114. Enantioselective Optode Membranes with Enantiomer Selectivity for (*R*)- and (*S*)-1-Phenylethylammonium Ions

by **Petr Holý**[1]), **Werner E. Morf**, **Kurt Seiler**, and **Wilhelm Simon***

Swiss Federal Institute of Technology (ETH), Department of Organic Chemistry, Universitätstrasse 16,
CH-8092 Zürich

and **Jean-Pierre Vigneron**

College of France, Chemistry of Molecular Interactions, 11, place Marcelin Berthelot, F-75005 Paris

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The recently developed general principle of ion-selective optode membranes, based on conventional ionophores, was employed to design a new approach to the determination of enantiomeric excess. Highly lipophilic chiral crown derivatives, exhibiting a high selectivity for a chiral ammonium guest cation, are combined with a H^+-selective chromoionophore in a plasticized PVC membrane. The enantiomer recognition process is translated by this sensor with an optical transduction into a signal easily measured by a conventional spectrophotometer, and enantiomeric excess can be determined without the need for polarized light. Characteristics and a theoretical description of these new optode membranes are given.

Introduction. – Enantiomeric ionophores that exhibit a remarkable enantiomer-selective recognition in membranes were pioneered mainly by the groups of *Prelog* [1–4], *Lehn* [5–11], *Cram* [12–18], and others [19–23]. These carrier molecules interact preferentially with one enantiomeric form of certain biogenic organic ammonium ions, when both forms are present in an aqueous solution in contact with the membrane.

A straightforward investigation of the selectivity behavior of enantiomeric ionophores was obtained by incorporating these molecules as active membrane components into liquid membranes, and by studying the emf response of corresponding potentiometric electrode cells to solutions of enantiomeric ions [24–31]. Of the many ionophores tested by this method, the highest selectivities were found for crown-ethers designed by *Cram*, having incorporated 1,1′-bi-2-naphthol as a chiral unit. The (*S*,*S*)-isomer of the crown ether containing two binaphthol units [15] has shown a preference for protonated methyl (2*S*)-2-amino-2-phenylethanoate over the (2*R*)-isomer by a factor of 4.2 [25]. More recently, the same enantiomeric ions have been even better recognized by a crown-ether with one 3,3′-diphenyl-1,1′-binaphthyl unit [18]. For this ligand, an enantiomer-selectivity factor of 13.1 has been potentiometrically determined [31]. With the (+)-isomer of a chiral tetracarboxamide derivative of 18-crown-6, synthesized by *Lehn* and coworkers [7], which incorporates two (*R*,*R*)-tartaric-acid units, a preference for (+)-(*R*)-1-phenylethylammonium ((+)-(*R*)-PEA) ions over the (–)-(*S*)-enantiomeric ions by a factor of 2.7 has been measured [26] [30].

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[1]) On leave from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo nám. 2, 16610 Prague 6, Czechoslovakia.

An especially interesting approach is the measurement of the potential difference between two membrane electrodes with the opposite enantiomer selectivity [28–30]. If the compositions of the electrode membranes differ only in the chiral form of the ionophore used, the measured electrode potential difference gives a nearly linear response to the enantiomeric excess (ee) of the selected substrate ions in the aqueous sample [29] [30].

Recently, we reported on the design and realization of novel ion-sensitive optode membranes based on ionophores [32–37]. They make use of so-called chromoionophores that drastically change their optical properties upon ion complexation. One class of these membranes, acting as competitive ion-exchange system, generally incorporates a combination of conventional ionophores, chromoionophores, and lipophilic ionic sites.

Here, we report, on the theoretical basis, the development and the response behavior of enantiomer-selective optode membranes. An optical sensing device for measuring the enantiomeric excess of PEA ions is presented. An intriguing feature is that such sensors exhibit an optical response to the ee value without the need for polarized light.

Theoretical. – An exhaustive treatment of the performance characteristics of optode membranes of the type discussed here was set forth already [34]. The membranes behave as ion-exchange systems which are based on the ion-selective action of conventional electrically neutral ionophores in combination and competition with chromoionophores selective for a different sort of ions. In the use of chromoionophores that drastically change their absorption spectrum upon ion complexation, the observed absorbance A at a given wavelength can be described by:

$$A = A_0 \cdot (1 - \alpha) + A_1 \cdot \alpha \tag{1}$$

or

$$\alpha = (A - A_0)/(A_1 - A_0) \tag{1a}$$

where α denotes the relative amount of uncomplexed chromoionophore, and A_0 and A_1 are the limiting absorbance values found for $\alpha = 0$ (full complexation) and for $\alpha = 1$ (no complexation), respectively. In the present case, the membrane phase contains, in addition to a H^+-selective chromoionophore, one optical isomer of a chiral ionophore which either prefers the (+)-(R) over the (–)-(S)-enantiomer of PEA ion (type **a**) or *vice versa* (type **b**).

