Design and Characterization of a Novel Ammonium Ion Selective Optical Sensor Based on Neutral Ionophores

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A novel principle of an optical sensor (optode) has been realized in practice by combining NH_4^+ -selective neutral ionophores of the macrotetrolide type, H^+ -selective neutral chromoionophores that drastically change their absorption spectrum upon protonation, and lipophilic anionic sites in the same plasticized PVC membrane. Such chemical sensors generally offer the advantages of a highly selective and reversible recognition of given ionic substrates and of a straightforward optical transduction of the recognition process. The present membrane exhibits the theoretically expected absorbance response to ammonium ions in pH-buffered samples. Dynamic range, selectivity coefficients, reproducibilities, and response times of the new optode system are promising in view of analytically relevant applications.

Keywords Optodo, ammonium ion selective, polyvinyl chloride membrane, neutral chromoionophore, nonactin, monactin

Since the pioneering contribution by Lübbers and Opitz¹, a growing interest has been directed towards the development of novel sensors with an optical transduction of the chemical recognition process¹⁻²⁶ (optical sensors, optodes¹). The often cited advantages of such devices, in comparison with corresponding electrochemical sensors, are their great versatility (use of fiber optics), their electrical safety and insensitivity to electrical interference, as well as the fact that no reference device is needed. On the other hand, the number of highly selective optodes accepted in analytical practice is unfortunately still limited. Most promising for successful applications so far are only the pH-sensitive and related optodes (e.g. for CO_2) based on immobilized fluorogenic substances, as well as the O_2 -selective devices utilizing fluorescence quenching.^{1,2}

In clear contrast, a great variety of ion selective membrane electrodes has become available, the majority of which are based on a selective recognition of a given ion by electrically neutral ionophores (neutral carriers for specific cations or anions; for a review see refs. 27-34). It is an analytical challenge to use these highly selective molecules without further structural modifications for the realization of a new generation of optical sensors. Yet, successfully applied optical measuring devices based on neutral carriers were so far not reversible (test strips for single use^{35,36}), and a few well praised reversible systems of this type^{10,22-24} were not sufficiently reliable and/or too exotic for analytical chemical demands. Somewhat more encouraging were

two attempts where electrically charged ionophores were incorporated into the sensing membranes of optodes.^{13,25} However, the resulting selectivities were rather poor in these cases.

Optodes which incorporate electrically neutral chromoionophores³⁷ or fluoroionophores³⁸, i.e. carriers that drastically change their optical properties in the UV/VIS region upon ion complexation, could ideally combine the most salient requirements. These are the highly selective and reversible recognition of a given ionic or ionogenic substrate and the straightforward optical transduction of the recognition process. Whereas some earlier attempts in this direction aimed at an immobilization of such ion selective ligands on the surface of appropriate supports^{10,22-24} or even at a water-solubilization of the ligands³⁹, it seems to be much more promising to make use of the well proved ion selective electrode membrane technology. The fundamental design features of corresponding novel optodes have been worked out very recently^{15,16} and were converted into practice in the case of different PVC membrane systems based on CO_3^{2-} -selective chromoionophores.¹⁵⁻¹⁷ A similar approach was realized with the recently introduced first example of an ion selective photodiode (ISPD).²⁶ Theoretical treatments of the response function, the selectivity behavior, the response time, the absorbance range, the life time, the signal stability, and the geometrical aspects of ionophore based optical sensors have also been presented earlier.¹⁷

A detailed study^{15,16} of the membrane morphology in the design of electroneutral optode membranes reveals an especially attractive version, namely the *neutral-carrier-supported ion exchange membrane system*, denoted as class IIb in refs. 15, 16. Here, we report on the first optical sensor of this type, which was realized by combining NH_4^+ -selective ionophores and H^+ -selective chromoionophores (all of these carriers being electrically neutral) together with lipophilic anionic sites in the same plasticized PVC membrane phase.

Experimental

Reagents

All chemicals used were of highest analytical grade available, and aqueous solutions were prepared with doubly quartz-distilled water. NaCl , KCl , NH_4Cl , NaH_2PO_4 and Na_2HPO_4 were obtained from E. Merck AG (Darmstadt, West Germany). For membrane preparation, nonactin/monactin (NH_4^+ ionophores), poly(vinyl chloride) (PVC; high molecular), potassium tetrakis(*p*-chlorophenyl)borate (KTpCIPB), bis(2-ethylhexyl)sebacate (DOS), and tetrahydrofuran (THF) were obtained from Fluka AG (Buchs, Switzerland). The synthesis of the H^+ -selective neutral chromoionophore ETH 5294 (3-octadecanoylimino-7-diethylamino-1,2-benzophenoxazine; a lipophilic, highly basic dye molecule related to *Nile Blue*) is described elsewhere.¹⁸

Membrane preparation

The optode membranes of composition I were prepared from a batch of 6 mg of nonactin/monactin, 4 mg of ETH 5294, 3.9 mg of the additive KTpCIPB (to create lipophilic anionic sites), 160 mg of the plasticizer DOS, and 80 mg of PVC. The optode membranes of composition II were prepared from a batch of 5 mg of nonactin/monactin, 3 mg of ETH 5294, 3.4 mg of the additive KTpCIPB, 160 mg of the plasticizer DOS, and 80 mg of PVC.

