Effect of the Sol-Gel Matrix on the Performance of Ammonia Fluorosensors Based on Energy Transfer

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We report on the effect of organomodified sol-gel materials on fluorosensors for ammonia. The fluorosensors are based on ion pairs composed of an inert fluorophore and a pH-sensitive absorber dye and are embedded in sol-gel glass. Upon exposure to ammonia, deprotonation of the pH-sensitive dye bromophenolblue occurs, and consequently, energy is transferred from the fluorophore rhodamine B or tetramethylrhodamine ethyl ester to the absorber. The response of the fluorosensors using different ratios of precursors, such as tetramethoxysilane and phenyltrimethoxysilane, is investigated. Detection limits for sol-gel layers composed of 50% tetramethoxysilane and 50% phenyltrimethoxysilane are as low as 0.1 mg/L of aqueous ammonia. Response times are of the order of 3 to 6 min for forward response. The reversibility of the sensor is related to the composition of the organically modified sol-gel glasses and is fastest for sensor layers composed of pure phenyltrimethoxysilane. Conditioning, regeneration, and storage of the layers are shown to be of vital importance for the performance of the sensor layers.

INTRODUCTION

Sol-gels in sensor applications offer several interesting features in that (a) they are optically transparent from the UV to the near-infrared, (b) their microstructure can be controlled, (c) they provide a perm selectivity in that only small ions and molecules can enter its network, and (d) they are chemically and mechanically stable. Most sol-gel techniques use water and low molecular weight alkoxysilanes such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) as sol-gel precursors. The entrapment of various indicators in porous, optically clear glasses prepared by sol-gel techniques

was reported [1–7]. However, there are no in-depth investigations on the use of sol-gel glasses which are organically modified and contain lipophilic alkyl or aryl groups.

The first integrated optic ammonia sensor based on evanescent field absorption was reported by Klein *et al.* [8]. The sensitive element of this sensor was a multimode strip waveguide spin-coated with a sol-gel-immobilized indicator such as bromocresol purple. The dye was incorporated in a porous SiO_2 matrix fabricated by the sol-gel technique; 5 mg/L ammonia was detectable, but only if the relative humidity was higher than 85%. The near-infrared absorbing dye oxazine 170 was used to measure ammonia and acids via optical waveguides [9]. Simon *et al.* reported on near-infrared absorbing dyes for sensing gaseous ammonia [10]. However, the sensors suffered from low stability in the silica matrix and poor reversibility. Fluorescein and rhodamine 6G doped sol-gel optical fibers as platforms for chemical and biosensors were published by Narang

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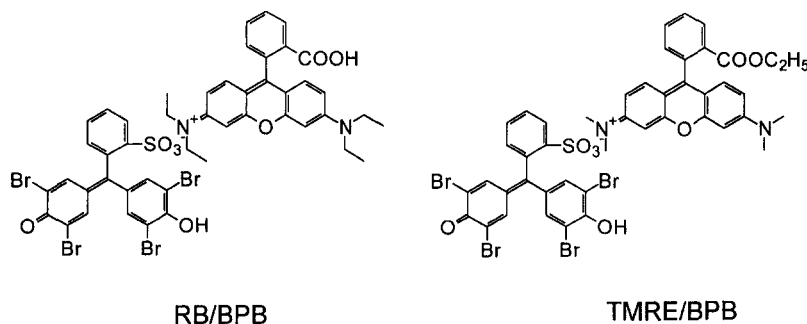[4] To whom correspondence should be addressed.

Fig. 1. Chemical structure of the RB/BPB and the TMRE/BPB ion pair.

et al. [11]. The fluorescein doped sol-gel fiber responded to ammonia and acid vapors within 12 s. Recently, optical sensors based on fluorescence energy transfer for the measurement of pH, carbon dioxide, and ammonia were reported [12–14]. They made use of the phenomenon that fluorescence energy transfer occurred from an inert fluorophore (donor) to an analyte-sensitive absorber (acceptor) when the emission of the fluorophore overlapped with the absorbance of the absorber molecule. These sensors measured both analyte-dependent changes in fluorescence intensity and in fluorescence lifetime.

In this work, we similarly made use of fluorescence energy transfer between fluorescent rhodamines and bromophenol blue to measure ammonia in aqueous solution. The effect of the organomodified sol-gel matrix on the characteristics of the ammonia sensors was investigated in detail. Furthermore, storage, conditioning, and regeneration were shown to exert a significant effect on the sensor performance.

