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Design of a Calcium-Selective Optode Membrane Based on Neutral Ionophores

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A large variety of new optical ion-sensor constructions (optodes) can now be realized by using a poly(vinyl chloride) (PVC) membrane that incorporates a cation-selective neutral ionophore, a specially tailored H^+-selective neutral chromoionophore, and lipophilic anionic sites in the same plasticized PVC membrane. Such membranes generally combine 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 Ca^{2+} activities in different pH-buffered samples. Dynamic range, reproducibility, response time, long-time stability, and selectivity of the new optode membrane are discussed.

INTRODUCTION

The development of sensors with an optical transduction of the chemical recognition process (optical sensors; optodes (*1*)) has become a highly relevant and rapidly expanding area in analytical chemistry (*1-26*). These optical sensing devices are based on absorption, reflection, fluorescence, or chemiluminescence measurements, and they commonly make use of chemical compounds that drastically change their optical properties as the result of the actual recognition process. With only few exceptions, immobilized indicator dyes, especially fluorigenic substances, have so far been applied as the active materials. Very often, optical fibers are employed for focusing the incident light onto the corresponding sensing area of the probe and for guiding it back to the detector, but this is not required for fundamental studies of the presented type of membranes (*26*).

Recently, we reported on the design features (*15*), the morphological aspects (*16*), and the theoretical description (*17*) of a new class of optodes which are based on conventional, electrically neutral ionophores. These highly selective compounds have been widely applied in membranes of a large variety of ion-selective electrodes (for a review, see refs 27-34). For the preparation of optical sensing layers, such neutral carriers for cations or anions can be combined in the same bulk membrane phase with a second sort of highly selective neutral ionophores, so-called chromoionophores (*18, 35*) which dramatically change their absorption spectrum in the UV-vis region upon complexation of the corresponding ionic species. The well-proved poly(vinyl chloride) (PVC) membrane technology can basically be adopted for these new optode systems, which is in clear contrast to some earlier approaches (*10, 22-24*).

Formally, two functional principles are possible for the present optical sensing devices (*15-17*): (I) a carrier-induced coextraction of cations and anions from the sample into the membrane phase, or (II) a carrier-induced exchange of cations or of anions between sample and membrane. In the first case the absorbance response depends under ideal conditions on the product of the respective ion activities in the sample solution, while in the second case the response is controlled by the corresponding ratio of ion activities.

Here, we report on the development of a novel optode membrane belonging to the group of *neutral-ionophore-based cation exchangers*. The present system was realized by combining a conventional Ca^{2+}-selective carrier (ETH 1001 (*27*)) and a newly prepared H^+-selective chromoionophore (ETH 5294; this work) together with lipophilic anionic sites in the same plasticized PVC membrane phase. A similar optical sensor utilizing the NH_4^+-selective carrier antibiotics nonactin/monactin was described previously (*18*). Membrane-coated glass plates instead of optical fibers were used for the present fundamental studies.

EXPERIMENTAL SECTION

Reagents. Aqueous solutions were prepared with doubly quartz-distilled water and salts of the highest purity available.

For membrane preparation, ETH 1001 (Ca^{2+} ionophore), poly(vinyl chloride) (PVC; high molecular), potassium tetrakis(4-chlorophenyl)borate (KTpClPB), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ($NaTm(CF_3)_2PB$), bis(2-ethylhexyl)sebacate (DOS), and tetrahydrofuran (THF) were obtained from Fluka AG (Buchs, Switzerland).

For the synthesis of ETH 5294 (H^+ chromoionophore), Nile Blue A chloride was obtained from Sigma Chemie GmbH (Deisenhofen, FRG) and stearoyl chloride (pract.) and ethyl acetate from Fluka AG (Buchs, Switzerland).

