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Optical Sensor for Ammonia Based on the Inner Filter Effect of Fluorescence

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A new indicator-immobilization technique for the development of a sensor material for monitoring ammonia gas in aqueous solutions is presented. It is based on the change in absorbance intensity of a lipophilized pH indicator homogeneously dissolved in silicone rubber. Exposure to ammonia leads to a deprotonation of the indicator and this increases the absorbance of the base form of the indicator bromophenol blue. The sensor responds over a 17 μg/L to 17 mg/L concentration range, with a detection limit of 12 μg/L.

KEY WORDS: Lipophilic indicator; optical ammonia sensing; fluorescent particles; inner filter effect.

INTRODUCTION

We describe the application of a homogeneous two-component chromophore–polymer-based optode for measurement of ammonia in liquids. The sensor consists of a lipophilic pH indicator contained in a thin silicone membrane cast on a transparent polyester support.

In previous work, ammonia sensors were described that use plain pH indicators contained in a silicone layer. Reichert *et al.* [1] used bromophenol blue in a silicone network but gave no experimental details on specificity and stability of the sensing layer. Gauglitz and Kraus report on problems related to the immobilization of bromocresol purple in silicone [2], because an inhomogeneous film was obtained and a poor indicator loading within the sensing layer was found, which was caused by partial crystallization of the indicator dye.

To overcome the solubility problem, we prepared a bromophenol blue derivative which was obtained by replacing the sodium counterion of its sulfo group with cetyltrimethylammonium bromide, thus forming a lipophilic ion pair. This ion pair is easily soluble in silicone rubber. Membranes were made which, on exposure to an aqueous ammonia solution, turned blue because ammonia diffuses into the membrane to deprotonate the indicator.

The optode membrane was optimized so as to have a useful range from 17 μg/L to 17 mg/L ammonia. Because silicone is impermeable to protons, it is unaffected by pH. However, a detection limit close to 10 μg/L ammonia is required for ammonia sensing in real samples such as in fish waters. We therefore designed a fluorosensor because of the intrinsic sensitivity of fluorescence and using the inner filter effect (IFE).

The IFE has been shown to be useful for optical pH sensing by Gabor and Walt [3], although cuvette experiments with dissolved pH indicators and fluorophores were reported. The IFE has also been applied for potassium sensing by He *et al.* [4]. We make use of a lipophilized indicator and fluorescent particles which we incorporate into the above (absorbance based) silicone membrane. The particles cannot be washed out because they are mechanically incorporated into the silicone network. This is a generic approach to make all absorbance-

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based sensors fluorescent, provided the analyte-dependent absorption overlaps with the excitation and/or the emission of the fluorescent particles.

EXPERIMENTAL

Ammonium chloride, trichloromethane ($CHCl_3$), and bromophenol blue sodium salt were purchased from Aldrich (Steinheim, Germany). Cetyltrimethylammonium (CTA) bromide was from Fluka Chemie AG (Buchs, Switzerland). The fluorophore polystyrene particles (Fluorescent Magenta) were purchased from American Colors, Inc. (Orlando, Fl). The excitation/emission maxima of the particles are at 570/605 nm, respectively. Mylar polyester film, Type GA 10, was from Du Pont (Bad Homburg, Germany), and transparent silicone elastosil (E4; splitting off acetic acid) from Wacker (Munich, Germany). A 0.5 *M* Sörensen buffer (sodium borate/HCl), pH 7.38, was used for preparation of sample solutions. The indicator ion pair was prepared as follows: 1 mmol (0.7 g) of the sodium salt of bromophenol blue was dissolved in 80 ml of water. The solution was treated with 1 ml of 0.1 *M* HCl and a solution of 0.36 g (1 mmol) of CTA bromide dissolved in 60 ml of water. The ion pair precipitated and was filtered by suction. After drying the crude material it was found to be of analytical purity.

