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Characteristics of new composite- and classical potentiometric sensors for the determination of pharmaceutical drugs

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Abstract

The characteristics of new types of coated wire potentiometric sensors based on composite coatings were compared with classical potentiometric sensors. The composite sensors contained respectively the clay montmorillonite (MM) and the zeolite NaY as the ionically conducting components, embedded in PVC based rubber phase membranes. The behavior of 20 basic medicinal drugs and 5 biogenic amines was studied on 9 potentiometric sensors of different composition. The behavior of 3 composite sensors, and 6 more classical PVC based sensors either of the "inner solution" or "coated wire" type were studied. The analytes were chosen to cover a wide log *P* range of, e.g. −1.54 (noradrenaline) up to +5.55 (promethazine). All sensors were tested using a high-throughput FIA-based method. The results were interpreted via statistical data analysis. The responses of all electrodes had a very high correlation to the log *P* of the analytes. This was also the case for the ion-pair based electrodes containing a specific cationic drug as the counterion. Classical ion-pair based sensors containing tetrakis (*p*-chlorophenyl) borate (TCPB) and a counterion with a high log *P* value (e.g. promazine) were the least sensitive. The composite-based sensors were the most sensitive. Coated wire electrodes were statistically shown to behave in the same way (selectivity and sensitivity) as inner solution electrodes. The results are discussed using a physico-chemical model. Practical applications of the most performant (composite) sensors are shown in HPLC detection of the pharmaceutical drugs and the biogenic amines. Detection limits in the 10^{-7} M regio (injected concentrations) are obtained for lipophilic drugs (log $P > 2$).

Keywords: Potentiometry; Ion selective electrodes; HPLC detection; Pharmaceutical drugs; Biogenic amines

1. Introduction

Potentiometric determination of organic ionic analytes is not yet widely exploited. In a review of 1998 [1], Buhlmann et al. cite some 90 publications on the topic, mostly from the 1980 to 1998 period. One reason is that many important classes of such compounds are rather hydrophilic. As we will show further, hydrophilic organic ions are presently hard to determine with the technique. For the determination of basic drugs, potentiometric electrodes have been constantly described in the literature since about 1980. As early as 1990, Vytras [2,3] lists some 90 basic drugs for which a potentiometric determination has been worked out. From that period on until now, we counted some 50 publications on the potentiometric determination of basic pharmaceutical drugs, and last years, there is clearly a renewed interest [4]. Some publications were even devoted to dissolution measurements [5,6]. Two types of electrodes are used, i.e. (mostly) "inner solution", and (to a lower extent) "coated wire" electrodes. Working models of the way of action of such electrodes are only vaguely or partly described in books on potentiometry [7–9] or on electrochemistry in general [10]. The ideas are scattered in the primary literature, where there is still discussion and controversy about the way of action of potentiometric membranes [11–13]. These thick polymeric materials are used in the rubber phase. PVC and plasticizer are the most frequently used basic materials (see [14] for a recent review on polymers used in these and other sensor applications). They are made ionically conductive by addition of a high molecular weight anionic component (for membranes responsive to cationic substances) and mobile countercations. The membranes therefore

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behave as cation exchangers. The anionic components used in cation-sensing membranes are mostly borate compounds. Also used, but to a much lower extent, are phosphomolybdic- (PMA), phosphotungstic-, silicomolybdic- and silicotungstic acid [15]. Zeolite and clay crystalline particles were used occasionally in potentiometry [16,17]. They were used more extensively (as nanocomposite materials, see e.g. [17]) in polymer electrolytes, for application in fuel cells. A classically composed potentiometric membrane is developed for the determination of one specific cation. Mostly, an ion-pair is dissolved in the membrane, with the cationic part being the analyte cation itself. Such ion-pair electrodes are claimed to be selective for the cation which is used in the ion-pair [18,19]. Our group uses potentiometric electrodes as detection element in HPLC and CE. In such conditions, no strict selectivity is required. We rather have to be able to tune the selectivity to a class of substances. We therefore wanted to examine the importance of the anionic and cationic components used in the membranes (ion-pair or no ion-pair) on the membranes' selectivity and sensitivity. A classical tetrakis (*p*-chlorophenyl) borate (TCPB) anion based electrode was therefore compared with less classical composite materials containing the inorganic MM, NaY, and PMA. The experiments were done with "inner solution" as well as with "coated wire" electrodes.

2. Experimental

2.1. Instrumentation

The flow injection analysis (FIA) was performed using an autoinjector (Thermo Separation Products) with a 200 μL injection loop, a Spectra Physics 8810 isocratic HPLC pump, and a PC 1000 data acquisition system from Thermo Separation Products. The eluent was acetonitrile/aqueous 1 mM nitric acid 10:90 (v/v), the apparent pH (pH*) was 3.02. The flow rate was 1 mL min^{-1}. The injector outlet peek tubing was directed perpendicularly towards the sensing membrane of the electrode in a flow cell [20]. The distance of the LC tubing outlet to the electrode was 100 μm. The membrane potential was measured against an Orion 800500 Ross® reference electrode using a high impedance (10^{13} Ω) amplifier.