The membrane components were dissolved in 1.5 ml freshly distilled THF. A portion of 0.2 ml of this solution was injected onto a rotating, dust-free glass plate of 35 mm diameter (Herasil quartz glass, W. Möller AG, Zürich, Switzerland) which was located in a THF-saturated atmosphere. A spinning device¹⁹ with a closed aluminium/plexiglass cell was used (rotating frequency: 600 min^{-1}). This allows the preparation of glass-supported membranes having controlled and highly reproducible thicknesses in the range between $0.5 \mu\text{m}$ and $7 \mu\text{m}$.¹⁹ After a spinning time of only about 4 s, the glass support with the optical sensing membrane was removed and stored in air during 10 min for further drying. Before the measurements, the membranes were conditioned for 10 min in 10^{-3} M HCl and for another 10 min in $0.1 \text{ M NH}_4\text{Cl}$ (buffered to pH 7.35).

Apparatus

UV/VIS absorbance measurements and spectra of

the PVC membranes were taken using a flow-through cell¹⁹ in a UVIKON Model 810 double-beam spectrophotometer (Kontron AG, Zürich). A schematic representation of the flow-through cell is shown in Fig. 1.

Experimental procedure

Two glass plates with identical membranes of about $4 \mu\text{m}$ thickness were mounted into the measuring cell of the flow-through system (see Fig. 1). The reference cell of the spectrophotometer contained two glass plates without membranes. The solutions of measuring and reference cell were identical and were changed simul-

Fig. 1 Schematic representation of the flow-through cell used for the present studies on optode membranes. 1, sample inlet (polypropylene support); 2, O-seal ring; 3, quartz glass support; 4, ion-sensing optode membrane; 5, plexiglass cell wall; 6, sample outlet (polypropylene support); 7, set screw.

Fig. 2 Absorption spectra of two $4 \mu\text{m}$ thick optode membranes of composition I after equilibration with pH-buffered solutions (pH 7.35) containing different concentrations of NH_4Cl . The deprotonated form of ETH 5294 shows an absorbance maximum at a wavelength of 535 nm, the protonated form shows two, at 611 nm and at 660 nm. (1), buffer; (2), $10^{-5} \text{ M NH}_4\text{Cl}$; (3), $3 \times 10^{-5} \text{ M NH}_4\text{Cl}$; (4), $10^{-4} \text{ M NH}_4\text{Cl}$; (5), $3 \times 10^{-4} \text{ M NH}_4\text{Cl}$; (6), $10^{-3} \text{ M NH}_4\text{Cl}$.

taneously from outside the spectrophotometer. Except for the recordings of the full absorption spectra given in Fig. 2, the absorbance measurements were made at a fixed wavelength of 660 nm. Steady-state readings for reproducibility studies were taken 2 min after sample change. Activity coefficients for NH_4^+ , Na^+ , K^+ and Li^+ ions in the different sample solutions were calculated according to ref. 40. Further details of the evaluation of results will be given elsewhere.¹⁹

Results and Discussion

It was shown previously¹⁵⁻¹⁷ that highly selective optical sensors of the type introduced here exhibit an absorbance response that depends on the ratio of the sample activities of the two ionic species which are involved in the ionophore-mediated ion exchange equilibrium. For the present system and ideal conditions, the absorbance A at steady-state is given by the relationship:^{15,17}

$$A = A_1\alpha + A_0(1 - \alpha) \quad (1)$$

with the following function for α :¹⁷

$$\alpha^2 / (1 - \alpha)^2 = K_{\text{exch}} a_{\text{NH}_4} / a_{\text{H}}. \quad (2)$$

Here α is the ratio of free to total chromoionophore concentration in the membrane, A_0 and A_1 are the limiting absorbance values for $\alpha=0$ and $\alpha=1$, respectively, a_{NH_4} and a_{H} are the activities of the two primary ions in the sample solution, and K_{exch} is the equilibrium constant of the basic ion exchange process including carrier complexation/decomplexation. When interfering ions M^+ of the activity a_{M} compete with NH_4^+ for the complexation of the corresponding ionophores and if the H^+ -selective ionophores are presumed to be not directly affected by these interferents, Eq. (2) must be extended as follows:¹⁷

$$\alpha^2 / (1 - \alpha)^2 = K_{\text{exch}} (a_{\text{NH}_4} + \sum_{\text{M}} K_{\text{NH}_4\text{M}}^{\text{opt}} a_{\text{M}}) / a_{\text{H}} \quad (3)$$

where $K_{\text{NH}_4\text{M}}^{\text{opt}}$ are the optical selectivity coefficients.¹⁷