Synthesis of 1,2-Benzo-7-(diethylamino)-3-(octadecanoylimino)phenoxazine (ETH 5294). A suspension of 1 g (2.8 mmol) of Nile Blue A chloride in 100 mL of water was stirred at 65 °C for 30 min. After the addition of 100 mL of 0.5 M NaOH, the resulting red precipitate was extracted three times with 100

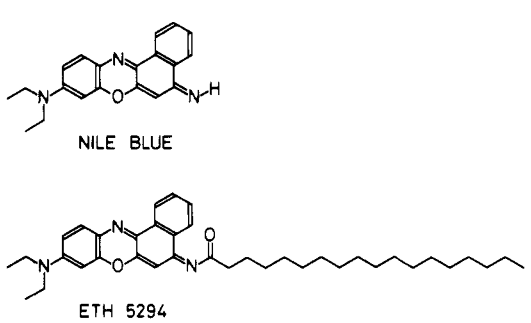

Figure 1. Constitutions of the basic (deprotonated) form of Nile Blue and of the new lipophilic isologue, the chromoionophore ETH 5294.

mL of CH_2Cl_2. The organic phase was filtered and the solvent evaporated to yield 510 mg (1.6 mmol) of the basic form of Nile Blue (see Figure 1).

To a solution of 317 mg (1 mmol) of basic Nile Blue in 13 mL of CH_2Cl_2, 242 mg (0.8 mmol) of freshly distilled stearoyl chloride dissolved in 2 mL of CH_2Cl_2 was added. After being stirred at room temperature for 15 min, the reaction mixture was diluted with 50 mL of CH_2Cl_2 and then washed with 50 mL of 0.1 M NaOH. The organic phase was filtered, the solvent evaporated, and the residue purified by flash chromatography (silica gel, ethyl acetate) and by recrystallization from ethyl acetate to yield 370 mg (0.63 mmol, 79.2%) of ETH 5294 (Figure 1).

The constitution of ETH 5294 was confirmed by ^1H NMR (300 MHz, $CDCl_3$), IR ($CHCl_3$), fast atom bombardment mass spectrometry (FAB-MS), and UV–vis spectra (see below). Elemental analysis: Calcd for $C_{38}H_{53}N_3O_2$ (583.9): C, 78.17; H, 9.15; N, 7.20. Found: C, 77.92; H, 9.35; N, 7.05.

Membrane Preparation. The optode membranes were prepared from a batch of 20 mg of ETH 1001, 5.5 mg of ETH 5294, 11.2 mg of the additive $NaTm(CF_3)_2PB$ (to create lipophilic anionic sites), 143 mg of the plasticizer DOS, and 70 mg of PVC.

The membrane components were dissolved in 2 mL of freshly distilled THF. A 0.2-mL portion 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 hand-made spinning device (*19*) with a closed aluminium/Plexiglas cell was used (rotating frequency, 600 rpm). This allows the preparation of glass-supported membranes having controlled and highly reproducible thicknesses in the range between 0.5 and 7 μm (*19*). After a spinning time of only about 4 s, the glass support with the optical sensing membrane was removed and stored at air for some minutes for further drying.

Apparatus. UV–vis absorbance measurements and spectra of the PVC membranes were taken with a flow-through cell (see Figure 2) (*19*) in a UVIKON Model 810 double-beam spectrophotometer (Kontron AG, Zürich).

The ion-selective electrodes were connected to FET operational amplifiers OPA 128 KM (Burr-Brown, Tucson, AZ). The analog signals were converted into digital ones with a Digital Multimeter Type 7150 (Solartron Schlumberger Instruments, Farnborough, UK). For the remote control, data storage, and handling, a personal computer, Apple IIe, was used with a GPIB IEEE 488 interface and an extended RAM memory (Apple Computer, Inc., Cupertino, CA), a real-time clock (Thunderware, Inc., Oakland, CA), a Model RX 80 matrix printer (Epson Corp., Nagano, Japan), and a graphic plotter, Color Pro 7440 (Hewlett-Packard, San Diego, CA), managed by a high-level language program (UCSD Pascal 1.3) written for this use. The pH values were determined with a pH glass electrode (Orion Ross Model 8103) and a Model SA 720 Orion pH meter (Orion Research AG, Küsnacht, Switzerland).