Since these ionophores form 1:1 complexes with the respective cations, and other ions which may interfere are absent, the basic results for the response of ideally composed optical membranes to solutions of both pure enantiomers (R and S) of PEA ions are given by [34]:

$$\alpha^2/(1 - \alpha)^2 = K_{\mathrm{exch}} \cdot a_{\mathrm{R}}/a_{\mathrm{H^+}} \tag{2a}$$

$$\alpha^2/(1 - \alpha)^2 = K_{\mathrm{exch}} \cdot a_S/a_{\mathrm{H^+}} \tag{2b}$$

K_{exch} is the overall equilibrium constant for the ionophore-induced ion-exchange process, a_R, a_S, and $a_{\mathrm{H^+}}$ are the activities of ions in the aqueous solution contacting the membrane.

In the case of a mixture of enantiomeric PEA ions contacting the membranes, the *Eqns. 2a* and *2b* must be extended:

$$\alpha^2/(1-\alpha)^2 = K_{\mathrm{exch}} \cdot (a_R + K^{\mathrm{opt}} \cdot a_S)/a_{\mathrm{H}^+} \tag{3a}$$

$$\alpha^2/(1-\alpha)^2 = K_{\mathrm{exch}} \cdot (a_s + K^{\mathrm{opt}} \cdot a_R)/a_{\mathrm{H}^+} \tag{3b}$$

$K^{\mathrm{opt}} < 1$ is the common coefficient for the selectivity exhibited by either of the optode membranes for the discriminated relative to the preferred enantiomeric ion. Such enantiomeric selectivity coefficients formally correspond to the optical selectivity coefficients for interfering ions [34] [35].

The value of K^{opt} can be determined in separate measurements with solutions of pure enantiomers for both membranes (separate-solution method). It has been shown earlier [34] [35] that the optical selectivity coefficients correlate favorably with the selectivity coefficients determined potentiometrically, if only 1:1 complexes with only monovalent ions are formed.

Due to the definition of K^{opt}, the same value should theoretically be obtained for both membranes. In such ideally matched optical systems, a direct measurement of ee can easily be performed. The ee value can be determined from the differential absorption of both membranes in contact with the same sample solution. For this purpose, *Eqns. 3a* and *3b,* which describe the membrane responses, can be rearranged to give the following expressions:

$$\alpha^2/(1-\alpha)^2 = B \cdot (1 + \kappa^{\mathrm{opt}} \cdot \mathrm{ee}) \tag{4a}$$

$$\alpha^2/(1-\alpha)^2 = B \cdot (1 - \kappa^{\mathrm{opt}} \cdot \mathrm{ee}) \tag{4b}$$

where ee is the enantiomeric excess in the aqueous sample, defined as

$$\mathrm{ee} = (a_R - a_S)/(a_R + a_S) \tag{5}$$

κ^{opt} is evidently a selectivity measure for the optode membrane in response to the enantiomeric excess, given by

$$\kappa^{\mathrm{opt}} = (1 - K^{\mathrm{opt}})/(1 + K^{\mathrm{opt}}) \tag{6}$$

and B is a weighted activity-ratio expression:

$$B = K_{\mathrm{exch}} \cdot (1 + K^{\mathrm{opt}}) \cdot (a_R + a_S)/(2\, a_{\mathrm{H}^+}) \tag{7}$$

In fact, according to *Eqns. 4a* and *4b*, the parameter B is directly related to the value α_0 of the absorbance function established for ee = 0, *i.e.* for racemic sample solutions. It can be demonstrated (see also below) that the highest sensitivity of the present optode membranes to the enantiomeric excess results, when the following experimental conditions are chosen:

$$B \approx 1, \alpha_0 \approx 1/2 \tag{8}$$

In this case, the following approximate description can finally be derived from *Eqns. 4a* and *4b* by expanding into a series and ignoring the higher-order terms. The results for membrane **a** reads:

$$\begin{aligned} \alpha \approx 1/2 &+ (1/8) \cdot \kappa^{\mathrm{opt}} \cdot \mathrm{ee} \\ &- (1/16) \cdot (\kappa^{\mathrm{opt}} \cdot \mathrm{ee})^2 \\ &+ (5/128) \cdot (\kappa^{\mathrm{opt}} \cdot \mathrm{ee})^3 \end{aligned} \tag{9a}$$

and for membrane **b**:

$$\begin{aligned}\alpha \approx 1/2 &- (1/8) \cdot \kappa^{\mathrm{opt}} \cdot \mathrm{ee} \\ &- (1/16) \cdot (\kappa^{\mathrm{opt}} \cdot \mathrm{ee})^2 \\ &- (5/128) \cdot (\kappa^{\mathrm{opt}} \cdot \mathrm{ee})^3 \end{aligned} \qquad (9b)$$

