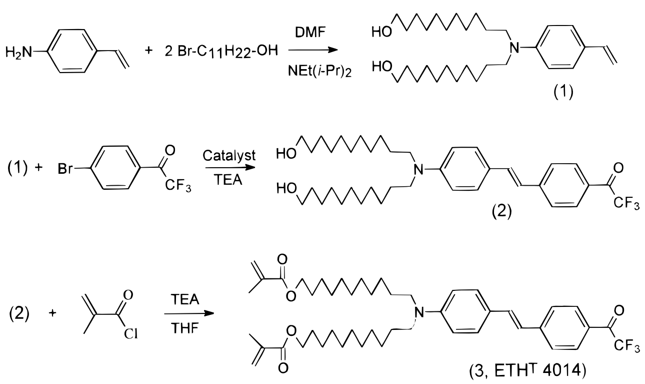

Figure 1. Synthesis of the fluorogenic monomer 4-[*N*,*N*-bis(11-methacroyloxyundecyl)amino]-4′-trifluoroacetylstilbene (ETH^T 4014).

methacrylates, rather than methyl methacrylate. The optical sensors obtained by copolymerization were investigated for their sensitivity to dissolved amines, as well as for their selectivity, stability, and response. Amines were the target analytes because a wide range of amines are pollutants in the pharmaceutical and chemical industry. They are used in the preparation of fertilizers, pharmaceuticals, surfactants, and colorants and can be found in agricultural areas. The presence of amines may also be indicative of food quality since they are produced during the degradation of fish products.

EXPERIMENTAL SECTION

Reagents. All reagents were of synthetic reagent grade. Flash chromatography was performed with silica gel 60 (63−200 μm) from Fluka (Buchs, Switzerland). *n*-Butyl methacrylate and *n*-dodecyl methacrylate were obtained from Fluka. *n*-Hexyl methacrylate came from Polysciences, Inc. (Warrington, PA).

Tyramine [4-(2-aminoethyl)phenol], spermidine [*N*-(3-aminopropyl)-1,4-diaminobutane], cadaverine (1,5-diaminopentane), histamine [2-(4-imidazolyl)ethylamine], and phenethylamine (2-phenylethylamine) were of analytical grade and were obtained from Fluka. Amine solutions were prepared by dissolving the appropriate amount of each amine in 0.1 M sodium hydroxide solution. Due to the high pH of 13.0, the amines were mainly present in the electrically neutral form and not in the ammonium form. The correct amine concentrations were calculated by using the Henderson−Hasselbach equation and the pK_a value of each amine.[17] The selectivity of the sensor layers to the different amines was calculated according to ref 1.

Synthesis of the Fluororeactand (see Figure 1). *N*,*N*-Bis-(11-hydroxyundecyl)-4-vinylaniline (1). A mixture of 4.0 g (30 mmol) of vinylaniline (90% purity), 15.2 g (60 mmol) of 11-bromo-1-undecanol, 15.0 g (116 mmol) of *N*-ethyldiisopropylamine, and 20 mL of dimethylformamide was stirred at 110 °C for 24 h under argon atmosphere. After cooling to room temperature, the product was poured onto 100 mL of distilled water, leading to a separation of the product from the aqueous phase. The product was put into 100 mL of dichloromethane, washed twice with distilled water, and dried over magnesium sulfate. After evaporation, the resulting oil was purified by flash chromatography using *n*-hexane/ethyl acetate (2:1 v/v) as the eluent, yielding 9.0 g of a pale yellow liquid: ^{1}H NMR (CDCl$_3$) δ (ppm) 7.27 (m, 2 H, =CH), 6.63 (d, 1 H, =CH), 6.57 (m, 2 H, =CH), 5.53 (d, 1 H, =CH), 4.96 (d, 1 H, =CH), 3.63 (t, 4 H, CH$_2$), 3.24 (t, 4 H, CH$_2$), 1.58 (m, 8 H, CH$_2$), 1.32 (m, 28 H, CH$_2$). Calcd for $C_{30}H_{53}NO_2$ (459.75): C, 78.37; H, 11.62; N, 3.05. Found: C, 78.20; H, 11.42; N, 3.03.

