Evanescent-Wave Spectroscopy on Bulk-Response Optode Membranes

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Abstract. The absorbance of a solvent polymeric bulk optode membrane in contact with sapphire as a wave-guide is studied with the attenuated total reflection (ATR) technique. Utilizing thin (≤ 1 μm) and, therefore, fast-responding membranes, high values of absorbance are obtained by multiple internal reflection (MIR). The approach has an advantage over conventional transmission studies in that the light beam does not pass through the sample solution. Sample-induced swelling of the membrane may, however, create problems.

1. Introduction

Recently, a quite generally applicable concept for sensing ions as well as certain neutral analytes by bulk optode membranes has been described [1–3]. It is fundamentally suitable for transmission and reflectance as well as luminescence measurements [1–3]. With only few exceptions, absorbance measurements have been carried out using conventional spectrophotometers [4–7] or photodiodes coated with such bulk optode membranes [8][9]. For practical applications, inaccuracies arising from sample-induced scattering and/or absorption changes in the transmission mode were compensated by referencing to a blank membrane [10–13]. Fluorescence studies on the same systems [14] and reflectance measurements on similar but not reusable reagent layers [15] have been described. Here, we report on a preliminary study of evanescent-wave spectroscopy (for further literature see [16–20]) applied to Ca^{2+}-selective optode membranes which were previously characterised in the transmission mode [7][11].

2. Experimental

2.1. Membranes

Membranes were prepared as described in [7] by dissolving in 2 ml of freshly dis-tilled THF, 15.4 mg of the ionophore (–)-(R,R)-N,N'-[bis(11-ethoxycarbonyl)undecyl]-N,N',4,5-tetramethyl-3,6-dioxaoctanediamide ($C_{38}H_{72}N_2O_8$; ETH 1001), 3.85 mg of the chromoionophore 11-[(1-butylpentyl)oxy]-11-oxoundecyl 4-{[9-(dimethylamino)-5H-benzo[a]phenoxazin-5-ylidene]-amino}benzeneacetate ($C_{46}H_{59}N_3O_5$; ETH 2439), 6.6 mg of anionic sites, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ($C_{32}H_{12}BF_{24}$Na; NaTm(CF$_3$)$_2$PB), 157 mg of plasticizer, bis(2-ethylhexyl) sebacate ($C_{26}H_{50}O_4$; DOS), and 75 mg of poly(vinyl chloride) (high molecular weight PVC), all *Selectophore*® from *Fluka AG*, Buchs, Switzerland.

A 0.2 ml portion of this soln. was brought onto a rotating, dust-free sapphire plate (single parallel MIR crystal, 52 mm x 10 mm x 1 mm, 30 internal reflections, angle of incidence 60°, refractive index 1.768 at 20°; *Victor Kyburz AG*, Safnern, Switzerland). This plate had been placed in a closed vessel saturated with THF, which formed part of a spin-on device [21] (rotating frequency 800 rpm) allowing the preparation of membranes with controlled and reproducible thickness of 0.5–2 μm. After removing the sapphire plate with the optode membrane from the spin-on device, it was left at r.t. for some h for further drying. The refractive index of the membrane was 1.474 as determined by an *Abbe* refractometer (*Carl Zeiss*, Oberkochen, Württ., Germany) at 20°.

2.2. Optical System

Optical measurements were performed using a *Fourier*-transform microscopic spectrophotometer in the transmission mode (*Hitachi* model U-6000 microscopic FT spectrophotometer, *Hitachi Ltd.*, Tokyo). The light beam, emitted from a 50-W halogen lamp, was focussed *via* a collimator lens on the sapphire crystal at an angle of incidence of 60° (see *Fig. 1*). In the attenuated total reflectance (ATR) mode (evanescent-wave), the absorbance of the sapphire-supported membrane in contact with different sample solns. was studied by measuring the output of the light intensity with the above spectrophotometer. For that purpose, the membrane (provided with a *Teflon*® spacer of 0.2 mm thickness) formed one wall of a thermostated (25°) flow-through cell of 36 μl volume, through which the sample soln. was driven (see *Fig. 1*) by a peristaltic pump (11 ml/h).