It may be inferred from Eqs. (1)–(3) that the present optode membranes formally respond to ammonia. Hence, for a selective determination of NH_4^+ ion activities, the pH value of the sample must either be measured simultaneously (e.g. by means of a pH-sensitive optode) or be adjusted by an appropriate buffer solution. Figure 2 shows the absorption spectra in the visible range, as obtained for the novel membrane system (composition I) after equilibration with pH-buffered solutions containing different concentrations of NH_4Cl . Evidently, the maximum change in absorbance with varying sample concentration is observed at a wavelength of 660 nm. Analogous spectra were recorded for membranes of composition II.

In Fig. 3, the absorbance values measured at 660 nm

Fig. 3 Absorbance values at 660 nm of different membranes (compositions I and II) as a function of $\log(a_{\text{NH}_4^+}/a_{\text{H}^+})$. The curves fitting the experimental points were calculated from Eqs. (1) and (3) using $\log K_{\text{NH}_4\text{Na}}^{\text{opt}} = -2.7$ and $\log K_{\text{exch}} = -3.36$ (membrane composition I) resp. $\log K_{\text{exch}} = -3.12$ (composition II).

Table 1 Selectivity coefficients $K_{\text{NH}_4\text{M}}^{\text{opt}}$ of the NH_4^+ -selective optode membranes for interfering ions M^+ , and comparison with corresponding potentiometric selectivities $K_{\text{NH}_4\text{M}}^{\text{pot}}$

Ion M^+	$\log K_{\text{NH}_4\text{M}}^{\text{opt}}$ (optode, this work)	$\log K_{\text{NH}_4\text{M}}^{\text{pot}}$ (electrode, refs. 27,28)
K^+	-1.2	-0.8
Na^+	-2.7	-2.9
Li^+	-3.4	-3.6

are given for membranes I and II as a function of $\log(a_{\text{NH}_4^+}/a_{\text{H}^+})$. A linear dependence of the absorbance is found in the NH_4^+ activity range between about 10^{-5} M and 10^{-3} M (at pH 7.35). The curves are not ideally symmetrical relative to the center of this range, which is at variance with the expectations from Eq. (2). Actually, the response functions are affected at very low NH_4^+ activities by the interfering Na^+ background concentration of 7×10^{-3} M. This is nicely demonstrated by the curve fits in Fig. 3, which are based on Eq. (3).

The selectivity factor $K_{\text{NH}_4\text{Na}}^{\text{opt}}$ determined from the calculated curves in Fig. 3 agrees well with the corresponding potentiometric selectivity coefficient reported for ion selective electrodes based on nonactin/monactin^{27,28} (see Table 1). Such a correlation was clearly predicted by a recent theoretical treatment.¹⁷ Optical selectivity coefficients were also obtained for K^+ and Li^+ by using similar curve fitting procedures (Table 1). Generally, the values compare favorably with potentiometric selectivities.

Figure 4 illustrates absorbance response vs. time recordings for a NH_4^+ -selective optode membrane (composition I) when it was exposed to repeated concentration step changes between 3×10^{-4} M and 3×10^{-3} M NH_4Cl solutions buffered to pH 7.35. The

Fig. 4 Short-time reproducibilities of the absorbance response of two $4\text{ }\mu\text{m}$ thick membranes (composition I) for sample changes between $3\times 10^{-4}\text{ M}$ and $3\times 10^{-3}\text{ M}$ NH_4Cl .

Fig. 5 Absorbance response vs. time curves for two $4\text{ }\mu\text{m}$ thick membranes (composition I) after concentration step changes in the sample solution from $3\times 10^{-4}\text{ M}$ to $3\times 10^{-3}\text{ M}$ NH_4Cl , and vice versa.

results document an excellent short-time stability and a remarkably high reproducibility of the optical signals. The mean absorbance values with standard deviations are 0.1526 ± 0.0009 for the five upper traces, and 0.1034 ± 0.0015 for the five lower traces.

The detailed response vs. time curves immediately after concentration step changes in both directions are presented in Fig. 5. It becomes evident (see also Fig. 4) that the novel optode systems exhibit a surprisingly fast establishment of the final steady-state absorbance values. The 95% response times are only about 5 s. This compares favorably with the theoretically expected value¹⁷ which is related to the typical diffusion time within a $4\text{ }\mu\text{m}$ thick plasticized PVC membrane.

The long-term stability and the life time of the present nonactin/monactin based membrane optodes appear to be limited by the insufficient solubility of the macroretrolide antibiotics. Thus, slow recrystallization

of the NH_4^+ -selective ionophores may lead to a successive reduction of the observable dynamic range. Research work aiming at the development of similar optical sensor systems with selectivity for other ions^{18,19} as well as of useful biosensors based on these¹⁹ is in full progress.

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