Experimental Procedure. *Absorption Experiments.* Two glass plates with identical membranes of about 2.5 μm thickness were mounted into the measuring cell of the flow-through system. The reference cell of the spectrophotometer contained two glass plates without membranes. The solutions of the measuring and the reference cell were changed from outside of the spectrophotometer. All measurements were made in the transmittance mode (see Figure 2).

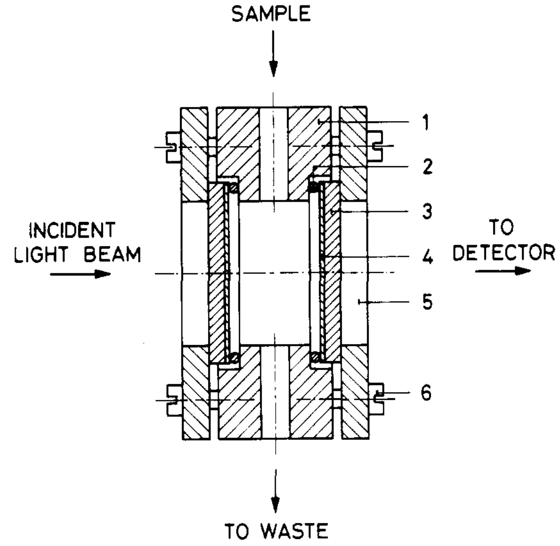

Figure 2. Schematic representation of the flow-through cell used for the present studies on optode membranes: (1) polypropylene support with sample inlet and outlet; (2) O-seal ring; (3) quartz glass support; (4) ion-sensing optode membrane; (5) Plexiglas cell wall; (6) screw for fixation.

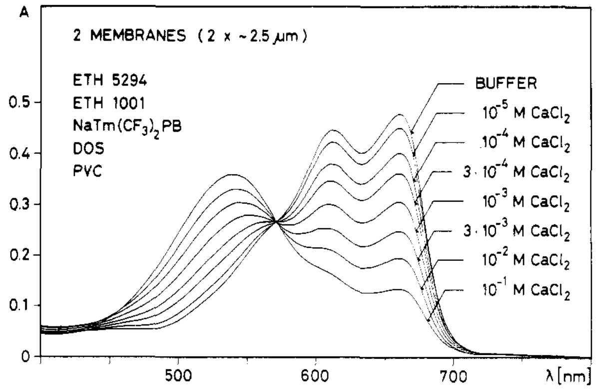

Figure 3. Absorption spectra of two 2.5 μm thick optode membranes after equilibration with pH-buffered solutions (acetate buffer, pH 5.3) containing different concentrations of $CaCl_2$. The deprotonated form of ETH 5294 shows an absorbance maximum at a wavelength of 545 nm and the protonated forms at 614 and 660 nm, respectively.

The buffer solutions used for the characterization of the optode membrane were (a) a citrate buffer (10^{-3} M citric acid, adjusted with 0.1 M NaOH to a pH of 6.5), (b) an acetate buffer of pH 5.3 (0.163 M sodium acetate adjusted with 1 M acetic acid), (c) an acetate buffer of pH 5.9 (0.190 M sodium acetate adjusted with 1 M acetic acid), and (d) a Tris buffer of pH 7.1 (0.005 M tris(hydroxymethyl)aminomethane adjusted with 0.5 M H_2SO_4).

Selectivity factors according to the separate solution method were determined by using 0.5 and 0.05 M solutions of the corresponding chloride salts in a citrate buffer (pH 6.5). For the determination of the Mg^{2+} selectivity factor a 0.1 M $MgCl_2$/Tris buffer solution (pH 7.1) was used as an additional sample. The selectivity coefficients were evaluated by comparing the response functions at $\alpha = 0.5$ (see eqs 6 and 7).