2.3. Reagents

Doubly quartz-distilled H_2O and chemicals of *p.a.* grade were used throughout. CaCl$_2$ solns. were prepared with a NaOAc buffer of pH 3.65.
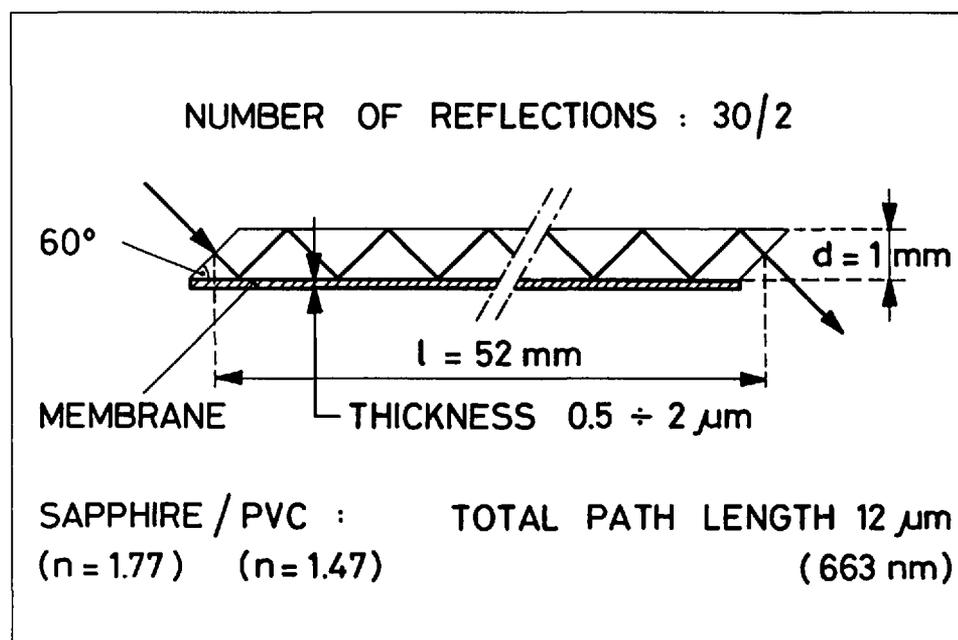
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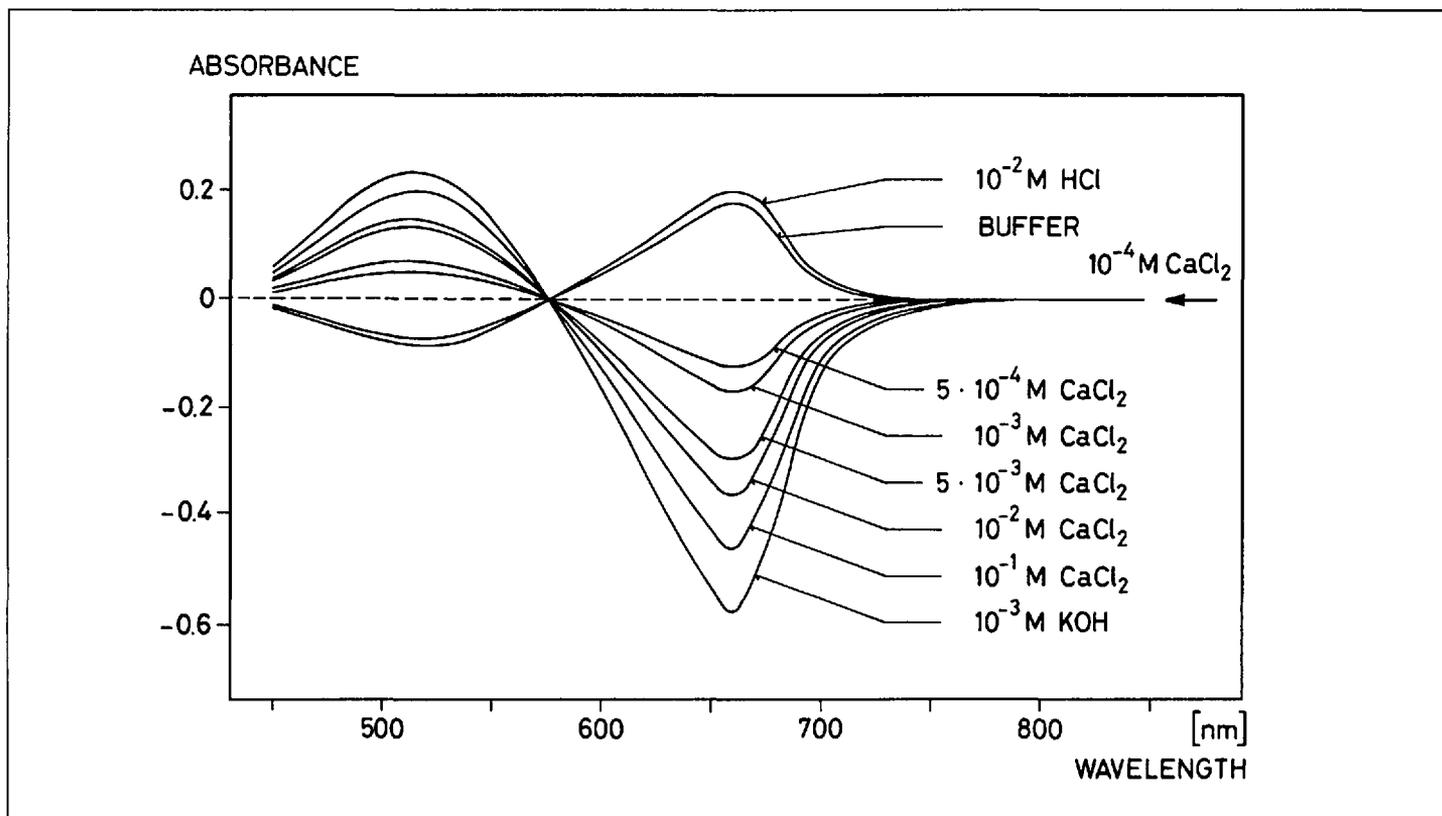Universitätstrasse 16
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Fig. 1. *Schematic diagram of sapphire wave-guide (ATR) in contact with an ion-selective PVC optode membrane*