Membrane cocktails were made by dissolving the lipophilic indicator and silicone in $CHCl_3$. The fluorescent particles were added immediately before the cocktail was spread on the support. Membranes were cast using a homemade spreading device. For preparing the membrane, a 20-μm spacer was used by applying the cocktail to the polyester support. The solvent was evaporated at room temperature. To complete the polymerization, the membranes were heated to 90°C in an oven for 2 days. The resulting membranes were calculated to be 10–13 μm thick. Before measurements, the membranes were conditioned for 1 day in 100 mmol Sörensen buffer, pH 7.38.

Membranes were mounted in homemade flow-through cells, and excitation and emission spectra measured using an Aminco SPF 500 spectrofluorometer equipped with a 250-W tungsten halogen lamp as a light source. Fluorescence intensity was observed at excitation/emission wavelengths of 570/605 nm. Standard solutions were pumped over the sensor membrane at a rate of 3 ml/min. All measurements were performed at 22°C. Ammonia solutions of defined concentration were prepared by adding ammonium chloride solutions to Sörensen buffer, pH 7.38. The ammonia concentration was calculated according to

$$\log[NH_3] = \log[NH_4^+] + (pH - pK)$$

RESULTS AND DISCUSSION

The NH_3 sensor is a yellow, fully transparent plastic foil without inhomogeneities. The membrane based on absorption measurements responds to 17 μg/L and 17 mg/L ammonia in water, giving a strong absorption at 607 nm. The silicone matrix has a high permeability for gases so we obtained relatively short response times, which are shown in Table I. There is no cross-sensitivity to pH, no swell, and the membrane does not get turbid, so we were able to do measurements without a further protection layer.

The IFE-based sensing approach requires the presence of an analyte-independent fluorophore whose excitation and emission intensity is modulated by the varying absorption of the ammonia-sensitive absorption indicator. This can happen when one form (normally the long-wave band) of the indicator overlaps with the excitation and/or emission band of the fluorophore. The improvement in the detection limit results from the fact that a low concentration of the fluorophore leads to a total filter effect, so the sensor can respond to smaller ammonia concentrations with increased signal change.

The excitation and emission spectra of the fluorescent particles are shown in Fig. 1. Both wavelengths (570 nm for excitation and 605 nm for emission) overlap the broad absorption band of the indicator dye. Conceivably, two mechanisms are operative, namely energy transfer (ET) and the IFE. Because the fluorophore is present in the form of fluorescent particles rather than in a homogeneous distribution, ET can be excluded because of the special separation of the donor and acceptor. Rather, the fluorescent particles act as a minute light source inside the membrane, and its emission is more or less screened by the absorber.

The IFE ammonia optode shows a fluorescence re-

Table I. Properties of the Different Types of Sensor Membranes

Technique	Absorbance	Inner filter effect
Fluorescent particles	—	Fluorescent magenta
Detection limit	12 μg/L	8 μg/L
Useful dynamic range	17 μg/L–17 mg/L	10 μg/L–1 mg/L
Response time t_{90}[a]	15–18 min	45–90 min

[a]At solutions from 85 to 170 μg/L.

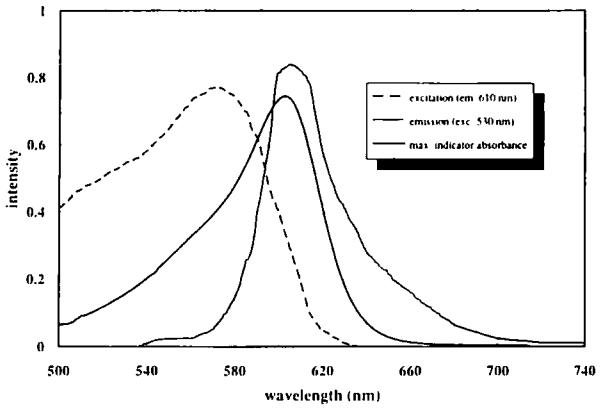

Fig. 1. Excitation and emission spectra of the fluorescent particles, as well as the absorbance of the base form of the lipophilized indicator.