Except for the recordings of the full absorption spectra given in Figure 3, the absorbance measurements were made at a fixed wavelength of 660 nm. Absorbance readings for reproducibility studies were taken 1 min after sample change.

emf Measurements. The membrane preparation and measuring technique of the emf measurements are described in detail elsewhere (*33*). All measurements were carried out on cells of the following type:

$Hg;Hg_2Cl_2,KCl(sat.)|1\ M\ LiOAc|sample\ solution||membrane||0.01\ M\ CaCl_2,AgCl;Ag$

The membrane composition of the calcium ion selective electrode used consisted of 3.4 wt % ETH 1001, 2.0 wt % KTpClPB, 31.2

wt % poly(vinyl chloride), and 63.4 wt % DOS.

RESULTS AND DISCUSSION

It was the primary aim to realize analytically useful optical sensors that are based on plasticized PVC membranes containing electrically neutral ion carriers of high selectivity. To this end, the H^+-selective chromoionophore ETH 5294 shown in Figure 1 was synthesized. This proton carrier is a lipophilic isologue of the highly basic oxazine-dye molecule Nile Blue (see Figure 1). The native compound usually exists in the protonated, singly charged form in aqueous solutions at pH values below about 10 (*36*). The high basicity of the corresponding neutral chromoionophore ETH 5294 (L) is required in view of an adequate competition between the mediated H^+-ion uptake and the complexation of the other primary ion M^{z+} (here Ca^{2+}) by the second neutral carrier (here ETH 1001 ($\bar{L}$)) of the optode membrane system.

Such a membrane is expected to favor the following ion-exchange reaction between the aqueous sample solution (aq) and the organic membrane phase (org), assuming a 1:2 ion/ligand complex for Ca^{2+} and ETH 1001 (*27*)

$$\mathrm{Ca\bar{L}_2}^{2+}(\mathrm{org}) + 2\,\mathrm{H}^+(\mathrm{aq}) + 2\mathrm{L}(\mathrm{org}) \rightleftharpoons \mathrm{Ca}^{2+}(\mathrm{aq}) + 2\,\mathrm{HL}^+(\mathrm{org}) + 2\mathrm{\bar{L}}(\mathrm{org})$$

The corresponding equilibrium constant K_{exch} depends on the stability constants β of the ionophore complexes and on the ionic distribution coefficients k (*17*)

$$K_{\mathrm{exch}} = \frac{(a_{\mathrm{Ca}^{2+}})[\mathrm{HL}^+]^2[\mathrm{\bar{L}}]^2}{[\mathrm{Ca\bar{L}_2}^{2+}](a_{\mathrm{H}^+})^2[\mathrm{L}]^2} = \frac{(\beta_{\mathrm{HL}^+}k_{\mathrm{H}^+})^2}{(\beta_{\mathrm{Ca\bar{L}_2}^{2+}}k_{\mathrm{Ca}^{2+}})} \quad (1)$$

where the activities a_i of species i refer to the aqueous solution and the concentrations [i] to the membrane which is assumed to behave as an ideal phase.

With $[\mathrm{R}^-]$ as the net concentration of negative sites in the membrane, the electroneutrality condition leads to the following concentration relationship:

$$[\mathrm{HL}^+] + 2[\mathrm{Ca\bar{L}_2}^{2+}] = [\mathrm{R}^-] \quad (2)$$

If the concentration ratios of uncomplexed relative to total ionophore present in the membrane phase are denoted by α and α', respectively, one can substitute

$$[\mathrm{L}] = \alpha L_{\mathrm{T}};\ [\mathrm{HL}^+] = (1 - \alpha)L_{\mathrm{T}} \quad (3a,b)$$

$$[\mathrm{\bar{L}}] = \alpha'\bar{L}_{\mathrm{T}};\ 2[\mathrm{Ca\bar{L}_2}^{2+}] = (1 - \alpha')\bar{L}_{\mathrm{T}} \quad (4a,b)$$

where L_{T} and $\bar{L}_{\mathrm{T}}$ are the respective total concentrations in the optode membrane.