The apparent pH value (pH*) of the mobile phase was controlled by a portable HI8314 pH meter (Hanna instruments, Germany) without corrections. The mobile phase was filtered through a 2 μm cellulose acetate filter (Alltech Associates). All experiments were done at room temperature (20–22 °C).

Electron microscope pictures of the composite materials were taken by a SEM 515 Philips Scanning Electron Microscope (FEI, Netherlands). Gold layers of 50 nm thickness were applied on the surface of electrode coatings by a Cressington sputter coater (Elektronen-Optik-Service GmbH, Dortmund, Germany), equipped with a Cressington mtm10 thickness monitor.

2.2. Chemicals

All chemicals were of analytical reagent grade. The eluent was prepared by dilution of a nitric acid solution and acetonitrile, both obtained from Acros Organics. One millimolar stock solutions of the different chemicals were prepared in the eluent. Different drugs and biogenic amines were obtained from the following sources (log *P* values are given between brackets): bromhexine (log *P* value = 4.03), cadaverine (−0.49), spermine (−1.11), adrenaline (−0.91), noradrenaline (−1.54), rimipramine (4.97), drofenine (4.83), promethazine (5.55), fluphenazine (2.82), diphenylpyraline (3.91), chlorpheniramine (3.57), pheniramine (3.12), ritodrine (1.22), ephedrine (1.02), salbutamol (−0.69), procaterol (−0.71) were purchased from Sigma. Clenbuterol (2.91) and dopamine (−0.48) were purchased from Fluka. Cocaine (2.00), heroin (1.91), atropin (1.59) and codeine (1.73) were purchased from Cerilliant, France. Etilefrine (0.22) was purchased from CRS, France. High-molecular mass PVC was obtained from Janssen Chimica. The other membrane components were of the highest quality grade available from Fluka. These included plasticizer bis(2-ethylhexyl)sebacate (DOS), potassium tetrakis (*p*-chlorophenyl) borate (TCPB), calix[6]arene-hexaethylacetate, dibenzo-18-crown-6, phosphomolybdic acid hydrate (PMA) and tetrahydrofuran (THF). Methyl red was obtained from Merck. The clay material montmorillonite, $Na(Al,Mg)_2Si_4O_{10}(OH)_2$, cation exchange capacity 0.94 mequiv./g, was obtained from Mineral Colloid BP (UK). The zeolite NaY, $Na_{56}[(AlO_2)_{56}(SiO_2)_{136}]$, cation exchange capacity 6.25 mequiv./g, was obtained from IOP (USA).

2.3. Electrode preparation

The PVC matrix liquid membrane coated electrodes based on the membrane solvent or plasticizer DOS and the cation-sensing elements were prepared. We used coated-wire electrodes with glassy carbon (3 mm diameter) as the substrate electrode material, and also inner solution electrodes with membrane diameters of 3 mm. The body (∼10 mm diameter) of an electrode consists of delrin for the coated-wire electrodes and PVC for the inner solution electrodes. The membranes had thicknesses varying between 185 and 215 μm. The thickness was measured with a micrometer screw (after peeling it off the substrate electrode in case of coated wire electrodes).

2.3.1. Preparation of the different ion-pairs

PMA-fluphenazine ion-pair: An 8 mL aliquot of 1×10^{-2} M PMA solution was mixed with a 12 mL 1×10^{-2} M fluphenazine solution [21]. *TPCB-cocaine ion-pair*: A 10 mL aliquot of 1×10^{-2} M TPCB was mixed with a 10 mL 1×10^{-2} M cocaine solution [18]. *TPCB-promazine ion-pair*: A 10 mL aliquot of 1×10^{-2} M TPCB was mixed with a 10 mL 1×10^{-2} M promazine solution [19]. The ion-pair precipitates were collected by filtration on a 2 μm cellulose acetate filter. The solid residue on the filter was washed thoroughly with deionised water, and then dried at room temperature for 24 h.

2.3.2. Preparation of the different electrodes

2.3.2.1. Coated wire electrodes. PMA-fluphenazine ion-pair electrode (PF(CW)): 7% ion-pair precipitate, 46.5% PVC and

46.5% DOS (wt.%) [21]. *Montmorillonite electrode (MM(CW)) and zeolite electrode (NaY(CW))*: 5% Montmorillonite or zeolite, 30% PVC and 65% DOS [16]. *TPCB electrode (BOR(CW))*: 2% TPCB, 32% PVC and 66% DOS [22]. *Electrodes with the receptors calix[6]arene-hexaethylacetate (CLX(CW)), dibenzo-18-crown-6 (CRW(CW))*: 2% TPCB, 5% CLX or CRW, 31% PVC and 62% DOS [22]. Three milliliters of THF was added to a 300 mg amount of these mixtures. For NaY membranes, this cocktail had a colloidal nature. In the case of MM and PF it contained both colloidal and suspended (precipitating on standing) material. The two latter cocktails were vortexed vigorously for one min, and deposited directly (before precipitation occurs) onto an electrode. Three layers of the membrane mixture were deposited consecutively on the electrode, at an interval of 20 min, using a Pasteur pipette. Each layer was formed by application of three drops of the membrane cocktail. The electrodes were allowed to dry for at least 2 h. Afterwards they were kept in deionised water overnight. Prior to use, the electrodes were conditioned with the running eluent in the FIA system until a stable baseline was observed (∼30 min).

