

Enantiomeric discrimination of chiral crown ether ionophores containing phenazine subcyclic unit by ion-selective potentiometry

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Abstract

In this paper the enantiomeric selectivity of two chiral phenazino-18-crown-6 ether hosts ((*R, R*)-**1** and (*R, R*)-**2**) is quantified. These hosts were incorporated into plasticized PVC membranes and used as recognition elements of ion-selective electrodes. The potentiometric response towards the two enantiomers of 1-phenylethylammonium ions (PEA^+) was measured. Potentiometric selectivity coefficients were calculated which reflect the ratio of the stability constants of the diastereomeric complexes. Ligand (*R, R*)-**1** does not show enantiomeric recognition, while ligand (*R, R*)-**2** has a slight preference for the (*S*)-(-) enantiomer over the (*R*)-(+) enantiomer manifested by a selectivity coefficient of 0.77. The results were compared to enantioselectivity patterns of the ligands towards α -(1-naphthyl)ethyl ammonium perchlorate ($NEA^+ClO_4^-$) enantiomers measured by circular dichroism and by 1H NMR titrations.

Keywords

chiral crown ether · phenazines · ionophore · ion-selective electrode · enantiomeric selectivity

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1 Introduction

Enantiomeric recognition is of great importance nowadays, when the pharmaceutical industry has adopted the new strategy of patenting enantiopure forms of certain optically active drugs. A widely used approach to distinguish between enantiomers is the application of high performance separation or electromigration techniques using chiral separation phases or forming diastereomers with chiral selectors prior to separation [1]. Another possibility is the use of enantioselective sensors or biosensors which allow the determination of the enantiomers without a preceding separation step [2, 3]. These sensors comprise potentiometric enantioselective membrane electrodes, amperometric biosensors and immunosensors.

Chiral selectors used in these systems, among others, are crown ethers, natural polysaccharides, cyclodextrins [4], maltodextrins [5], polyether and macrocyclic type antibiotics, antibodies and molecularly imprinted polymers [6]. It is of utmost importance to find or synthesize chiral selectors with as high selectivity as possible. An important step of this process is testing the selectivity of the resulting compounds in the targeted chiral system.

Enantiomeric selectivity can be assessed in numerous ways like calorimetric, UV-visible and NMR titrations, solvent extraction, transport through different membrane systems, mass spectrometry [7], circular dichroism (CD) measurements [8, 9] and chromatography [10].

Chiral selectors can be incorporated into a plasticized PVC based electrode membrane serving as an ionophore. Their enantioselectivity can be easily established by immersing the electrode into the solutions of the pure enantiomers separately, and measuring the potential difference formed at the membrane-solution interface. From this experiment the potentiometric selectivity coefficient can be easily calculated. The latter corresponds to the ratio of the stability constants of the two ionophore host-enantiomer guest complexes. This simple procedure has been elaborated and first applied for the determination of enantioselectivity of chiral crown ether compounds in the laboratory of W. Simon [11]. Later Horvath et al. demonstrated the feasibility of the method, i.e. that the enantioselectivity coefficient