4-[*N*,*N*-Bis(11-hydroxyundecyl)amino]-4′-trifluoroacetylstilbene (2). A mixture of 2.11 g (4.6 mmol) of **1**, 1.17 g (4.6 mmol) of 4-bromo-α,α,α-trifluoroacetophenone, 10 mg of palladium diacetate, 25 mg of tri-*o*-tolylphosphine, and 4 mL of triethylamine was heated at 115 °C for 22 h in a capped heavy-wall Pyrex tube that was flushed with dry nitrogen. Water and dichloromethane were added to the cooled solution, and the aqueous layer was extracted twice with 100 mL of dichloromethane. The combined dichloromethane solutions were washed three times with distilled water, dried over magnesium sulfate, and evaporated to dryness. After purification by flash chromatography (*n*-hexane/ethyl acetate, 2:1 v/v), a pure fraction of 2.1 g of **2** as an amorphous red solid, mp 53−57 °C was obtained: ^{1}H NMR (CDCl$_3$) δ (ppm) 8.03 (d, 2 H, =CH), 7.58 (d, 2 H, =CH), 7.44 (d, 2 H, =CH), 7.25 (d, 1 H, =CH), 6.88 (d, 1 H, =CH), 6.63 (d, 2 H, =CH), 3.64 (m, 4 H, −CH$_2$), 3.31 (m, 4 H, −CH$_2$), 2.17 (s, 2 H, OH), 1.58 (m, 8 H, CH$_2$), 1.32 (m, 28 H, CH$_2$). Calcd for $C_{38}H_{56}NO_3F_3$ (631.86): C, 72.23; H, 8.93; N, 2.22. Found: C, 72.38; H, 9.07; N, 2.31.

4-[*N*,*N*-Bis(11-methacryloxyundecyl)amino]-4′-(trifluoroacetyl)stilbene (3; ETH^T 4014). A solution of 0.15 g of methacryloyl chloride in 0.4 mL of tetrahydrofuran (THF) was added drop by drop to a mixture of 0.32 g (0.5 mmol) of **2**, 0.28 g of triethylamine, 2.5 mg of hydroquinone, and 6 mL of dry THF (cooled to 0 °C) and stirred under nitrogen. The reaction mixture was heated to 45 °C for 4 h. Then, a solution of 0.15 g of methacryoyl chloride in 0.4 mL of THF was added at 0 °C and the reaction mixture was kept at 45 °C for 12 h. After evaporation of the tetrahydrofuran, the residue was dissolved in dichloromethane and washed once with saturated aqueous sodium hydrogen carbonate solution and three times with water. The resulting solution was dried over sodium sulfate, and the solvent was removed under reduced pressure. The remaining liquid was purified by flash chromatography (*n*-hexane/ethyl acetate, 95:5 v/v), yielding 0.24 g of the pure viscous compound: ^{1}H NMR (CDCl$_3$) δ (ppm) 8.05 (d, 2 H, =CH), 7.58 (d, 2 H, =CH), 7.44 (d, 2 H, =CH), 7.24 (d, 1 H, =CH), 6.88 (d, 1 H, =CH), 6.63 (d, 2 H, =CH), 6.10 (s, 2 H, =CH), 5.55 (d, 2 H, =CH), 4.17 (t, 4 H, CH$_2$), 3.28 (m, 4 H, CH$_2$), 1.93 (s, 6 H, CH$_3$), 1.58 (m, 8 H, CH$_2$), 1.30 (m, 28 H, CH$_2$). Calcd for $C_{46}H_{64}NO_5F_3$ (768.01): C, 71.94; H, 8.40; N, 1.82. Found: C, 72.06; H, 8.52; N, 1.82.

Copolymer Synthesis. CP1, CP2, and CP3. The copolymerizations were carried out in degassed acetone or benzene solutions in an argon atmosphere at 60 °C in the presence of 2,2′-azobisisobutyronitrile (AIBN). For **CP1**, 30 mmol of freshly distilled butyl methacrylate, 30 mg of AIBN, and 9.2 mg of ETH^T 4014 were dissolved in 30 mL of acetone, and the resultant mixture was refluxed for 23 h. In the case of **CP2**, 30 mmol of freshly distilled hexyl methacrylate, together with 11 mg of ETH^T 4014 in acetone, were used. For **CP3**, 16.4 mg of ETH^T 4014, and 30 mmol of dodecyl methacrylate were reacted in benzene. The resulting copolymer solutions were cooled to room temperature

---

(16) Spichiger, U. E.; Demuth, C. *Anal. Chim. Acta* **1997**, *355*, 259.