EXPERIMENTAL

Apparatus

Fluorescence measurements were performed using an Aminco SPF 500 spectrofluorimeter equipped with a 250-W tungsten halogen lamp as the light source and a red sensitive detector. Sensor layers were mounted in the fluorimeter and fluorescence intensity was measured at excitation/emission wavelengths of 560/580 nm [15]. Buffered solutions of ammonia were passed over the sensor layer at a flow rate of 2 ml/min. The response to ammonia was measured as a decrease in relative fluorescence.

The AFM pictures were taken with a Nanoscope III (Digital Instruments, Santa Barbara, CA) in standard contact mode. Cantilevers were commercially available soft silicon nitride cantilevers with oxide-sharpened tips (Nanoprobe; Digital Instruments). Imaging was performed in a glass fluid cell. In contact mode the deflection of the cantilever while touching and scanning the sample was recorded. An analog feedback circuit adjusted the sample height to keep the deflection constant.

Chemicals

Rhodamine B chloride (RBCl) and Triton X-100 were obtained from Merck (Darmstadt, Germany) and bromophenol blue sodium salt from Aldrich (Steinheim, Germany). Tetramethoxysilane (TMOS), phenyltrimethoxysilane (PhTMOS), and octyltrimethoxysilane (OcTMOS) were from Fluka (Buchs, Switzerland). The synthesis of the ion pairs rhodamine B bromophenol blue (RB/BPB) and tetramethylrhodamine ethyl ester bromophenol blue (TMRE/BPB) has already been described in detail [15,16] (Fig. 1). Double-distilled water was used throughout.

Ammonia solutions were prepared by dissolving ammonium chloride in phosphate buffer of pH 6.8. The concentration of free ammonia was calculated by the Henderson–Hasselbach equation, which, at pH 6.8 and 25°C, was

$$\log [NH_3] = \log [NH_4^+] - 2.44. \tag{1}$$

Preparation of the Ammonia-Sensitive Layers

Glass plates (11 × 35 mm) were dip-coated in a cocktail consisting of 3 mg of the respective indicator (RBCl, RB/BPB, or TMRE/BPB), 0.5 ml of 0.1 N HCl, 2.5 ml of ethanol, 2 ml of the respective silane, and 50 μl of Triton X-100, 4 days after cocktail preparation. The compositions of cocktails for the preparation of layers L-1 through L-7 are listed in Table I. A tape was

Table I. Composition of Cocktails Used for Making Sol-Gel Layers L-1 to L-7[a]

Layer	Indicator (3 mg)	Silane (2 ml)
L-1	RBCl	100% TMOS
L-2	RBCl	50% TMOS, 50% PhTMOS
L-3	RB/BPB	100% OcTMOS
L-4	RB/BPB	100% TMOS
L-5	RB/BPB	100% PhTMOS
L-6	RB/BPB	50% TMOS, 50% PhTMOS
L-7	TMRE/BPB	50% TMOS, 50% PhTMOS

[a]The cocktails consist of 0.5 ml of 0.1 N HCl, 2.5 ml of ethanol, and 50 μl of Triton X-100.
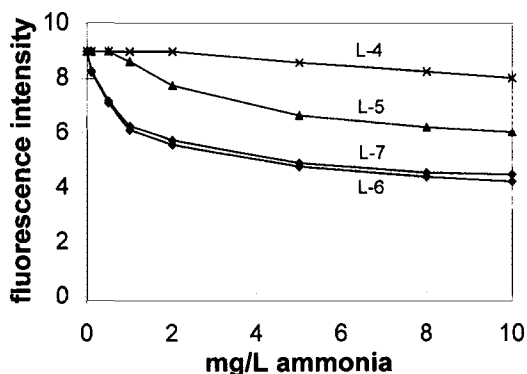

Fig. 2. Calibration plots of sol-gel layers L-4 to L-7.