Fig. 2. *Normalized evanescent-wave spectra taken in the ATR approach on a Ca^{2+}-selective optode membrane in contact with different sample solutions* (see *Experimental*)
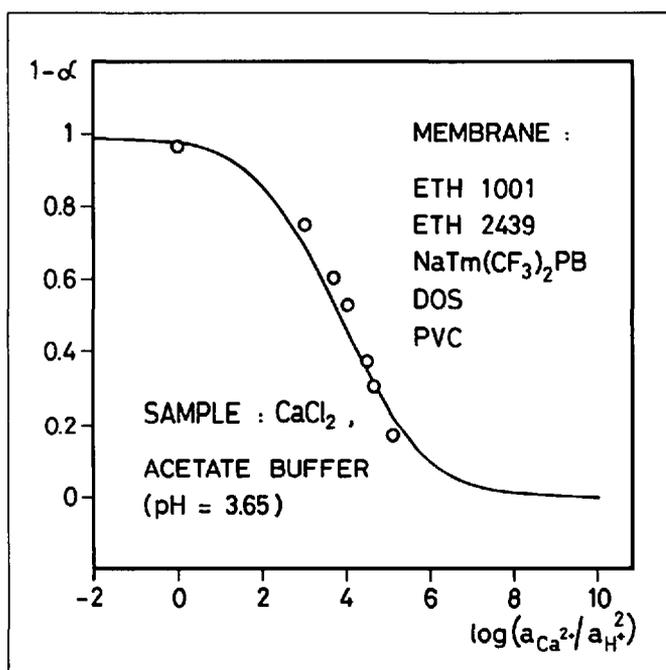

Fig. 3. *Experimental evanescent-wave response in comparison with computed curve (solid line).* 1–α: Degree of protonation.