(17) Lide, D. R. *Handbook of Chemistry and Physics*, 74th ed.; CRC Press: Boca Raton, 1993−1994; Vol. 8, pp 43−45.

Table 1. Compositions of the Chromogenic Copolymers CP1−CP3

copolymer	monomer	ETH^T 4014 (%)	T_g (°C)	M_n	M_w/M_n
CP1	butyl methacrylate	0.21	41	42 000	2.1
CP2	hexyl methacrylate	0.18	−4	53 000	2.7
CP3	dodecyl methacrylate	0.13	< −20	120 000	3.7

and poured into 150 mL of *n*-hexane (**CP1**), acetonitrile (**CP2**), or acetone (**CP3**) to precipitate the copolymers. The solvent was decanted, and the copolymers were redissolved and reprecipitated twice in the respective solvents. The dye content was calculated by dissolving a defined amount of the copolymer in di-*n*-butyl ether and correlating the observed absorbance with the molar absorbance of ETH^T 4014 in di-*n*-butyl ether. The amounts of ETH^T 4014 in the copolymers, **CP1**−**CP3**, were then found to be 0.21, 0.18, and 0.13 wt %, respectively (Table 1).

Membrane Preparation. Sensor membranes **M1**−**M3** were obtained by dissolving 100 mg of the corresponding copolymers **CP1** and **CP2** in 1.0 mL of ethyl acetate, and **CP3** in 1.0 mL of cyclohexane. A dust-free glass plate was placed in a spin-coating device[18] with a solvent-saturated atmosphere. Then, 0.3 mL of the solution was transferred onto the rotating glass support. The resulting membranes were placed in ambient air for drying.

A peeling-off of the copolymer layers was observed after several hours of measurement in the flow-through cell, especially in the case of **M1**. Therefore, glass plates cleaned in a mixture of concentrated sulfuric acid and 30% hydrogen peroxide (3:1 v/v) were silanized by exposing them to hexamethyldisilazane vapor for 15 h in a desiccator at room temperature and at ambient pressure. After the silanization process, the lipophilic copolymer layers adhered well to the lipophilized glass plates and no peeling-off upon exposure to aqueous solutions was observed. The thickness of the layers was found to be 0.65, 0.30, and 0.15 μm, respectively, for **M1**−**M3**. The partial diol formation of the copolymer layers upon exposure to water requires conditioning of the sensor layer in aqueous solution before measuring dissolved aqueous amines.

Apparatus. Melting points were measured on a Büchi melting point apparatus (Flawil, Switzerland) and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL 200 Gemini spectrometer with chemical shifts given in δ (ppm) relative to TMS. The fluorescence measurements were performed on a Perkin-Elmer LS 50 B spectrofluorometer by using a specially designed flow-through cell[16] and pumping the sample solutions at a flow rate of 1.5 mL min^{-1} using a Perpex peristaltic pump (Jubile, Switzerland). The differential scanning calorimetry (DSC) measurements were performed on a Perkin-Elmer DSC 7; all the samples were first heated to 140 °C and then the inflection points in a second heating scan at 10°/min were reported as glass transition temperature values with an accuracy of ±0.5 °C. The molecular weights and molecular weight distributions were determined using polystyrene standards in THF polymer solutions by GPC on a Knauer HPLC System with a PLGel mixed C 5-μm column, a viscosimetry detector (Viscotek Differential model 502), a differential refractometry detector (Knauer DRI), and a small-angle light scattering detector (Chromatix KMX-6). The thickness of the sensor layers on the glass plates was measured by using an Alpha-Step 200 surface profiler from Tencor Inst. (Mountain View, CA) with an accuracy of 0.05 μm.