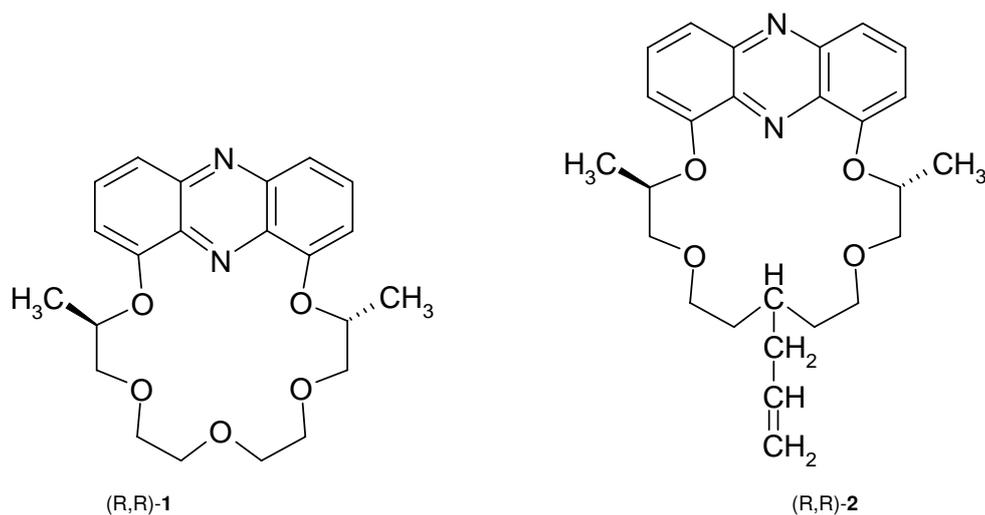


Fig. 1. Schematics of ligands (*R, R*)-1 and (*R, R*)-2

obtained by this method does not depend on experimental variables, like the type of plasticizer or the addition of lipophilic salts to the membrane [12]. The potentiometric method has several advantages in the evaluation of enantiomeric selectivity. It requires very small amount of the chiral selector, typically in the order of 1 milligram. The procedure and the instrumentation is very simple and cheap and requires little time.

Therefore we selected this option for the determination of the enantioselectivity of two phenazino-18-crown-6 ether ligands. These ligands were synthesized earlier [8, 13] and their enantioselectivity for α -(1-naphthyl)ethylammonium perchlorate ($\text{NEA}^+\text{ClO}_4^-$) was probed by circular dichroism spectroscopy, resulting in qualitative information, and by $^1\text{H-NMR}$ titration that provided stability constants of the host-guest complexes [9].

2 Experimental

2.1 Chemicals

Poly(vinyl)chloride (Corvic S704) was purchased from ICI. The plasticizers 2-nitrophenyl octyl ether (oNPOE) bis(2-ethylhexyl) sebacate (DOS) were products of Fluka, Selectophore grade. Selectophore grade lipophilic salts, potassium tetrakis(4-chlorophenyl)borate (KTPClPB) and sodium tetraphenylborate (NaTPB) were also purchased from Fluka. Racemic 1-phenylethylamine, and the two pure enantiomeric forms (*S*)-(-)-1-phenylethylamine and (*R*)-(+)-1-phenylethylamine were obtained from Aldrich. (*R*)-(+), (*S*)-(-) and racemic phenylethylammonium chloride solutions ((*R*)-(+), (*S*)-(-) and racemic PEA^+Cl^-) were prepared from the corresponding amine by titrating it with hydrochloric acid using a glass indicator electrode. The final concentration of these solutions were 0.1 M and their pH was 4.36, 4.35 and 5.20, respectively.

Phenylethylammonium tetraphenylborate (PEA^+TPB^-) was prepared from racemic phenylethylammonium chloride and NaTPB by precipitation from aqueous solutions. Inorganic

chemicals, hydrochloric acid (HCl), potassium chloride (KCl), sodium chloride (NaCl), ammonium chloride (NH_4Cl) and lithium acetate were obtained from Reanal Fine Chemicals, Hungary. Tetrahydrofuran was purchased from Fluka.

Solutions were prepared with double-distilled water.

2.2 Synthesis of the chiral crown ether ionophores

Synthesis of the phenazino-18-crown-6 ligands is described elsewhere [8, 13].

The schematics of ligand (*R, R*)-1

((*3R,13R*)-(-)-3,13-dimethyl-2,5,8,11,14-pentaoxa-20,26-diazatetracyclo[13.9.3.0^{19,27}.0^{21,25}]heptacos-

1(25),15,17,19,21,23,26-heptaene) and ligand (*R, R*)-2

((*3R,13R*)-(-)-3,13-dimethyl-8-(2-propenyl)-2,5,11,14-tetraoxa-20,26-diazatetracyclo[13.9.3.0^{19,27}.0^{21,25}]heptacos-

1(25),15,17,19,21,23,26-heptaene) are shown in

Fig. 1.

2.3 Electrode preparation

PVC based ion-selective electrode membranes were prepared by weighing the appropriate amount of PVC, plasticizer, lipophilic salt and chiral crown ether ionophore into a glass vial and dissolving them in tetrahydrofuran. The solution was cast into a teflon mould. After evaporation of the solvent a translucent membrane was obtained with an approximate thickness of 150-200 μm and a diameter of 21 mm. 7 mm circular disks were cut from the membranes and mounted into a Philips electrode body. 0.1 M racemic PEA^+Cl^- (pH=5.20) was used as an internal solution and Ag/AgCl as an internal reference electrode.