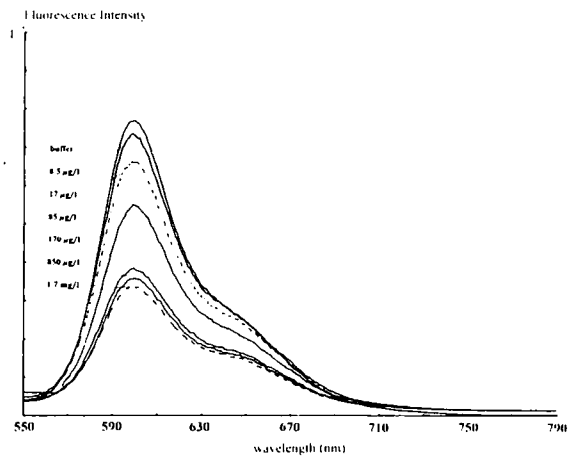

Fig. 2. Emission spectra of the IFE-based ammonia optode at various concentrations of ammonia (excitation at 570 nm).

sponse to solutions of ammonia as shown in Fig. 2. The signal is fully reversible, but at low ammonia levels response times of about 45–90 minutes are observed. Table I gives figures of note for the sensor membrane. The absorption of the membrane at levels up to 1 mg/L filtered the fluorescence of particles totally.

There is a considerable improvement in the detection limit of the fluorescent sensor as reported previously in other cases [3–5]. We attribute this to the relative excess of primary absorber over the fluorophore. As a result, small changes in the fraction of the acid-to-base form of the dye cause a substantial change in the optical density of the membrane and in its permeability for the fluorescence of the particles. Figure 3 gives the normalized work function.

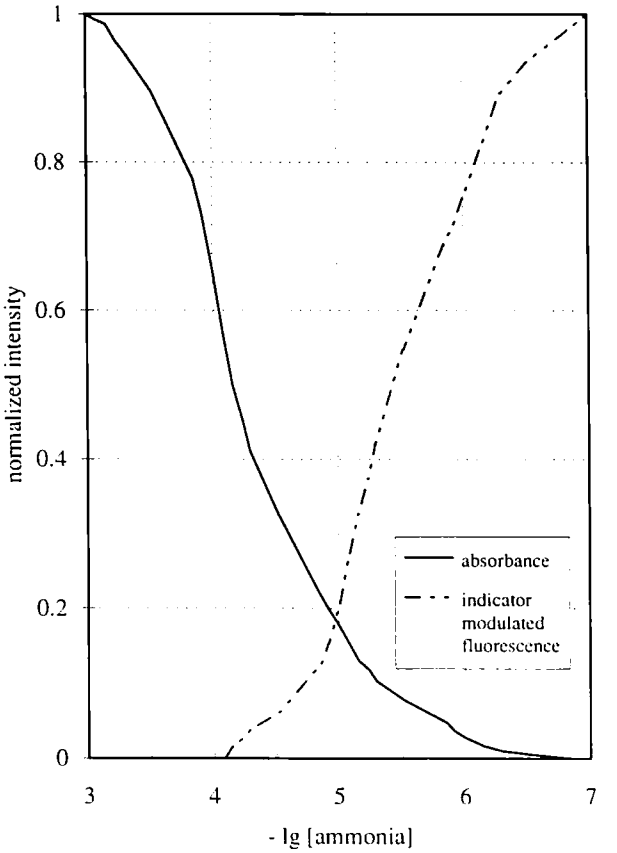

Fig. 3. Normalized steady-state response curves of ammonia-sensitive membranes. Absorbance measurements of silicone membrane without fluorescent particles at 605 nm (——); fluorescence measurements of membrane at 605 nm (excitation, 570 nm) (—).

CONCLUSION

We think that the IFE-based sensor has all the advantages of a fluorescence optrode and a lower detection limit. Further advantages include its high sensitivity, simplicity, chemical and mechanical stability, reproducibility, and long-term stability. Furthermore, the sensor overcomes the problem of poor dye loading and all the problems associated with an inhomogeneous indicator distribution.

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