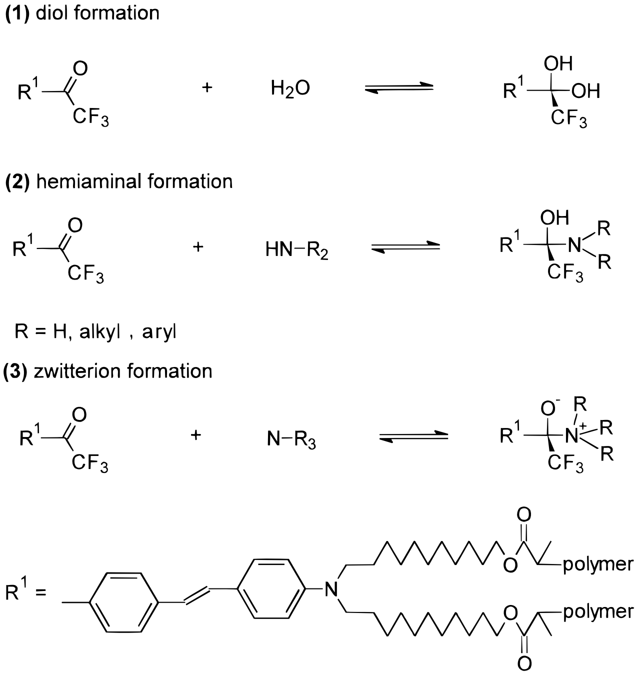

Figure 2. Schematic representation of the chemical reactions of trifluoroacetophenone derivatives with water and amines.

RESULTS AND DISCUSSION

Optical Properties of the Fluorogenic Reactand ETH^T 4014 in Solution and in the Copolymer. The synthesis of the reactand was performed via alkylated 4-vinylaniline, which was coupled to 4-bromo-α,α,α-trifluoroacetophenone by a palladium-catalyzed Heck reaction (Figure 1). This synthetic procedure provided the methacrylate derivative of a reactand within a simple three-step reaction. This finding contradicts the claim that such a synthesis would be impossible because 4-vinylaniline derivatives polymerize too readily during palladium-catalyzed coupling.[19] The reactand resulting from the synthetic procedure consisted of a stilbene chromophore and terminal donor−acceptor substituents. The alkylamino group served as an electron donor, and the long alkyl chains attached to the nitrogen atom were used for the introduction of the polymerizable methacrylate groups. The acceptor part of the fluorescent reactand was the trifluoroacetyl group, where the C-nucleus is attacked selectively by nucleophilic reagents, such as alcohols and amines (see Figure 2). The change in fluorescence intensity of ETH^T 4014 was based on the nucleo-

---

[18] Seiler, K. *Ionenselektive Optodenmembranen*; Fluka Chemie AG: CH-9470 Buchs, Switzerland, 1991.

[19] Hassner, A.; Birnbaum, D.; Loew, L. M. *J. Org. Chem.* **1984**, *49*, 2546.

Table 2. Optical Properties of ETH^T 4014 in Solution

solvent	λ_{max} (abs) (nm)	ϵ (L mol^{-1} cm^{-1})	λ_{max} (em) (nm)	$E_T(30)$ (molar transition energy) (kcal mol^{-1})[20]
cyclohexane	430	27 000	487	30.9
di-*n*-butyl ether	436	34 300	523	33.0
toluene	441	23 900	530	33.9
diethyl ether	437	35 600	545	34.5
ethyl acetate	444	34 800	597	38.1

Table 3. Optical Properties and Response Characteristics of Copolymer Membrane Layers, M1−M3

polymer	λ_{max} (nm) (HAM)[a]	λ_{max} (nm) (TFA)[b]	max sensitivity to 1-butylamine (mM)	LOD (mM)	forward response[c] (min)	reverse response[d] (min)
M1	422	528	2.5−250	0.8	4−8	8−15
M2	421	522	3.1−310	1.0	6−10	10−15
M3	420	514	5.0−500	1.6	8−12	15−20

[a] Emission maximum of the hemiaminal (HAM) form of the fluoro reactand. [b] Emission maximum of the trifluoroacetyl (TFA) form of the fluoro reactand. [c] Time for 95% of the signal change to occur in changing from lower to higher amine concentrations (Δc typically 50 mM). [d] Time for 95% of the signal change to occur in changing from higher to lower amine concentrations (Δc typically 50 mM).

philic addition of amines to the trifluoroacetyl group of the reactand, thereby forming a hemiaminal or zwitterion and bringing about a change in the degree of electron delocalization.