Different membrane compositions used throughout the study are shown in Table 1.

2.4 Apparatus

A Radelkis OP-208/1 type precision digital pH meter was used in the potentiometric measurements. All e.m.f. measurements were carried out in stirred solutions. A double-junction

Tab. 1. Composition of the different electrode membranes

Nr. membrane	ionophore [w/w%]	PVC [w/w%]	Plasticizer [w/w%]	lipophilic salt additive [w/w%]
1	1.0 ((<i>R, R</i>)-1)	32.7	66.3 (DOS)	–
2	0.9 ((<i>R, R</i>)-1)	31.9	66.7 (DOS)	0.5 (38 mol% of the ligand) PEA ⁺ TPB ⁻
3	1.0 ((<i>R, R</i>)-1))	33.1	65.3 (DOS)	0.6 (48 mol% of the ligand) KTpCIPB
4	1.0 ((<i>R, R</i>)-1)	32.7	66.3 (oNPOE)	–
5	1.0 ((<i>R, R</i>)-2)	33.1	65.9 (DOS)	–
6	1.0 ((<i>R, R</i>)-2)	32.7	65.8 (DOS)	0.5 (38 mol% of the ligand) PEA ⁺ TPB ⁻
7	1.0 ((<i>R, R</i>)-2)	32.8	65.6 (DOS)	0.6 (53 mol% of the ligand) KTpCIPB
8	1.0 ((<i>R, R</i>)-2)	32.6	66.4 (oNPOE)	–

reference electrode (Ag/AgCl/1 M KCl/0.1 M lithium acetate) was used throughout the study. Selectivity coefficients were obtained by the separate solution method.

3 Results and discussion

3.1 Calibrations in racemic phenylethylammonium chloride solutions

As a first step it had to be confirmed whether the electrode membranes containing ligand (*R, R*)-1 and (*R, R*)-2 are sensitive to phenylethylammonium ions. Therefore all the electrode membranes prepared were calibrated in the solution of racemic PEA⁺Cl⁻. The concentration range used was 10⁻¹ to 10⁻⁶ M. Characteristic data of the calibration curves are shown in Table 2. Calibration curves are shown in Fig. 2 for ligand (*R, R*)-1 and in Fig. 3 for ligand (*R, R*)-2.

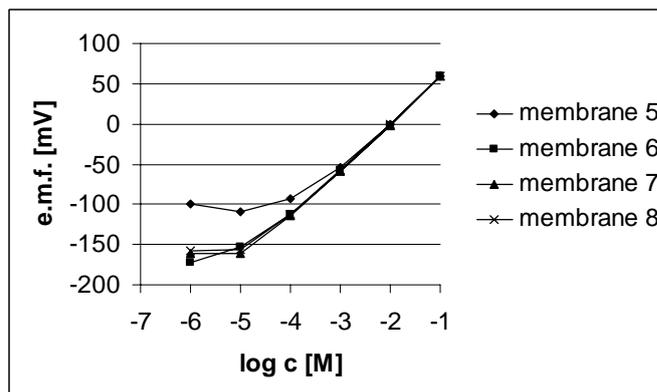


Fig. 3. Calibration curves of electrodes 1 – 4 containing ligand (*R, R*)-2 in racemate solution of PEA⁺Cl⁻

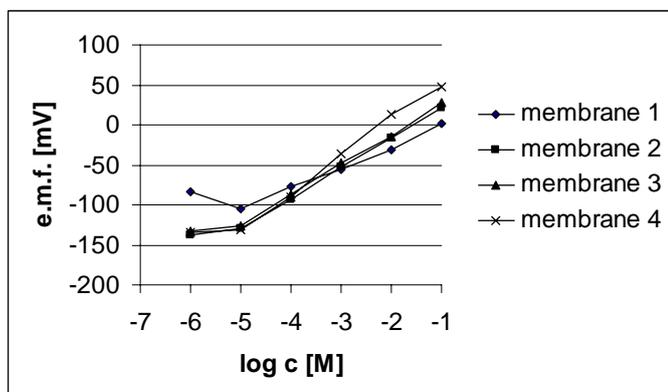


Fig. 2. Calibration curves of electrodes 1 – 4 containing ligand (*R, R*)-1 in racemate solution of PEA⁺Cl⁻