Table II. Figures of Merit of Sol-Gel Layers L-1 to L-7

Layer(s)	Response time (min) Forward	Response time (min) Reverse	Dynamic Range (mg/L)	LOD (mg/L)
L-1, L-2, L-3	—	—	—	—
L-4	7	—	5.0–10.0	5.0
L-5	5	7	1.0–5.0	1.0
L-6	6	>40	0.1–1.0	0.1
L-7	6	>40	0.1–1.0	0.1

glued on one side of the glass plates in order to prevent both sides from being coated. Because polycondensation of the silane and formation of the sol-gel caused changes in the polymer network, the sensor layers were stored dry for 2 weeks before measurements. The concentration of the catalyst was varied from 0.001 to 0.01 and 0.1 N HCl. Homogeneous layers were cast from cocktails containing 0.1 N HCl after 3–5 days. In the case of less acidic catalysts (0.001 and 0.01 N HCl), stable sensor layers were obtained after typically 10 days. However, the concentration of HCl did not affect the response to
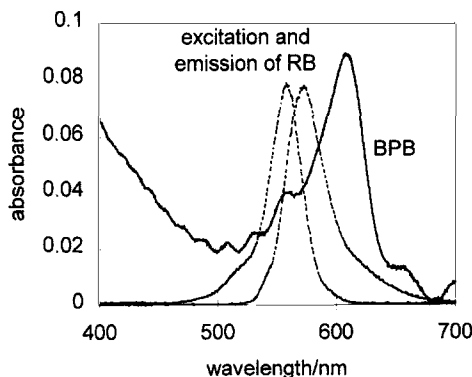

Fig. 3. Excitation and emission spectra of rhodamine B (dashed line) and absorbance of deprotonated bromophenol blue (solid line).

ammonia. Consequently, 0.1 N HCl was chosen in order to reduce the required time for the layer preparation.

RESULTS

Effect of the Layer Composition on the Signal Magnitude

Rhodamine B (RBCl) (L-1, L-2) and the ion pairs RB/BPB (L-3 to L-6) and TMRE/BPB (L-7) were incorporated in organomodified sol-gel glasses. The response of sol-gel layers L-1 to L-7 to aqueous ammonia was measured as a change in relative fluorescence. The calibration plots of layers L-4 to L-7, are shown in Fig. 2 and the sensor characteristics are listed in Table II.

Layers L-1 and L-2, containing only RBCl, did not respond to ammonia. Octyl-modified sol-gel layer L-3 was heterogeneous and not stable on the glass support. Layer L-4, containing the ion pair RB/BPB, showed a linear response between 3 and 10 mg/L ammonia and a maximum relative signal change of 10%. Layer L-5, containing the same indicator but an organically modified silane, was more sensitive and provided a sensitive range of 0 to 5 mg/L of aqueous ammonia with relative signal changes as high as 35%. L-6 and L-7, made from RB/BPB and TMRE/BPB, respectively, were most sensitive to ammonia concentrations in the range of 0–2 mg/L, with maximum signal changes of around 55%.

The response to ammonia depended strongly on the type of silane used as the sensor matrix. The relative signal change of the optode layer increased with increasing content of organically modified silane (<50%) but decreased with an excess of modified silane (>50%). As a result, the response of L-6 (50% TMOS, 50%

PhTMOS) to 10 mg/L ammonia was 1.6 times higher than the response of L-5 (100% PhTMOS) and 5.5 times higher than that of L-4 (100% TMOS).

The reproducibility was investigated 2 weeks after layer preparation using five layers of type L-6. The standard deviation for 5 mg/L ammonia was 1.9%, and the standard error 1.1%, respectively ($n = 10$).

Effect of the Matrix on the Response Time

The forward response of layer L-4 to aqueous ammonia was in the range of 5–10 min. The reverse response, however, was irreversible. RB/BPB- and TMRE/BPB-doped sol-gel layers (L-6, L-7) showed forward response times similar to those of L-4, but were not fully reversible as well. Only 70–80% of the initial fluorescence was achieved when exposed to buffer. In contrast, the response of L-5, based on pure PhTMOS as the matrix, was fully reversible.

Effect of Storage, Conditioning, and Regeneration on the Response

The highest sensitivity was obtained for L-6. Thus, layers of this type were used to study the effects of storage, conditioning, and regeneration on the response to ammonia. The sol-gel layers were stored dry in ambient air, in distilled water, and in phosphate buffer of pH 6.8. Measurements were performed 2 weeks after layer preparation and after overnight conditioning in the respective environment. Storage in distilled water resulted in signal enhancement by 10 and 30% compared to dry storage and storage in buffer.

After measurements, sol-gel layers L-6 were stored dry, in water, and in buffer for 16 h and again exposed to ammonia. The calibration plot of L-6 after regeneration in water is similar to the calibration plot of a new layer. However, a 60% loss in the relative signal change was observed for storage in air and a 20% loss for storage in buffer.