It becomes immediately evident that the constant and the quadratic terms cancel, if a differential absorbance measurement between the (*S*)-enantiomer-selective membrane and its (*R*)-enantiomer-selective counterpart is carried out. Therefore, neglecting the cubic terms, such combinations of ideally matched optode membranes directly exhibit linear responses to the enantiomeric excess in the sample solutions:

$$\Delta\alpha \approx (1/4) \cdot \kappa^{\mathrm{opt}} \cdot \mathrm{ee} \qquad (10)$$

This behavior is completely analogous to the results reported earlier for corresponding enantiomer-selective electrode assemblies [28–30]. Moreover, the effects caused by the presence of additional interferents in the sample can be treated in the same way. It should finally be noted that the linearity of the response function, $\Delta\alpha$ *vs.* ee, is also maintained, even when the conditions specified in *Eqn. 8* are not adequately adjusted, although, in such cases, a certain reduction in the sensitivity must be taken into account. The general relationship obtained on the basis of *Eqns. 4a* and *4b* after expansion into a series and omission of cubic and higher-order terms assumes the form:

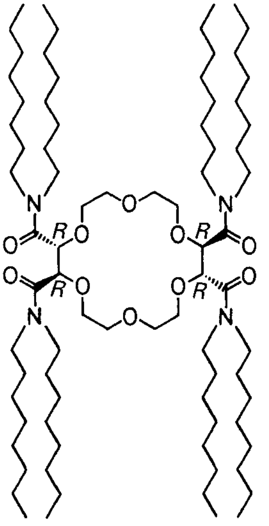
$$\Delta\alpha \approx \alpha_0(1 - \alpha_0) \cdot \kappa^{\mathrm{opt}} \cdot \mathrm{ee} \qquad (11)$$

Hence, the requirement to adjust the value α_0 (for racemic solutions) to the center of the total absorbance range $A_1 - A_0$ turns out to be not that stringent. For example, if $\alpha_0 = 0.4$ or 0.6 instead of 0.5, the numerical factor of 0.25 in *Eqn. 10* is reduced only to 0.24.

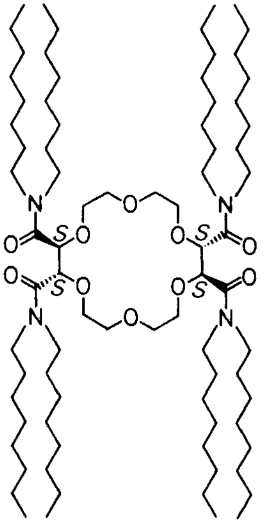
We must keep in mind that the linear response of the membranes to the enantiomeric excess according to *Eqns. 10* and *11*, is an approximation neglecting cubic and higher-order terms in the series (see *Eqns. 9a* and *9b*). The maximum range of $\Delta\alpha$ (for ee from + 1 to − 1) calculated from the original *Eqns. 4a* and *4b* is slightly higher than its approximation by the *Eqn. 10*, but the difference should only be taken into account for ligands with a good enantiomer-selective recognition. The simple linear approximation (*Eqn. 10*) brings the error higher than 0.0028 (this value corresponds to the experimental error coming from the inaccuracy in absorbance reading $\Delta A \pm 0.001$) for $\kappa^{\mathrm{opt}} > 0.32$, that means for ligands with $K^{\mathrm{opt}} < 0.51$. For a narrower range of ee, for example ee ± 0.5, *Eqn. 10* is sufficiently precise for all ligands accessible.

Results and Discussion. – As pointed out in *Theoretical*, the absorbance response of an ion-exchange optode membrane, which incorporates two competing ionophores, depends on the ratio of the corresponding ion activities, in the special case described here on the ratio of the activities of PEA and H^+ ions (*Eqns. 2a* and *2b*). By the use of pH-buffered solutions with an appropriate and constant pH, the absorbance is directly related to PEA acitivities. A Li^+-phosphate buffer was used throughout to minimize an interference by additional buffer cations and, therefore, to avoid a contraction of the limited dynamic range of an optical membrane. Indeed, no interference by Li^+ ions ($c_{Li^+} = 0.02$M) was observed, since these small cations do not meet the requirements for a strong complexation by the crown-ether ionophore with its rather well-defined cavity of coordinating ligand atoms.