The structurally related reactands ETH^T 4003 and 4004, which have already been used in plasticized PVC layers, were shown to exhibit a pronounced solvatochromism. Similar behavior was found for the methacrylate derivative ETH^T 4014 (Table 2). This behavior allowed us to investigate the decreasing polarity of the copolymers by comparing the fluorescence emission maximums of the copolymers with the reactand in solution. The emission maximums decreased from 528 to 522 and 514 nm, respectively, in going from butyl and hexyl methacrylate to dodecyl methacrylate. These values were at shorter wavelengths than the maximum of ETH^T 4014 in PVC plasticized by bis(2-ethylhexyl)sebacate (561 nm). The fluorescence maximums of the copolymers were comparable to the emission maximums of the reactand in toluene (530 nm) and di-*n*-butyl ether (523 nm) and, consequently, gave an indication of the low polarity inside the copolymer layer (Tables 2 and 3). The well-known $E_T(30)$ scale, based on the molar transition energies of the pyridinium-*N*-phenoxide betaine dye, which was developed by Dimroth, Reichardt, and co-workers, is helpful in discussing solvent polarity in the vicinity/microenvironment of a solvatochromic dye.[20] It constitutes a more comprehensive measure of polymer polarity than the dielectric constant of single components since it reflects more reliably the complete picture of all intermolecular forces acting between solute and solvent. When the observed fluorescence maximums of ETH^T 4014 in different solvents (see Table 2) were linearly correlated with the $E_T(30)$ values of the respective solvents, then the $E_T(30)$ values for the membrane bulk using ETH^T 4014 as an indicator were calculated to be 33.5 kcal mol^{-1} in **M1**, 33.1 kcal mol^{-1} in **M2**, and 32.6 kcal mol^{-1} in **M3**.

Performance of Amine-Sensitive Membranes M1−M3. The fluorescence excitation maximums of the fluorogenic reactand in the copolymers **M1**−**M3** were all located at around 453 and 468 nm, whereas the emission maximums were at 528 nm in the case of **M1**, and at 522 nm for **M2** and 514 nm for **M3** (Table 3). Exposure to aqueous amines resulted in a decrease in fluorescence intensity of the reactand due to the conversion of the trifluoroacetyl group of the stilbene into a hemiaminal (Figure 2). At the same time, the conversion of the reactand resulted in an increase in the fluorescence of the hemiaminal form of the stilbene with an excitation maximum at ~390 nm and an emission maximum at ~420 nm (Table 3, Figure 3). Since the fluorescence intensity of the hemiaminal was at a shorter wavelength, the fluorescence of the trifluoroacetyl form was chosen for further investigations.

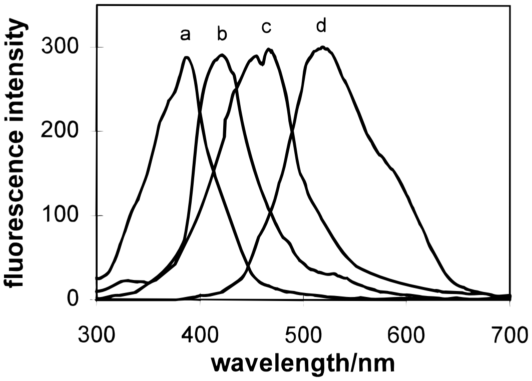

Figure 3. Fluorescence excitation and emission spectra of **M2** after (a, b) and before (c, d) contact with 500 mM 1-butylamine at pH 13.0. When changing from 0.1 M NaOH to aqueous 1- butylamine, the trifluoroacetyl form (c, d) is converted into the hemiaminal (a, b).

The fluorescence emission of all three copolymer membranes (**M1**−**M3**) decreased significantly at ~530 nm on exposure to solutions containing 1-butylamine (when excited at 450 nm) and was fully reversible. Due to the favorable mechanical stability and low T_g value, **M2** was characterized in more detail. The relative decrease in the fluorescence of **M2** in changing from 0 to 0.5 M 1-butylamine was 90%. The forward response time, t_{95}, was in the

---

(20) Reichardt, C. *Solvents and solvent effects in organic chemistry*, 2nd ed.; Wiley VCH: Weinheim, Germany, 1990.