3. Results and Discussion

For the present study, a membrane was used containing a selective ionophore for Ca^{2+} and another one for H^+ (chromoionophore) in a relatively thick organic phase [7]. Upon extraction of one Ca^{2+} from the sample solution, two H^+ are released from the membrane phase. Consequently, the chromoionophore changes its optical spectrum according to the degree of protonation [1–3].

Such a bulk-response optode membrane, extracting analytes from the sample solution, was put in contact with a sapphire crystal acting as an optical waveguide (see *Fig. 1*). For a light beam ($\lambda = 663$ nm) at an angle of incidence of 60° and refractive indices of 1.768 and 1.474 for sapphire and PVC membrane, respectively (see *Experimental*), an effective path length of 831 nm (hypothetical thickness averaged for perpendicular and parallel polarized light) is calculated for each re-

flectance [18–20]. A crystal of 52 mm length therefore provides an effective path length of 15 times 831 nm ≡ 12 μm (one side of sapphire plate; number of reflections: 30/2). With membranes of *ca.* 1 μm thickness, membrane-inherent response times of *ca.* 1 s have been obtained [7]. The response time of a 12 μm thick membrane in the transmission mode would be at least 100 times higher, although the absorbance observed in the ATR approach described here remains comparable. Therefore, thin membranes can be used in the MIR mode to produce sensors of fast response, simultaneously exhibiting high absorbances. Furthermore, in the evanescent-wave spectroscopy, the light beam does not pass through the sample solution if the membrane is sufficiently thick (in the present case: ≥ 253 nm, penetration depth of the electromagnetic field vector in a bulk membrane phase [20]).

From measurements in the transmission mode on a DOS/PVC membrane containing the chromoionophore ETH 2439, molar decimal absorption coefficients of $7.1 \cdot 10^4 \, l \, mol^{-1} \, cm^{-1}$ (at 656 nm) and $3.2 \cdot 10^4 \, l \, mol^{-1} \, cm^{-1}$ (at 512 nm) were calculated for the absorption maxima of protonated and deprotonated form, respectively. Based on these values, such a membrane of only 1 μm thickness should give an absorbance of *ca.* 0.15.

Evanescent-wave spectra obtained with a Ca^{2+}-selective optode membrane of similar thickness (see *Experimental*) in contact with different sample solutions are

given in *Fig. 2*. They are normalized for 10^{-4} M $CaCl_2$ solutions and the isosbestic point at 570 nm. The absorbance difference between protonated (in 10^{-2} M HCl) and deprotonated (in 10^{-3} M KOH) form of the chromoionophore is 0.859 for the ATR system described here (*Fig. 1*), corresponding to 0.072 for a membrane of *ca.* 1 μm thickness in the conventional transmission mode.

When plotting the degree of protonation, $1-\alpha$, of the chromoionophore against the ratio of activities, $a_{Ca^{2+}}/a_{H^+}$ (for details, see [7][21]), the sigmoidal shape of the theoretically predicted response curve [1–3] is corroborated (*Fig. 3*), although the deviations of the experimental data points from the computed curve are larger than those observed in conventional absorption studies [4][7].

In contrast to transmission measurements, where a swelling of the membrane due to contact with different sample solutions does not cause problems, in the evanescent-wave approach a decrease in absorbance is expected with increasing membrane volume. Indeed, baseline shifts of up to 6% in absorbance can be observed when changing from 10^{-1} to 10^{-4} M $CaCl_2$ sample solutions. In the conventional transmission mode, however, the corresponding shifts are well below 1%.

At a sample flow rate of 11 ml/h (10^{-4} M $CaCl_2$, buffered at pH ~3.6), the signal stability (at 663 nm) for the system described here amounts to ± 0.002 and ± 0.005 in absorbance with and without optode membrane, respectively. The reproducibility of the signal is 1.6% (SD, $n = 7$) when changing from 10^{-4} to 10^{-3} M $CaCl_2$ solutions, and the signal stability within 1 h is 0.8%.

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