It is known from earlier experiments that dummy membranes containing no ionophore can also respond to lipophilic cations even without lipophilic salt additives [12]. Their linear range for PEA⁺, however, is relatively narrow (down to 5 · 10⁻³ M). They behave as low capacity ion-exchangers and a suitably lipophilic cation can enter into the membrane, creating an interfacial potential. The addition of lipophilic salts to the membrane creates liquid ion-exchanger type ion-selective electrodes that can measure PEA⁺ cations down to 10⁻⁵-5 · 10⁻⁶ M concentration. Their selectivity is dictated by the lipophilicity of the cations. In our experiments all the electrodes studied measure the PEA⁺

ion in a relatively broad concentration range.

Membranes prepared from ligand (*R, R*)-1, however, have poorer performance characteristics. They show a sub-Nernstian behavior and a relatively large drift during calibration. The course of the calibration curves of membranes 1 and 4 i.e. the ones without lipophilic salt additive is quite different from the others, but still have much lower detection limit, than dummy membranes. Membranes 2 and 3 have similar characteristics, but this more reproducible behavior is probably due to the added lipophilic salts.

Tab. 2. Characteristic data of the potentiometric calibration curves taken in racemic PEA⁺Cl⁻ solutions

Ligand	Nr. Membrane	Slope ^a [mV/decade]	E ₀ [mV]	Linear range [M]
<i>(R, R)</i> -1	1	32.4	34.4	10 ⁻¹ -10 ⁻⁵
	2	39.2	61.2	10 ⁻¹ -10 ⁻⁵
	3	42.1	69.8	10 ⁻¹ -10 ⁻⁵
	4	33.9	81.5	10 ⁻² -10 ⁻⁵
<i>(R, R)</i> -2	5	60.1	119.7	10 ⁻¹ -10 ⁻³
	6	61.3	120.9	10 ⁻¹ -10 ⁻⁵
	7	61.8	120.6	10 ⁻¹ -10 ⁻⁵
	8	60.5	119.9	10 ⁻¹ -10 ⁻⁵

^a the numbers reflect the initial slopes of the calibration curve between 10⁻¹ and 10⁻² M concentrations.

The electrodes containing (*R, R*)-1 show large drift which

can be attributed to the relatively low lipophilicity of ligand (*R, R*)-1. This can cause the slow leaching of this ionophore from the membrane phase into the aqueous phase resulting in non-reproducible response of the electrode.

Electrode membranes prepared from ligand (*R, R*)-2 show much better calibration behavior. They show Nernstian response with response times below 1 minute and the drift is negligible. All four membranes have very similar calibration curves. The type of plasticizer or the different added lipophilic salts do not have an influence on the shape of the curves, except for membrane 5, prepared with DOS without lipophilic salt, the linear range of which is somewhat smaller.

3.2 Determination of selectivity coefficients for (*S*)-(-)-PEA⁺Cl⁻ over (*R*)-(+)-PEA⁺Cl⁻

Selectivity coefficients were determined by the separate solution method.

(*S*)-(-)-PEA⁺ ion was considered as the measured ion and (*R*)-(+)-PEA⁺ ion was regarded as the interfering ion. The electrodes were immersed into 0.1 M solution of one enantiomer and the e.m.f. was recorded for ten minutes. After rinsing and drying, the electrodes were immersed into the solution of the other enantiomer and the e.m.f. was recorded similarly. The procedure was repeated four times. The difference between the electromotive forces in (*S*)-(-)-PEA⁺Cl⁻ solution and in (*R*)-(+)-PEA⁺Cl⁻ solution was calculated. The selectivity coefficient of the electrode for the (*S*)-(-) enantiomer over the (*R*)-(+) enantiomer was obtained from the following equation:

$$\text{e.m.f.} = E_0 + s \log(a_{(S)-(-)} + K_{(S)-(-), (R)-(+)}^{\text{pot}} a_{(R)-(+)}),$$

where

- e.m.f. is the potential difference in the measuring cell,
- E_0 is the standard potential of the cell
- s is the Nernst factor or slope of the calibration line
- $a_{(S)-(-)}$ is the activity of the measured ion and
- $a_{(R)-(+)}$ is the activity of the interfering ion (the other enantiomer).