To ensure long-term stability of the response of the optical membranes, two new chiral ionophores, *(+)-(2*R,*3*R,*11*R,*12*R*)-*N,N,N′,N′,N″,N″,N‴,N‴*-octaoctyl-1,4,7-10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxamide* (**1a**; *(+)-OOA-18C6*) and its (2*S*,3*S*,11*S*,12*S*)-isomer (**1b**; *(−)-OOA-18C6*), with extremely high lipophilicities were synthesized and combined in the membrane phase with the highly lipophilic chromoionophore *7-(diethylamino)-3-(octadecanoylimino)-1,2-benzophenoxazine* (*ETH 5294*). Two pairs of optical membranes, one incorporating the chiral ligand **1a** and the other the ligand **1b**, were equilibrated with pH-buffered solutions containing different concentrations of (+)-PEA ((*R*)-configuration) and (−)-PEA ((*S*)-configuration) ions. *Fig. 1a* and *1b* represent the absorption spectra for each pair of membranes. The protonated form of the chromoionophore *ETH 5294* shows two absorption bands with maxima at 610 nm and 660 nm and the deprotonated form absorbs at *ca.* 540 nm. A higher concentration of PEA ions leads, therefore, to a decrease in absorbance at 610 and 660 nm. Obviously (+)-(*R*)-PEA ion is prefered by (+)-ionophore **1a** (in the membrane **a**, *Fig. 1a*), whereas (−)-(*S*)-PEA ion is prefered by (−)-ionophore **1b** (membrane **b**, *Fig. 1b*). The recorded spectra are similar to those of the first reported and reversible cation-exchange optical membrane incorporating the same chromoionophore *ETH 5294* and nonactin/monactin as a NH_4^+-selective ligand [35].



1a *((+)-OOA-18C6)*



1b *((−)-OOA-18C6)*

In *Fig. 2a* and *2b*, the changes in absorbance (in $1-\alpha$ values calculated from absorbances at 660 nm using *Eqn. 1a*) for membranes **a** and **b** are given as a function of $\log(a_{PEA}/a_{H^+})$ for both enantiomers of PEA ion. The reliability of the measured values and the theoretical analysis are documented by the nearly perfect fit of the experimental points on the theoretical response according to *Eqns. 2a* and *2b*.