The absorbance A of this system obviously is related to the fraction α of the uncomplexed species L

$$A = A_1\alpha + A_0(1 - \alpha) \quad (5)$$

$$\alpha = (A - A_0)/(A_1 - A_0) \quad (5a)$$

where A_1 and A_0 are the limiting absorbance values for $\alpha = 1$ and $\alpha = 0$, respectively.

Equations 1–3 and R_{T} as the total net concentration of negative sites are used for deriving the final response function

$$\frac{(\alpha^2/2)\{R_{\mathrm{T}} - (1 - \alpha)L_{\mathrm{T}}\}}{[1 - \alpha]^2[\bar{L}_{\mathrm{T}} - \{R_{\mathrm{T}} - (1 - \alpha)L_{\mathrm{T}}\}]^2} = \frac{1}{K_{\mathrm{exch}}}\frac{(a_{\mathrm{Ca}^{2+}})}{(a_{\mathrm{H}^+})^2} \quad (6)$$

As was shown previously (*15–18, 26*) in a more general description this highly selective optical membrane exhibits an absorbance response that depends on the ratio of sample activities for the two ionic species involved.

It is evident from eq 6 that, even when ideally selective ionophores could be applied in the present optode membranes, an optical determination of Ca^{2+} activities becomes possible only if the pH value of the sample is either measured simultaneously (e.g. by means of a pH-sensitive optode) or adjusted by an appropriate buffer solution. Correspondingly, as in the case of ion-selective electrode measurements, the determined activities depend on nonthermodynamic assumptions, conventions, or standardization procedures involved in the establishment of ion activity values for calibration and buffer solutions.

Table I. Selectivity Coefficients $K^{\mathrm{opt}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$ of the Ca^{2+}-Selective Optode Membrane for Interfering Ions N^{z+} in Comparison with Corresponding Potentiometric Selectivities $K^{\mathrm{pot}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$ Using the Separate Solution Method (SSM) Approach

ion N^{z+}	log $K^{\mathrm{opt}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$ (optode, this work)	log $K^{\mathrm{pot}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$ (electrode, ref 27)
K^+	−3.8	−3.9
Na^+	−3.6	−3.5
Li^+	−3.1	
Mg^{2+}	−4.1	−5.5

When interfering cations (N^{z+} and $J^{\nu+}$) compete with the primary ions for the complexation of the corresponding ionophores, the activities in eq 6 have to be replaced by sums of selectivity weighted activities (*17, 18*)

$$\mathrm{LHS\ [eq\ 6]} = \frac{1}{K_{\mathrm{exch}}}\frac{[a_{\mathrm{Ca}^2} + \sum_{\mathrm{N}} K^{\mathrm{opt}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}a_{\mathrm{N}^{z+}}^{2/z+}]}{[a_{\mathrm{H}^+} + \sum_{\mathrm{J}} K^{\mathrm{opt}}_{\mathrm{H}^+\mathrm{J}^{\nu+}}a_{\mathrm{J}^{\nu+}}^{1/\nu+}]^2} \quad (7)$$

This semiempirical extension of eq 6 is in analogy to the terms arising in the Nicolsky–Eisenman equation for ion-selective electrode potentials (*27–34*). The selectivity factors of the present optode membrane ($K^{\mathrm{opt}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$, separate solution method (SSM)) are presented in Table I in comparison with the corresponding selectivities of a Ca^{2+}-selective electrode ($K^{\mathrm{pot}}_{\mathrm{Ca}^{2+}\mathrm{N}^{z+}}$) based on the same ionophore. The agreement is convincing, except for the optical Mg^{2+} measurement, which is apparently affected by a Tris buffer interference. In contrast to a previously described optode membrane (*18*) which formally exhibited a response to ammonia, the present system is not expected to be sensitive to electrically neutral, ionogenic species.