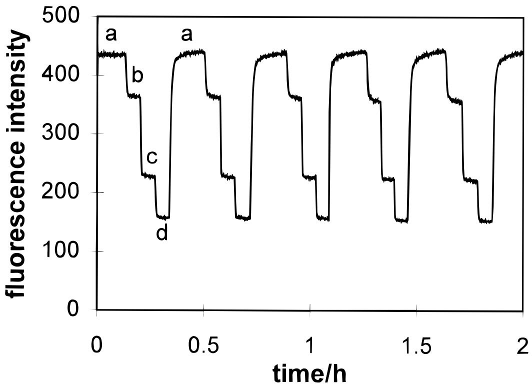

Figure 4. Short-time repeatability of the response of **M2** on exposure to 1-butylamine: a, 0.1 M sodium hydroxide solution; b, 10 mM 1-butylamine; c, 50 mM 1-butylamine; d, 100 mM 1-butylamine (at pH 13.0), showing the reproducibility and the response time measured at an excitation and emission wavelength of 450 and 520 nm, respectively.

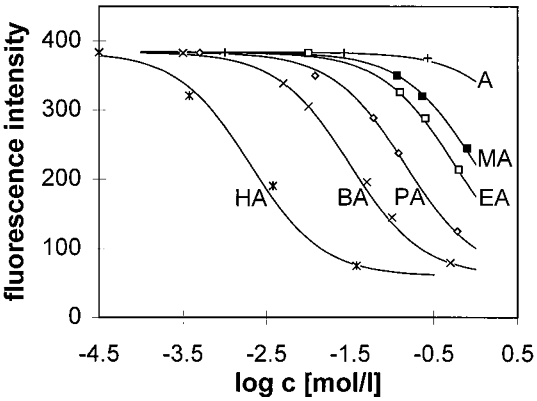

Figure 5. Response function of **M2** on exposure to aqueous amines at pH 13.0 measured at an excitation wavelength of 450 nm and an emission of 520 nm: (A) ammonia; (MA) methylamine; (EA) ethylamine; (PA) 1-propylamine; (BA) 1-butylamine; (HA) 1-hexylamine. The solid lines represent the response functions calculated according to ref 1, which gives the symbols for the experimental results.

Table 4. Selectivity (log K^{opt}) of M1−M3 for Amines, Ethanol, and Carbonate

analyte	**M1**	**M2**	**M3**
1-hexylamine	0.0	0.0	0.0
1-butylamine	−1.2	−1.2	−1.2
1-propylamine	−1.8	−1.9	−1.9
ethylamine	−2.3	−2.4	−2.5
methylamine	−2.6	−2.7	−2.8
ammonia	−3.5	−3.5	−3.7
diethylamine	−2.7	−2.9	−2.9
triethylamine	−2.1	−2.1	−2.0
aniline	−1.7	−1.8	−1.8
ethanol	−4.2	−4.2	−4.2
carbonate	<−4.5	<−4.5	<−4.5
spermidine		−3.5	
histamine		−3.8	
cadaverine		−2.1	
tyramine		−2.5	
benzylamine		−0.9	
phenethylamine		−0.8	

range of 6−10 min, whereas the time for the reverse response was in the range of 10−15 min. The short-time reproducibility of **M2** is shown in Figure 4. The relative standard deviations for 10, 50, and 100 mmol 1-butylamine were found to be 1.9%, 1.3%, and 1.4% ($n = 20$), respectively, during 48 h of continuous measurement. The limit of detection (LOD) of **M2** for 1-butylamine was 1.0 mM. The selectivity of **M2** for primary amines is shown in Figure 5 and the log K^{opt} values (logarithm of the relative selectivities between the interfering amines obtained by calculating the horizontal distance between the amine response curves at the point of inflection; see also Figure 5)[1] in Table 4. As has been shown for amine sensor layers based on ETH^T 4001 and 4004, exposure to water results in the partial formation of a diol which brings about small changes in both absorbance and fluorescence.[1] However, due to the significantly higher nucleophilicity of amines, exposure to aqueous amines results in the formation of a hemiaminal or zwitterion. This causes a large decrease in absorbance and fluorescence of the trifluoroacetyl form of the reactand as well as an increase in the hemiaminal form.