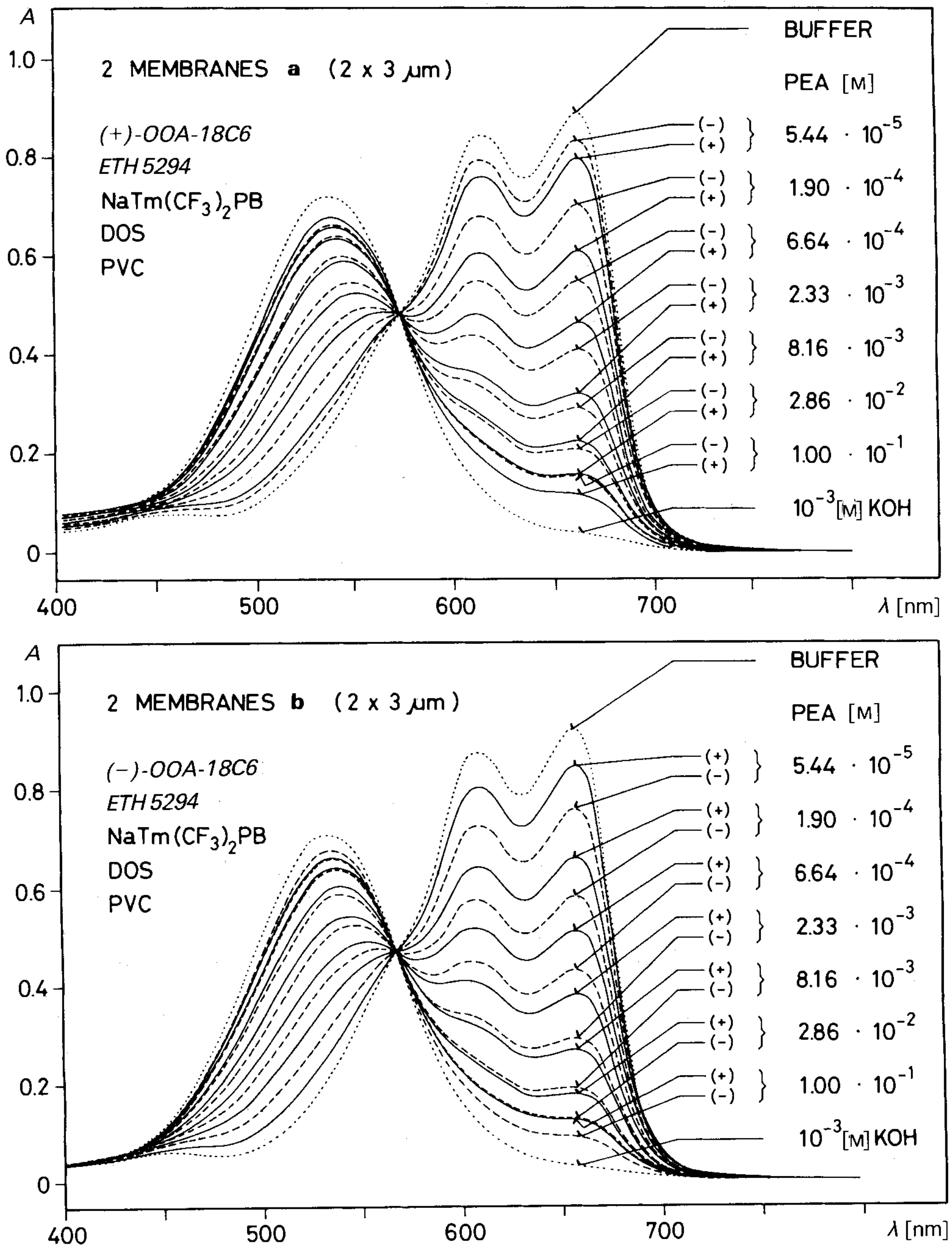


Fig. 1. *Absorption spectra of two 3-μm thick optode membranes* **a** *and* **b**, *after equilibration with pH-buffered solutions* (lithium phosphate buffer, pH 7.0) *containing different concentrations of optically pure (+)-(*R*)- or (−)-(*S*)-PEA chloride*. The protonated form of *ETH 5294* shows two absorbance maxima at 660 and 610 nm and the deprotonated form one band at 540 nm.

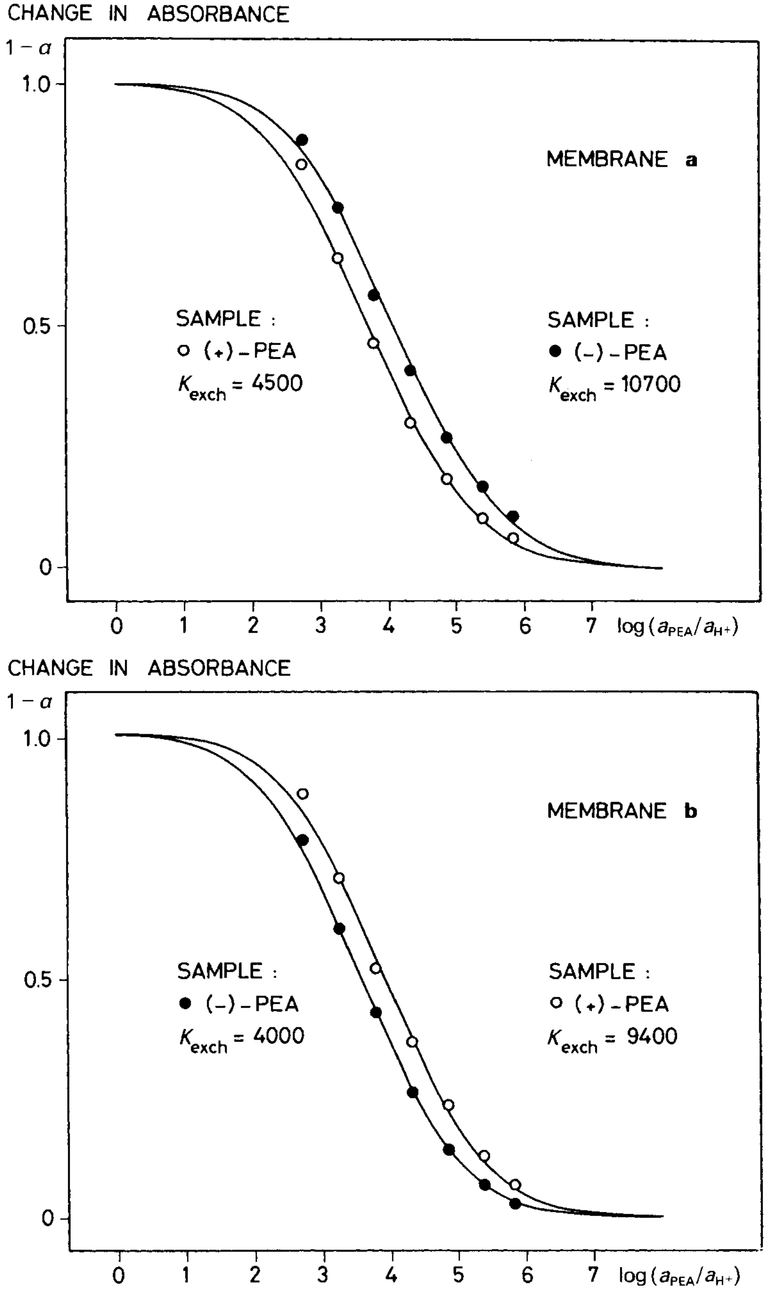


Fig. 2. *Response curves of optode membranes* **a** *and* **b** *on different concentrations of (+)-(R)- and (−)-(S)-PEA ions* (lithium phosphate buffer, pH 7.0). The experimental points were calculated from absorbance values at 660 nm. The activities of PEA ions were obtained using a *Debye-Hückel* formalism and the pH of the solutions was measured by a pH-glass electrode. The K_{exch} values have been obtained by fitting *Eqns. 2a* and *2b* to the experimental points.