If a membrane cannot differentiate between the enantiomers there is no difference in the electrode potentials obtained in the two solutions. Enantioselective membranes show different e.m.f. responses in the solutions of the two enantiomers, the difference in e.m.f.s being larger with higher enantioselectivity. A typical measurement record is shown in Fig. 4 for membrane 7. Potentiometric selectivity coefficients calculated for all the electrodes studied are shown in Table 3.

All electrode membranes containing ligand (*R, R*)-2 show higher potential values in (*S*)-(-)-PEA⁺Cl⁻ than in (*R*)-(+)-PEA⁺Cl⁻. It can be seen that the potentiometric selectivity coefficients obtained with different membrane compositions i.e. with different plasticizers or different or no lipophilic salt additives are not differing within the limits of the standard error. The average potentiometric selectivity coefficient is 0.77. This conforms to the ratio of the stability constants of the two complexes

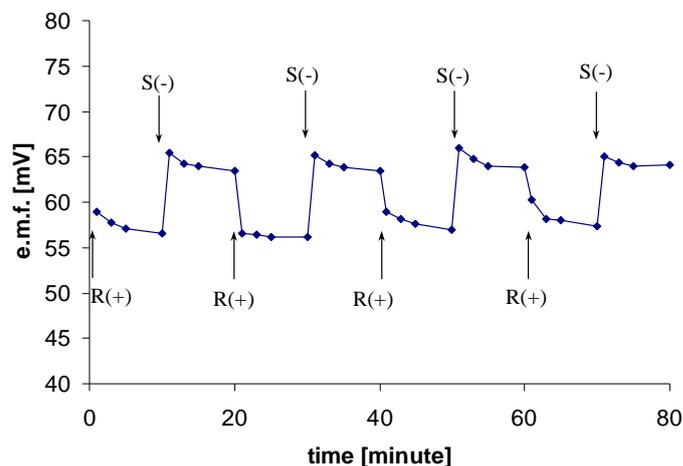


Fig. 4. Change in the potential response of membrane 7 during alternating immersions into 10⁻¹ M (*R*)-(+)-PEA⁺Cl⁻ and (*S*)-(-)-PEA⁺Cl⁻ solutions

that the crown ether forms with the two PEA⁺ enantiomers. The electrodes slightly prefer the (*S*)-(-) enantiomer over the (*R*)-(+) enantiomer i.e. (*R, R*)-crown ether forms more stable complex with the (*S*)-(-) enantiomer. This is in agreement with earlier results measured by circular dichroism spectroscopic studies, which qualitatively confirmed that ligand (*R, R*)-2 forms more stable complex with (*S*)-(-)-NEA⁺ClO₄⁻ in acetonitrile [9]. ¹H NMR titrations were also carried out [9] to determine the stability constants in CDCl₃/CDOD₃ (1:1; v/v) and resulted in a complex stability constant ratio of 3.72, (*R, R*)-2 forming the more stable complex with (*S*)-(-)-NEA⁺ClO₄⁻.

Electrode membranes containing ligand (*R, R*)-1 do not show an explicit preference of one enantiomer of PEA⁺ over the other. The average $K_{(S)-(-), (R)-(+)}^{\text{pot}}$ is 1.07. Therefore this ligand hardly discriminates between the two enantiomeric forms of PEA⁺Cl⁻. This conforms the findings of Farkas et. al. who also found unexpectedly poor discriminating power of this ligand in enantiomeric solutions of NEA⁺ClO₄⁻ [9]. They explained this phenomenon with the overcompensation of the steric repulsion between the small methyl group of the host and the naphthalene hydrogens of the guest by the strong $\pi - \pi$ interaction.