Figure 3 shows the absorption spectra in the visible range, as obtained for the present Ca^{2+}-selective optode membrane after equilibration with pH-buffering solutions containing different concentrations of $CaCl_2$. Evidently, the maximum change in absorbance with varying sample concentration is observed at a wavelength of 660 nm.

In Figure 4 the absorbance values measured at 660 nm are given as a function of log $(a_{\mathrm{Ca}^{2+}}/a_{\mathrm{H}^+}{}^2)$. The hydrogen ion activities were measured with a pH glass electrode. The Ca^{2+} ion activities in Figure 4a were determined with a Ca^{2+}-selective electrode based on the ionophore ETH 1001. The calibration curves of the optode membrane show no significant differences between two buffer systems of different pH values and different ionic strengths I (citrate buffer pH = 6.5, I = 0.0087; acetate buffer pH = 5.3, I = 0.163). In Figure 4b, which exhibits the absorbance values measured in the presence of a constant sodium acetate background, the Ca^{2+} activities were calculated by using a Debye–Hückel formalism (*37*). Curve II was calculated according to eq 6 using 7.1 for log K_{exch}, whereas the calculation of curve I is based on eq 7, taking the Na^+ interference (log $K^{\mathrm{opt}}_{\mathrm{Ca}^{2+}\mathrm{Na}^+} = -3.6$; fixed interference method (FIM)) into account.

Apparently, the new chromoionophore ETH 5294 does not fulfil the basicity requirements for a sufficiently strong competition with the highly selective Ca^{2+} ionophore at a physiological pH. This is in contrast to the previously described