The overall equilibrium constants K_{exch} in these equations include the complex formation constants of the H^+-chromoionophore and PEA-ionophore complexes in the membrane phase and the distribution coefficients of the involved ions between the aqueous measuring solution and the membrane phase. For each optode membrane, the K_{exch} values were used to deduce the optical selectivity coefficients K^{opt}, which are exclusively established by the ratio of complex formation constants of the two diastereoisomeric crown ether complexes. For membrane **a**, it reads:

$$K_{RS}^{\mathrm{opt}} = K_{\mathrm{exch}}(_{+,R})/K_{\mathrm{exch}}(_{-,S}) = 4500/10\,700 = 0.421 \tag{12a}$$

and for membrane **b**:

$$K_{SR}^{\mathrm{opt}} = K_{\mathrm{exch}}(_{-,S})/K_{\mathrm{exch}}(_{+,R}) = 4000/9400 = 0.426 \tag{12b}$$

This surprising conformity of the two selectivity coefficients corroborates the symmetry of this system. Hence, this result was promising in view of a determination of ee in the solution of PEA ions. Nine solutions with known ee were prepared volumetrically from pH-buffered solutions of pure enantiomers of PEA chlorides. To ensure an α value close to 0.5 for both pairs of membranes (**a** and **b**) in contact with the racemic solution, a total PEA chloride concentration of $6.36 \cdot 10^{-4}$ M was chosen. The differences in absorbance for both types of membranes were related to the values for racemic solution (α_0^a and α_0^b):

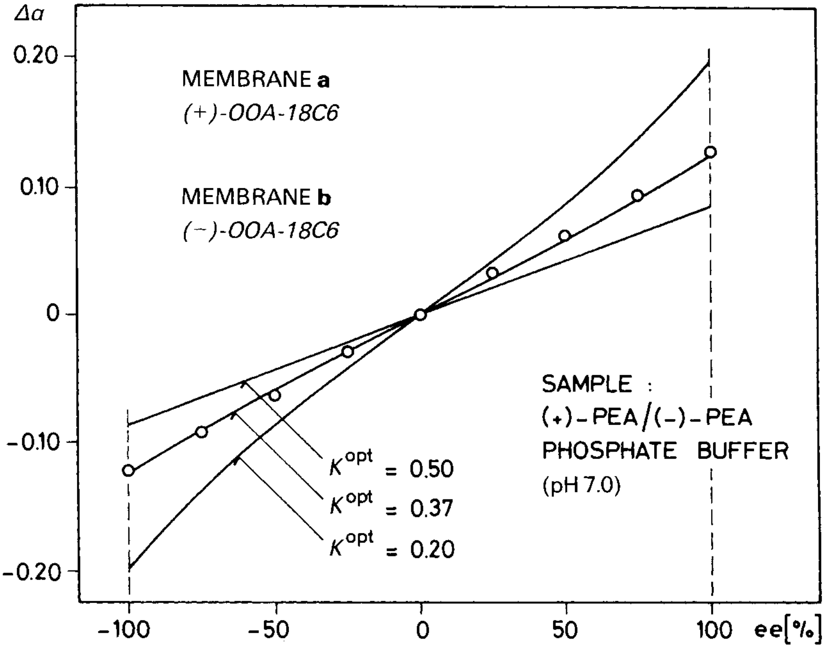


Fig. 3. *Absorbance differences in* $\Delta\alpha$ *(Eqn. 14) of two pairs of optode membranes as a function of the enantiomeric excess* (ee, in %) *of PEA ions*. The solutions of PEA ions were prepared volumetrically from solutions of optically pure PEA chlorides in lithium phosphate buffer (pH 7.0), so that the total concentration in all solutions was $6.36 \cdot 10^{-4}$ M. The theoretical curves were calculated on the basis of *Eqns. 7a* and *7b* and for $K^{\mathrm{opt}} = 0.2$, 0.37 and 0.5.