3.3 Determination of selectivity coefficients in the solutions of hydrophilic interfering cations

Selectivity coefficients for PEA⁺ over potentially interfering common cations were determined by the separate solution method. These data can provide useful information when enantiomer selectivity is determined in buffered solutions. Table 4 shows the results obtained for H⁺, Na⁺, K⁺ and NH₄⁺-ions.

Among all the cations studied, H⁺-ion is the only one showing substantial interference in the membranes containing ligand (*R, R*)-1 as ionophore, and only in those compositions, that do not have any added lipophilic cation (Membrane 1 and 4). The other electrode membranes measure PEA⁺ selectively over hydrophilic cations with a selectivity coefficient ranging from 6.8·10⁻² to 1.3·10⁻³. This implies that dilute buffer solutions containing the above cations do not interfere with the enan-

Tab. 3. Potentiometric selectivity coefficients of the membranes for (S)-(-)-PEA⁺Cl⁻ over (R)-(+)-PEA⁺Cl⁻

Ligand	Nr. Membrane	Δ e.m.f. ^a [mV] (n=4)	Slope [mV/decade]	CV [%] [14]	$\log K_{(S)-(-),(R)-(+)}^{pot}$	$K_{(S)-(-),(R)-(+)}^{pot}$	$K_{average}$	CV [%]
(R, R)-1	1	-4.04	32.4	15	0.125	1.33	1.07	18.6
	2	-0.28	39.2	5.0	0.00714	1.02		
	3	-1.70	42.1	0.36	0.0404	1.10		
	4	2.34	33.9	1.5	-0.0690	0.853		
(R, R)-2	5	5.65	60.1	10	-0.0940	0.805	0.773	4.16
	6	6.40	61.3	0.11	-0.104	0.786		
	7	6.95	61.8	2.4	-0.112	0.772		
	8	8.28	60.5	6.4	-0.137	0.730		

^a difference in the e.m.f.s measured in (S)-(-)-PEA⁺Cl⁻ and (R)-(+)-PEA⁺Cl⁻ solutions

Tab. 4. $\log K_{PEA^+,j}^{pot}$ values of the membranes studied in the solution of different hydrophilic cations

Ligand	Nr. Membrane	$\log K_{PEA^+,j}^{pot}$			
		H ⁺	Na ⁺	K ⁺	NH ₄ ⁺
(R, R)-1	1	0.83	-1.17	-1.47	-1.22
	2	-1.68	-1.66	-1.62	-1.48
	3	-2.69	-2.86	-1.82	-2.30
	4	-0.10	-1.91	-1.50	-1.82
(R, R)-2	5	-1.57	-1.83	-2.05	-2.27
	6	-2.04	-1.90	-2.23	-2.38
	7	-2.08	-1.93	-2.28	-2.45
	8	-1.78	-2.91	-2.80	-2.76

tiomer selectivity determination.

4 Conclusion

Two chiral phenazino-18-crown-6 ligands ((R, R)-1 and (R, R)-2) were incorporated into solvent polymeric electrode membranes in order to measure their enantiomeric selectivity towards (S)-(-) and (R)-(+)-PEA⁺ ions. Different membrane compositions were used changing the type of plasticizer and the type of lipophilic salt additive. After verifying that the potentiometric membrane electrodes are measuring PEA⁺ ion, the electrode responses were recorded in (S)-(-)-PEA⁺Cl⁻ and in (R)-(+)-PEA⁺Cl⁻ solutions, successively. The potential differences showed in the solution of the two enantiomeric forms were used to calculate the potentiometric selectivity coefficients, which in turn correspond to the ratio of the complex stability constants of the two diastereomeric crown ether-organic ammonium salt complexes. Ligand (R, R)-1 did not show enantiomeric differentiating ability, while ligand (R, R)-2 had a slight preference for the (S)-(-) enantiomeric form over the (R)-(+) one. The average potentiometric selectivity coefficient that approximates the ratio of the two stability constants was 0.77 with a 4.16% CV. This is in good agreement with the earlier results obtained by circular dichroism and ¹H NMR measurements, i.e. ligand (R,R)-2 preferably forms heterochiral complexes (host-guest complexes with opposite configurations) with enantiomers of protonated primary ammonium salts.

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