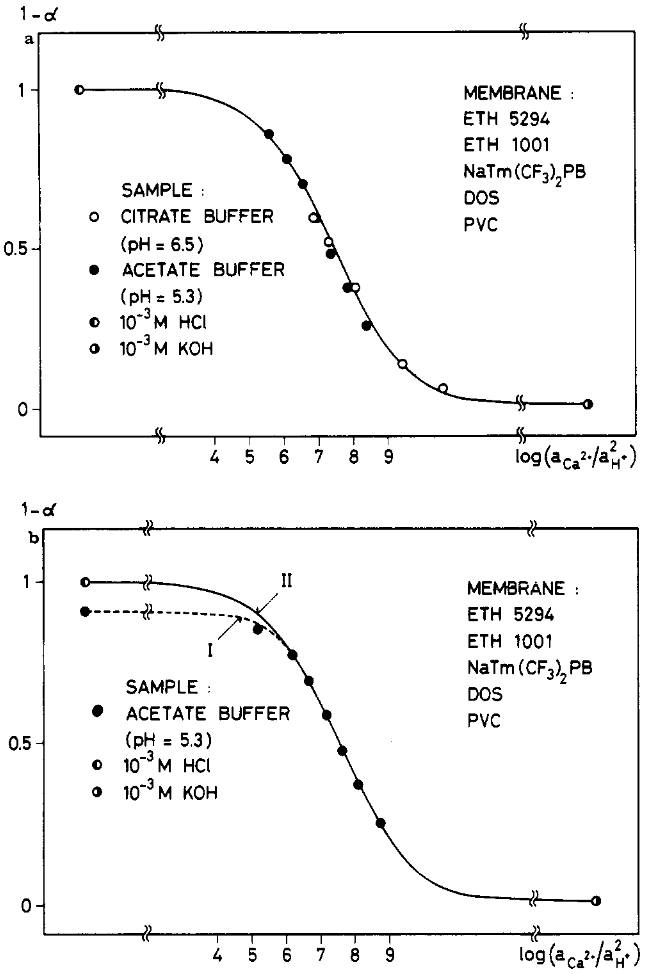

Figure 4. (a) Absorbance values at 660 nm of the optode membrane as a function of log ($a_{Ca^{2+}}/a_{H^+}{}^2$) in two different buffer systems (citrate buffer, pH 6.5; acetate buffer, pH 5.3). The ion activities were derived from measurements with a Ca^{2+}-selective electrode and a pH glass electrode, respectively. The curve fitting the experimental points was calculated from eqs 5 and 6 by using log K_{exch} = 7.1. (b) Absorbance values at 660 nm of the optode membrane as a function of log ($a_{Ca^{2+}}/a_{H^+}{}^2$) in a sodium acetate buffer solution of pH 5.3. The Ca^{2+} activities were calculated according to a Debye–Hückel formalism. The hydrogen-ion activity was estimated with a pH glass electrode. Curve I was calculated from eqs 5 and 7 by using log K_{exch} = 7.1 and log $K^{opt}_{Ca^{2+}Na^+} = -3.6$; curve II was calculated as in part a from eqs 5 and 6 with log K_{exch} = 7.1.

optode membranes based on the macrotetrolides nonactin/monactin in combination with the same chromoionophore (*18*). For that sensor system a linear response range was achieved for NH_4^+ activities between about 10^{-5} and 10^{-3} M (at pH 7.35). Obviously, the extent of complexation of the ionophores involved should as far as possible be matched for an improvement of the response characteristics.

Figure 5 illustrates the absorbance response vs time recordings for the Ca^{2+}-selective optode membrane when it was exposed to repeated concentration step changes between 10^{-3} and 3×10^{-4} M $CaCl_2$ solutions (acetate buffer of pH 5.9). The results document a remarkably high reproducibility of the optical signals. The mean absorbance values with standard deviations, as obtained from the measured signals after 1 min, are 0.2013 ± 0.0005 for the four upper traces, and 0.1604 ± 0.0003 for the five lower traces. The precision of these absorbance determinations would correspond to a standard deviation of the derived activity values $a_{Ca^{2+}}$ of <1.5%.

On the other hand, Figure 5 also shows that the equilibration times for the Ca^{2+}-selective membranes are in the second-range and therefore comparable to those found for a previously described NH_4^+-selective optode membrane of the same type (*18*). The response time compares favorably with the theoretically expected value (*17*) which is related to the typical diffusion time within a 2.5 μm thick plasticized PVC membrane.

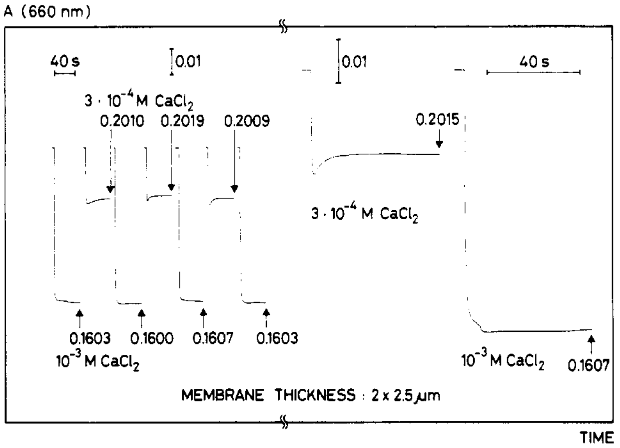

Figure 5. Short-time reproducibilities of the absorbance response of two 2.5 μm thick membranes for sample changes between 10^{-3} and 3×10^{-4} M $CaCl_2$ (acetate buffer, pH 5.9).

The absorbance signal at a wavelength of 660 nm for the optode membrane in contact with a 3×10^{-4} M $CaCl_2$ acetate buffer solution (pH 5.3) was recorded during a time period of 15 h. The absorbance values for the calculation of the average value 0.3367 and the standard deviation 0.0005 (which correlates with the instrument stability) were taken every 30 min ($n = 31$). Before and after this long-time measurement the absorbance spectra taken were nearly identical; a slight decrease in intensity of only about 1% was observed at 660 and at 614 nm.

Further fundamental studies as well as research work aiming at the development of similar optical membranes with selectivity for other ions and their application in different sensor systems are in full progress.