$$\Delta\alpha^{a} = \alpha^{a} - \alpha_{0}^{a} \tag{13a}$$

$$\Delta\alpha^{b} = \alpha^{b} - \alpha_{0}^{b} \tag{13b}$$

$$\Delta\alpha = \Delta\alpha^{a} - \Delta\alpha^{b} \tag{14}$$

In *Fig. 3*, the differential response of two optical membranes $\Delta\alpha$ is given as a function of the ee (in %), and the experimental points are compared with theoretical curves calculated from *Eqns. 9a* and *9b* for three values of K^{opt}. The experimental data correspond to a value $K^{opt} = 0.37$. This value is comparable to the selectivity coefficient $K^{opt} = 0.42$ determined by the separate-solution method described before and to the potentiometric selectivity coefficient $K^{opt} = 0.39$ found for the both new chiral ionophores used here.

An enhancement of the precision of this ee measurement, which is influenced mainly by the error in absorbance reading ($\Delta A \pm 0.001$ means Δ(ee) ± 2.5%), may be achieved by ionophores with better enantiomer selectivities leading to an extension of the dynamic range due to the higher value of κ^{opt} (see *Theoretical*).

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Experimental Part

Reagents. For aq. solns. doubly quartz-distilled H_2O was used. Buffer solns. were made from $LiOH \cdot H_2O$ (*p.a.*) and orthophosphoric acid (*p.a.*), both from *Fluka AG* (CH–Buchs). Pure enantiomers of 1-phenylethylamine and racemic amine were obtained in *purum* qualities from *Fluka AG*, and their hydrochlorides were prepared by a usual procedure [33]. For membrane preparation, poly(vinylchloride) (PVC, high molecular), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate, bis(2-ethylhexyl)sebacate (DOS), and THF were obtained from *Fluka AG* as well as all reagents and solvents for the synthesis of the chiral ligands (PCl_5, dioctylamine, Et_3N, CH_2Cl_2, pentane, toluene, EtOH).

Apparatus. ^{1}H-NMR spectra were measured on the *Bruker WM-300* spectrometer (frequency 300 MHz), IR spectra were taken on *Perkin-Elmer PE 125*, and FAB-MS spectra on the *VG ZAB2-SEQ* instrument. UV/VIS absorbance measurements and spectra of the PVC membranes were recorded by a *Uvikon* Model *810* double-beam spectrophotometer (*Kontron AG*, CH–Zürich).

Synthesis. Both ligands used in this study, **1a** and **1b**, were prepared according to the same procedure, which is a slightly modified version of an earlier described method [7]. As a starting material for the preparation of **1a**, (+)-(2*R*,3*R*,11*R*,12*R*)-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid was used, and for **1b** the (2*S*,3*S*,11*S*,12*S*)-isomer was taken. The preparation of chiral tetraacids was described in [7].

The preparation of the H^+-selective neutral chromoionophore 7-(diethylamino)-3-(octadecanoylimino)-1,2-benzophenoxazine *(ETH 5294)* is described in [36].

*(+)-(2*R*,3*R*,11*R*,12*R*)-*N,N,N′,N′,N″,N″,N‴,N‴*-Octaoctyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxamide* (**1a**; *(+)-OOA-18C6*). To the stirred soln. of the corresponding (+)-tetraacid (40 mg, 0.084 mmol) in dry CH_2Cl_2 (2 ml), freshly sublimed PCl_5 (72 mg, 0.346 mmol) was added gradually during 12 h. After stirring at r.t. overnight, a clear soln. was obtained, and volatile components were evaporated *in vacuo*. The crude acyl chloride formed a crystalline deposit. Without purification, the crude acid chloride was dissolved in 2 ml of dry CH_2Cl_2 and added dropwise to 2 ml of dry CH_2Cl_2 containing dioctylamine (80 mg, 0.331 mmol) and Et_3N (35 mg, 0.346 mmol). After stirring for 1 h, the soln. was evaporated to dryness, the residue was partitioned between pentane (3 ml) and H_2O (2 ml) and the org. phase washed with dil. HCl and H_2O. Removal of the solvent gave crude **1a** as an oil. The crude product was purified by flash chromatography (silica gel, column 1 × 15 cm, eluent toluene/2% EtOH) to yield pure **1a**. The yield after exhaustive drying (60 h at 313 K, 13 Pa) was 67 mg (60%, oil). $[\alpha]_D^{25} = +35.8$ ($c = 0.504$, $CHCl_3$). IR ($CHCl_3$): 1112, 1468, 1639, 2860, 2932, 2960. ^{1}H-NMR ($CDCl_3$) 0.88, 1.26,

3.3 (3*m*, 136 H, $CON(C_8H_{17})_2$); 3.7 (*m*, 16 H, CH_2O); 4.81 (*s*, 4 OCH). FAB-MS (matrix: 3-nitrobenzyl alcohol): 1334 (3, M^+), 795 (10), 322 (13), 268 (36), 266 (14), 242 (14), 240 (29), 142 (14), 98 (10), 73 (15), 72 (14), 71 (93), 69 (39), 67 (13), 58 (12), 57 (100), 55 (43). Anal. calc. for $C_{80}H_{156}N_4O_{10}$ (1334.14): C 72.02, H 11.79, N 4.20; found: C 72.23, H 11.90, N 3.96.