The response of the sol-gel layers to ammonia was measured 2, 3, 5, 7, and 24 weeks after layer preparation and dry or humid storage. After storage of 1 week in the respective environment (3 weeks after layer preparation), no loss in sensitivity for layers stored in distilled water was observed. Storage in ambient air or in buffer caused a decrease in sensitivity. In the latter case, the response to ammonia decreased by 70–80%, probably because of crystallization of buffer salts in the pores of the sol-gel. However, the sensitivity to ammonia remained constant over a period of 6 months for sol-gel layers stored in distilled water. For sol-gel layers stored in ambient air,

a sensitivity loss was observed only after the first week. Afterward, the sensitivity also remained constant for 1 month.

Effect of pH on the Response

The effect of pH on the response of sol-gel layers L-6 to ammonia was studied using phosphate buffers of pH 6.8, 5.7, and 7.8. In buffer of pH 7.8 the fluorescence decreased by 1.5% relative to the fluorescence at pH 6.8. In buffer of pH 5.7, however, the fluorescence increased by 2.9%. The response to a low pH was fast (1.5% min^{-1}); exposure to a high pH resulted in a slow drift reaching a steady state after typically 6 min (0.48% min^{-1}).

DISCUSSION

Recently, an ammonia sensor membrane based on rhodamine B chloride (RBCl) immobilized in plasticized poly(vinyl chloride) (PVC) has been reported [15,17]. Its response to ammonia was based on the deprotonation of neutral (fluorescent) RBCl by ammonia to form a fluorescent zwitterion and the consequent conversion of the zwitterion into a nonfluorescent lactone [15,17]. For the formation of the lactone a highly lipophilic and unpolar matrix such as plasticized PVC was required.

In the present case, RBCl is similarly entrapped in sol-gel glass based on the precursors TMOS and the more lipophilic PhTMOS (L-1, L-2). Both types of membranes do not respond to ammonia because the lipophilicity of the sol-gel matrices is insufficient for the conversion of the zwitterion into the lactone. However, RB incorporated in sol-gel as fluorophore/absorber pairs RB/BPB and TMRE/BPB exhibits a significant response to ammonia (see Table II). The principle of both ion pairs is based purely on energy transfer from the fluorescent rhodamine dyes to bromophenol blue, which is deprotonated upon exposure to ammonia (Fig. 3).

In the hydrophilic and proton-permeable TMOS, the response of RB/BPB is relatively low, with maximum signal changes of around 10%. This is due to the fact that under the experimental conditions (in buffer of pH 6.8), the absorber BPB becomes deprotonated and a reaction with ammonia does not further change the absorbance of BPB (and consequently the fluorescence of the rhodamine). The response of sensors based on PhTMOS–TMOS mixtures to pH is much slower because the more lipophilic matrix restricts diffusion of ions into the matrix. Consequently, these layers can be used for the measurements of gaseous species such as
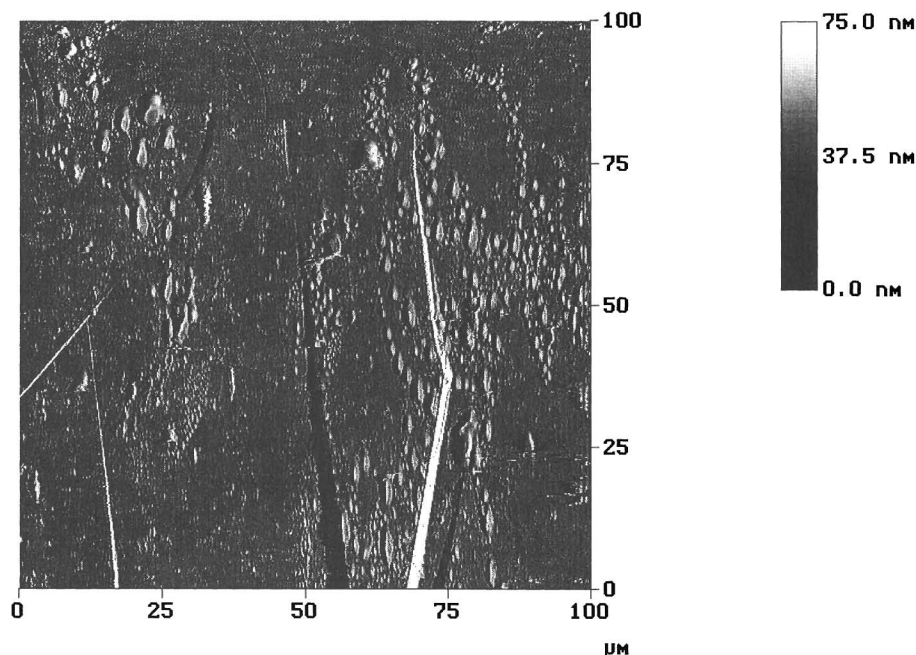
Fig. 4. Atomic force microscopy (AFM) image of sol-gel layer L-4 showing the rough nonporous surface.

ammonia. The stability of the response and the cross-sensitivity to pH can be further reduced by employing pure PhTMOS as the matrix.