M2 exhibited neither bleaching of the dye, chemical decomposition, nor peeling-off of the copolymer layer from the glass support for a period of longer than 6 months when stored in the dark at room temperature. The membrane exhibited remarkable operational stability, and no leaching of the copolymerized ETH^T 4014 was observed during 1 week of continuous measurement.

The sensitivity to the amines was mainly governed by the balance between lipophilicity and sterical hindrance at the reactive trifluoroacetyl site. The first factor determined the distribution of the amines between the polymer and the aqueous phase, and the second factor influenced the ease of reaction. Consequently, lipophilic primary amines easily interacted with the dye, whereas the signal changes with bulky secondary and tertiary amines were much lower. The sensitivity of **M1**−**M3** to primary amines decreased with decreasing polarity of the copolymer. In going from **M1** to **M3**, the points of inflection of the calibration plots to 1-butylamine (not shown) shifted from 25 to 31 and 50 mM, respectively (compare the figures for sensitivity in Table 3). The copolymer membranes also showed large signal changes upon exposure to aromatic amines. In the case of aniline, it was assumed that fluorescence quenching accounted for the enhanced sensitivity because not only was the fluorescence of the trifluoroacetyl form decreased but the fluorescence of the hemiaminal form was quenched and was much smaller than in the case of the aliphatic amines. This behavior has already been observed with amine sensor membranes based on trifluoroacetyl dyes in plasticized PVC.[1]

The sensitivity of **M2** to biogenic amines was very low in the case of histamine, spermidine, tyramine, and cadaverine, although spermidine exhibited three amino groups. We assume that the high polarity of the amines prevented their extraction from the aqueous phase into the highly unpolar copolymer membranes. However, **M2** was extremely sensitive to the more lipophilic and unpolar benzylamine and phenethylamine. In the case of tyramine, the high pH of the sample solution deprotonated the phenolic hydroxy group, and the resulting anionic molecule was not extracted into the lipophilic polymer matrix. This lack of sensitivity to the anion was due to the lack of lipophilic cationic sites within the membrane (e.g., lipophilic ammonium compounds), which would compensate for the extracted anion and enable the anion exchange.

Cross-Sensitivity of M1−M3 to Carbonate and Ethanol. All three copolymer membranes were investigated for their selectivity to potential interferences. Trifluoroacetophenone derivatives are well-known for their selective interaction with carbonate in ion-selective electrodes[21] and they respond to alcohols.[2-4] Consequently, the response of **M1**−**M3** to a 0.1 M solution of carbonate at pH 13.0 was investigated. No signal change was observed. All three membranes exhibited a significant response to ethanol, albeit with more preference for amines than ethanol due to the higher nucleophilicity of amines (Table 4). The sensitivity of membranes to alcohols is low compared with the sensitivity to amines. Whereas the sensor membranes used for ethanol measurement respond to ethanol in the molar range,[3,4] the response of **M1**−**M3** to amines is in the millimolar range. Furthermore, the sensor membranes for ethanol only show sufficient response in the presence of a catalyst albeit at neutral pH. In the present investigation, no such catalyst was used.

There is a significant effect of pH in that the concentration of amines in aqueous solution is pH-dependent. The pK_a value of aliphatic amines is generally ∼10.5.[17] At higher pH, virtually all amines are present in the electrically neutral form and large changes in the signals of the sensor membranes on exposure to amines are observed. At lower pH values than 8.0, there is no response to amines because they are present in the "unreactive" protonated ammonium form. Nevertheless, the presence of basic amines in water can increase the pH up to ∼12 unless the analyte solutions are buffered, and thus, they can be investigated by the presented sensor membranes.