*(−)-(2S,3S,11S,12S)-*N,N,N′,N′,N″,N″,N‴,N‴*-Octaoctyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxamide* (**1b**; *(−)-OOA-18C6*). The pure compound **1b** is an oil. $[\alpha]_D^{25} = -36.1$ ($c = 0.350$, $CHCl_3$). Spectral characteristics of **1b** are completely identical with those of **1a**. Anal. calc. for $C_{80}H_{156}N_4O_{10}$ (1334.14): C 72.02, H 11.79, N 4.20; found: C 71.87, H 11.86, N 4.07.

Membrane Preparation. The optode membranes were prepared from batch solns. Ligand (5.7 mg; **1a** for membrane **a** and **1b** for membrane **b**), 2.0 mg of chromoionophore *ETH 5294*, 3.5 mg of $NaTm(CF_3)_2PB$ (sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate), to create lipophilic anionic sites, 40 mg of PVC, and 80 mg of DOS (bis(2-ethylhexyl) sebacate, plasticizer) were dissolved in 0.75 ml of freshly distilled THF. This soln. (0.2 ml) was injected onto a rotating, dust-free glass plate of 35 mm diameter, which was located in a THF-saturated atmosphere. A home-made spinning device with a closed Al/*Plexiglass* cell was used, which allows the preparation of glass-supported membranes having controlled and reproducible thickness in the range of 0.5–7 µm [39]. After a spinning time of about 4 s, the membrane with the glass support was removed and after drying for several min at air mounted into a measuring flow-through cell *(Fig. 4)*.

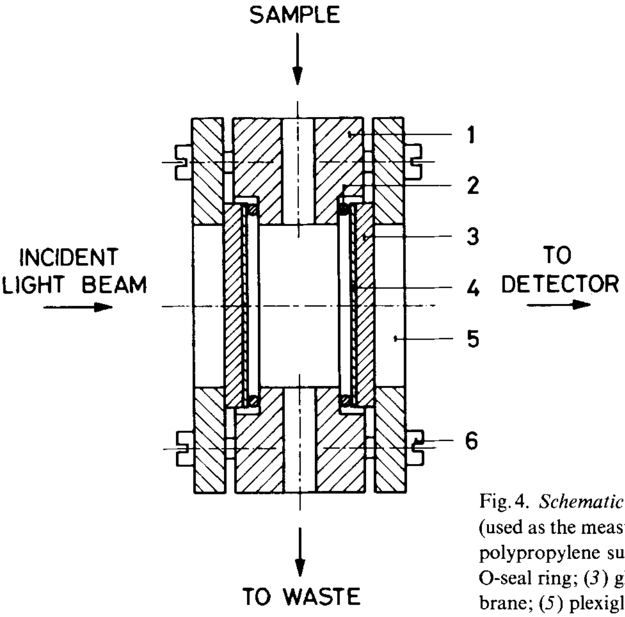

Fig. 4. *Schematic representation of the flow-through cell* (used as the measuring device for the present studies). (*1*) polypropylene support with sample inlet and outlet; (*2*) O-seal ring; (*3*) glass plate; (*4*) ion-sensing optode membrane; (*5*) plexiglass sheet; (*6*) fixing screws.

Experimental Procedure. Two glass plates with membranes of the same composition were mounted into the measuring cell *(Fig. 4)*. The reference cell contained two glass plates without membranes. The measuring and the reference cell were filled simultaneously by the same soln. Except for the recording of the full absorption spectra in the range 800–400 nm (*Fig. 1a* and *1b*), the absorbance measurements were made at a fixed wavelength of 660 nm.

The response functions of membranes **a** and **b** were measured separately in pH-buffered solns. of enantiomerically pure salts ((+)-(*R*) and (−)-(*S*)-PEA chlorides) of different concentrations in the range from 10^{-5} to 10^{-1} M. The possibility of measurement of enantiomeric excess was checked in solns. of PEA ions with known ee (from +1 to −1 with increments of 0.25) prepared volumetrically from buffered solns. of pure enatiomeric salts. The total concentration of PEA chlorides was $6.36 \cdot 10^{-4}$ M throughout. The calculation of the activities of PEA ions was performed according to a *Debye-Hückel* formalism using the parameters for NH_4^+ ions [40].

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