Apart from affecting the cross-sensitivity to pH, the matrix also affects the response times for forward and reverse response. Whereas mixtures of PhTMOS and TMOS gave a fast forward response (5–10 min), the reverse response was irreversible. However, by increasing the content of organomodified PhTMOS up to 100%, sensors with a fast reverse response were obtained. The effect of the matrix is based on the fact that polar matrices enable the formation of a deprotonated and, therefore, negatively charged species of the absorber in the sol-gel matrix. This causes large signal changes but an irreversible response. Less polar and more lipophilic sol-gel materials, in contrast, are less able to solvate the deprotonated form of the absorber because there is a serious dielectric constraint to deprotonation in the nonpolar matrix. Consequently, signal changes are smaller but the response is reversible because the uncharged protonated form of the dye is stabilized in the matrix. A recent investigation has shown a similar behavior with ammonia sensors based on pH indicator dyes in plasticized PVC [18].

When using organomodified sol-gel glasses, one has to take into account that the sol-gel process is much slower than with common TMOS. However, despite this limitation, sol-gels still are simple in preparation and provide sensor layers whose indicators do not leach out. The enhanced lipophilicity of the sol-gel affects the response of these sensor layers in that ions are restricted in their diffusion, whereas gases are not. Consequently, organomodified sol-gel glasses are most appropriate for gas sensors for both the gas and the aqueous phase. Organomodified sol-gel glasses still exhibit much better mechanical stability than most polymer materials such as plasticized PVC and ethyl cellulose. Due to their rigid structure, swelling effects which can cause drift are not observed. Finally, their storage stability is superior since they do not contain plasticizers, which are generally necessary for proper functioning of most optical sensors.

To understand further the response of the ammonia sensors, atomic force microscopy (AFM) images of organically modified and unmodified sol-gel layers L-4 and L-5 were taken. L-4 does not contain any organically modified precursor, but pure TMOS. This sensor shows a poor and irreversible response to ammonia (see Fig. 2 and Table II). The surface of L-4 is rough and exhibits little porosity (Fig. 4). In contrast, the images of the organically modified sol-gel layers show pores of 4-μm depth. Porosity increases with an increasing content of PhTMOS, which allows better diffusion of ammonia into and inside the layer and is likely to cause the reversible response to ammonia. Figure 5 shows an im-
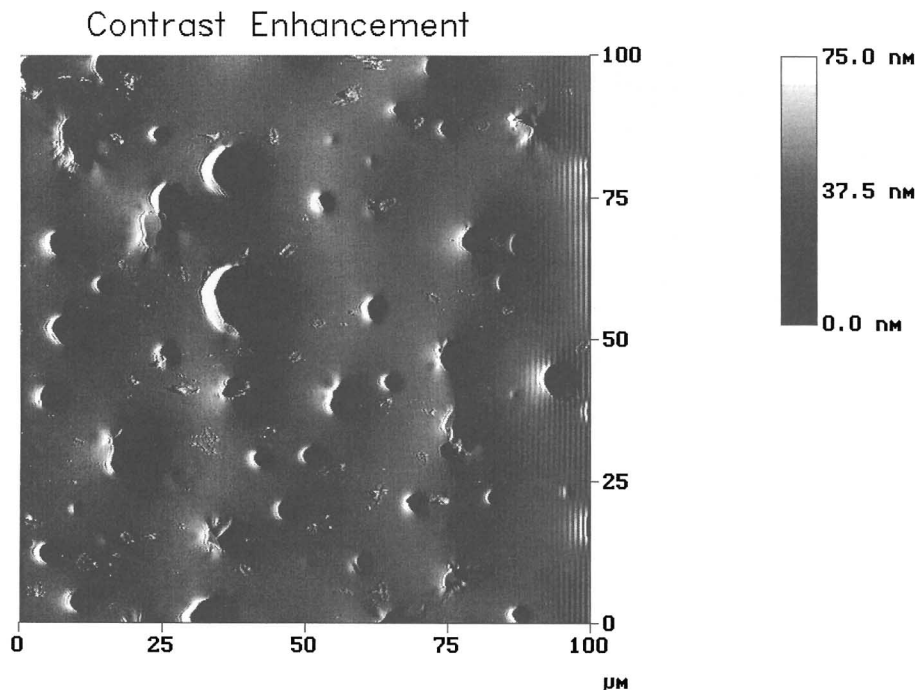
Contrast Enhancement