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Fabrication of Pyrolytic Carbon Film Electrodes by Pyrolysis of Methane on a Machinable Glass Ceramic

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Pyrolytic carbon film electrodes were fabricated by low-temperature (850 °C) pyrolysis of methane onto a machinable glass ceramic material called Macor. As determined by cyclic voltammetry, good charge transfer characteristics were displayed by the electrodes without pretreatment. For the ferro/ferricyanide redox couple, heterogeneous rate constants on the order of 2×10^{-3} cm/s were obtained. Slight rate enhancements were observed after electrochemical pretreatment. Results obtained for the oxidation of dopamine and the reduction of methyl viologen were comparable to those obtained with a commercially available glassy carbon electrode. Electrodes were also fabricated at ca. 1000 °C with a simple Fisher burner arrangement, but electrochemical pretreatment was required before reliable performance was obtained. Electrode surfaces were renewable, since films could be burned off at temperatures greater than 650 °C and a fresh film could then be deposited on the substrate. Scanning electron microscopy indicated similar surface morphologies for films fabricated at 850 and 1000 °C. A pronounced change in surface morphology of the substrate was apparent after heating to 1000 °C.

INTRODUCTION

Carbon films, useful as electrode surfaces, can be produced by pyrolysis of a carbonaceous gas onto a suitable substrate. A variety of materials have been employed as the substrate, including glassy carbon (*1*), graphite (*2–5*), porcelain ceramics (*6*), and quartz (*7, 8*). Deposition parameters believed to be important to the properties of the pyrolytic carbon film are time allowed for pyrolysis, temperature, partial pressure, and flow rate of the carbonaceous gas (*3*). Pyrolysis times as brief as a few minutes have been employed (*7*), although periods of several hours are more routine. The temperature of pyrolysis is typically between 1000 and 1200 °C. Pyrolysis is ordinarily performed in a tube furnace although a methane/oxygen torch (*7*) and a Bunsen burner (*8*) can also be used for quartz substrates. Recently, the low-temperature pyrolysis of ethylene on nickel has been reported (*9*). Apparently, nickel acts as a catalyst, allowing carbon film formation at temperatures as low as 550 °C. Frequently, pure methane or ethylene has been used as the source gas, although successful pyrolysis has been reported for 10% methane in argon (*3*). The flow rate of the source gas over the heated substrate, when noted in the literature reports, has been relatively fast, i.e. 200–500 mL/min.

The charge transfer characteristics of pyrolytic carbon film (PCF) electrodes have been found to be similar but slightly inferior to those of glassy carbon (GC) electrodes. Differences between PCF and GC electrodes may be due to structural differences at the atomic level, such as differences in orientation of the graphite crystallites and/or the identity and extent of formation of surface functional groups. It is widely believed that these factors affect the charge transfer characteristics of all types of carbon electrodes (*10–20*). Like other carbon electrodes, the activity of PCF electrodes is sensitive to electrochemical pretreatment.

We report the fabrication of PCF electrodes by pyrolysis of methane onto a glass ceramic substrate called Macor. Macor is a unique ceramic material, in that it can be machined to tight tolerances by ordinary high-speed tools. Macor can be used immediately after machining, since no subsequent firing is required, unlike other ceramics, which are machinable only in the "green" or unfired state. Macor is nonporous and has a very low helium permeation rate in a vacuum. It is a good insulator material and is chemically inert (*21*).

Two slightly different pyrolysis methods were employed. One method involved deposition in a tube furnace at 850 °C (the maximum which was provided by the available furnace). This temperature is significantly lower than pyrolysis temperatures reported in the literature for noncatalytic surfaces. Pyrolysis times were relatively long, on the order of 10–20 h. Alternatively, a simple Fisher burner arrangement was employed. In this case, the temperature of pyrolysis was fixed at ca. 1000 °C by the flame temperature. Shorter pyrolysis times of 1–5 h were employed. In both methods, natural gas from the common laboratory line was employed as the source gas.

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