Comparison between Copolymerized Reactands and Reactands in Plasticized PVC. Generally, copolymers with good flexibility and low T_g values are obtained by using methyl methacrylate and adding different amounts of alkyl acrylates. The latter lowers the T_g of the resulting copolymers and increases the flexibility of the material. The disadvantage of such an approach is that the polymerization reactivity of methacrylates and acrylates is different, producing copolymers whose composition may be different from the composition of the monomer feed.[22] Thus, the products of the copolymerizations may exhibit unpredictable properties. As a consequence, we chose to prepare copolymers from single methacrylates whose T_g values are already low, i.e., butyl, hexyl, and dodecyl methacrylate. These monomers were then copolymerized with small amounts of ETH^T 4014 (typically 0.1−0.2%), resulting in copolymers whose physical properties were practically undisturbed by the presence of the reactand. This meant that the properties of the copolymers (such as the T_g value) were predictable.

In summary, the response behavior of membranes incorporating the reactands linked to the polymer (**M1**−**M3**) is comparable to the structurally related reactands (ETH^T 4001 and 4004) dissolved in PVC/bis(2-ethylhexyl) sebacate. However, immobilizing the reactands brings about response times that are by a factor of 1.5−2 slower although **M1**−**M3** are ∼10 times thinner than layers from plasticized PVC. The forward response of **M1**−**M3** was in the range of 4−12 min and the reverse response within 8−20 min. The response of membranes composed of ETH^T 4004 and PVC plasticized with bis(2-ethylhexyl)sebacate was 5−7 min for the forward and 5−10 min for the reverse response. At the same time, the LODs decreased: the LOD of PVC-based membranes for 1-butylamine was 3 mM, whereas the LOD of **M2** for the same analyte was 1 mM. The selectivity pattern of **M1**−**M3** was similar to membranes based on plasticized PVC, with a preference for primary amines over secondary and tertiary amines.

However, the copolymer-based sensor layers exhibited several advantages over PVC-based membranes, namely: (a) all components were copolymerized and thus no leaching was observed; (b) due to the covalent linkage, no crystallization, migration, or aggregation of the dye was found, a behavior often observed with optical sensor membranes based on plasticized PVC; (c) the copolymers did not require plasticizers, thus enhancing the operational and shelf lifetimes, and (d) the present approach is not limited to sensing neutral analytes because copolymerizable additives, such as methacrylate derivatives of quaternary ammonium ions and borates, are available.[23,24] These cationic and anionic sites are necessary for the proper functioning of ion-selective optodes based on the mechanisms of ion exchange and coextraction. Consequently, selective ion sensors with high operational and shelf lifetimes are conceivable.[6]

The mechanical stability of membranes based on copolymers and of those based on plasticized PVC is comparable. The only significant difference is the lower adherence of the copolymers on glass plates, due to the copolymers' high hydrophobicity. The problem can be overcome by silanization of the glass supports, and the use of supports such as polyester or transparent polymethacrylates is also conceivable.

The fluorescent reactands ETH^T 4003 and 4004 have already been shown to respond to amines in organic solvents,[1] but since the copolymer layers **M1**−**M3** are soluble in almost any organic solvent except water and alcohols, they cannot be applied for monitoring amines in organic solvents. An application of copolymers for amine-sensing in organic solvents will, however, be feasible by preparing highly cross-linked copolymer layers which are insoluble in organic solvents.[8,23,24]

ACKNOWLEDGMENT

We thank Bosch Telecom GmbH, Germany, for their financial support for part of the work. We also thank Profs. Elizabeth A. H. Hall (University of Cambridge, Institute of Biotechnology) and Renate Dworczak (Karl-Franzens Universität, Graz, Österreich) for stimulating discussions.

Received for review November 24, 1998. Accepted February 2, 1999.

AC9812983

---

(21) Behringer, C.; Lehmann, B.; Haug, J. P.; Seiler, K.; Morf, W. E.; Hartman, K.; Simon, W. *Anal. Chim. Acta* **1990**, *233*, 41.

(22) Mohr, G. J.; Tirelli, N.; Lohse, C.; Spichiger, U. E. *Adv. Mater.* **1998**, *10*, 1353.

(23) Antonisse, M. M. G.; Lugtenberg, R. J. W.; Egberink, R. J. M.; Engbersen, J. F. J.; Reinhoudt, D. N. *Anal. Chim. Acta* **1996**, *332*, 123.

(24) Brunink, J. A. J.; Lugtenberg, R. J. W.; Brzozka, Z.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Electroanal. Chem.* **1994**, *378*, 185.