Fig. 5. AFM image of sensor layer L-5 showing the smooth, porous sensor surface.

age of layer L-5. The response of L-5 is fully reversible. However, after repeated exposure to buffered ammonia, the surface becomes rough and unporous, similar to layer L-4.

CONCLUSION

Ammonia-sensitive membrane layers are presented which make use of an absorbance-based pH indicator dye incorporated in organomodified sol-gel glasses. The use of the pH indicator dye together with inert fluorophores in the form of ion pairs allows us to apply the membranes in fluorescence rather than in absorbance mode. This is especially important if the membranes are to be used for optical fiber sensors. Organomodified sol-gel glasses provide advantages over common sol-gel glasses in that they are more lipophilic and improve the response times and relative signal changes to neutral (gaseous) analytes. Furthermore, cross-sensitivity to pH is reduced and leaching becomes negligible. The lipophilicity of the organomodified sol-gel glasses enables the fast diffusion of gases in and out of the material, whereas the diffusion of ions is restricted. The FRET-based sensors allow the measurement not only of fluo-

rescence intensity but also of fluorescence lifetime, which is currently under investigation.

ACKNOWLEDGMENTS

REFERENCES

1. A. M. Buckley and M. Greenblatt (1994) *J. Chem. Educ.* **71**, 599–602.
2. R. Reisfeld and C. K. Jorgensen (1992) *Struct. Bond.* **77**, 207–265.
3. D. Avnir, S. Braun, O. Lev, D. Levy, and M. Ottolenghi (1994) *Sol-Gel Optics—Processing and Applications*, Kluwer, Boston
4. B. C. Dave, B. Dunn, and J. S. Valentine (1994) *Anal. Chem.* **66**, A1120–A1127.
5. O. Lev, M. Tsionsky, L. Rabinovich, V. Glezer, S. Sampath, I Pandratov, and J. Gun (1995) *Anal. Chem.* **67**, A22–A30.
6. B. D. MacGraith, G. O'Keeffe, C. McDonagh, and A. K. McEvoy (1994) *Electron Lett.* **30**, 888–889.
7. M. Forster, J. Eberle, S. Strassler, and G. Pfister (1990) *Inst. Phys. Conf. Ser.* **111**, 479–480.
8. R. Klein and E. Voges (1993) *Sens. Actuators B* **11**, 221–225.

9. V. Chernyak, R. Reisfeld, R. Gvishi, and D. Venezky (1990) *Sens. Mater.* **2**, 117.

10. P. Simon, S. Sekretar, B. D. MacCraith, and F. Kvasnik (1997) *Sens. Actuators B* **38–39**, 252–255.

11. U. Narang, R. Gvishi, F. V. Bright, and P. N. Prasad (1996) *J. Sol Gel Sci. Technol.* **6**, 113–119.

12. S. B. Bambot, J. Sipior, J. R. Lakowicz, and G. Rao (1994) *Sens. Actuators B* **22**, 181–188.

13. Q. Chang, J. Sipior, J. R. Lakowicz, and G. Rao (1995) *Anal. Biochem.* **232**, 92–97.

14. J. Sipior, S. Bambot, M. Romauld, G. M. Carter, and J. R. Lakowicz (1995) *Anal. Biochem.* **227**, 309–318.

15. C. Preininger and G. J. Mohr (1997) *Anal. Chim. Acta* **342**, 207–213.

16. G. J. Mohr, T. Werner, I. Oehme, C. Preininger, I. Klimant, B. Kovacs, and O. S. Wolfbeis (1997) *Adv. Mater.* **9**, 1108–1114.

17. C. Preininger, G. J. Mohr, I. Klimant, and O. S. Wolfbeis (1996) *Anal. Chim. Acta* **334**, 113–123.

18. G. J. Mohr, K. Trznadel, S. Draxler, F. Lehmann, and M. E. Lippitsch (1998) *Anal. Chim. Acta* **360**, 119–128.