2.3.2.2. Inner solution electrodes. TPCB electrode (BOR(IS)): 2% TPCB, 32% PVC and 66% (wt.%) DOS [22] with an inner 1×10^{-3} KCl solution. *TPCB-cocaine ion-pair electrode (CC(IS))*: 2% ion-pair precipitate, 28.3% PVC and 69.7% DOS with a 5×10^{-3} M KCl + 5×10^{-3} M cocaine inner solution [18]. *TPCB-promazine ion-pair electrode (PMZ(IS))*: 2% ion-pair precipitate, 36.5% PVC and 61.5% DOS with a 5×10^{-3} M KCl + 5×10^{-3} M promazine inner solution [19]. A 300 mg amount of these mixtures was dissolved in 3 mL of THF. The membrane was first prepared onto a glassy carbon substrate electrode as described above for coated wire type electrodes. Then the membrane was gently peeled off the glassy carbon electrode by means of a pincet. This membrane was then glued onto an inner solution electrode PVC body (3 mm diameter opening) by means of a few drops of THF, and left to dry for at least 2 h. The inner compartment was then filled with the inner solution in which a Ag/AgCl reference electrode was placed. The BOR(IS) was stored in deionized water. The CC(IS) and PMZ(IS) electrodes were stored in respectively 1×10^{-3} M cocaine solution and 1×10^{-3} M promazine solution. Prior to use, the electrodes were conditioned with the running eluent in the FIA system until a stable baseline was observed (∼30 min).

2.4. Log P calculations

For the calculation of log *P* values of the analyte substances the internet site http://www.logp.com from ChemSilico LLC (Tewksbury, MA, USA) was used.

2.5. Statistical analysis

The statistical analysis performed on the results was done by SPSS version 12.0 for Windows Xp (SSPS Inc., Chicago). The statistical tests performed were a non-parametric bivariate correlation analysis where Kendall's tau-b coefficients were calculated, and a calculation of the standard deviations for all responses (average of six measurements) measured on all electrode types.

3. Results and discussion

3.1. Structural characteristics of the membranes used

The inorganic materials MM, NaY, and ion-paired PMA form particles with μm and sub-μm dimensions in the membrane preparations obtained as described in the experimental part. The three particles are chemically very different in nature, but show many common properties. MM, NaY, and PMA are polyelectrolytes and are very well known for their cation-exchange properties. MM is a clay-type material. NaY has a zeolite structure, resembling faujasite. MM and NaY are mineral structures with nanometer to micrometer dimensions. Their size and shape depends on the environment and conditions in which they are formed. PMA's smallest mineral dimensions correspond to so-called "Keggin" structures. When ion-paired with a lipophilic cation, larger ionic aggregates form, whose dimensions also depend on the environment and conditions of formation. In the present study, it was ion-paired with fluphenazine. The three materials are expected to form aggregates with sizes in the nanometer to micrometer scale when mixed with polymers. SEM pictures of these three electrode materials are shown in Fig. 1.

It is clear from these pictures, that the largest particles present vary from micrometer size (PF) up to multi-micrometer size (NaY). Electrode materials based on ion-pairs with the organic TCPB, like cocaine-TCPB and promazine-TPCB, are expected to form loosely bound ion–ion interactions in the rubber phase plastic. No particulate matter was observed in these materials, or in other materials used in the present study.

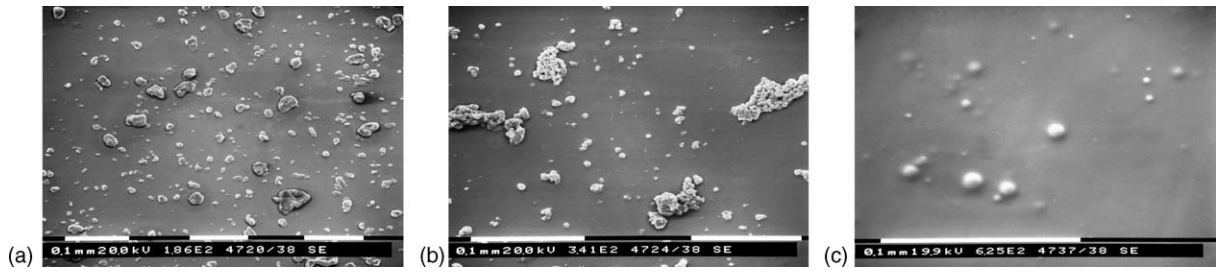

Fig. 1. SEM pictures of the membranes doped with inorganic materials. Montmorillionite (a), zeolite NaY (b) and the phosphomolybdic acid–fluphenazine ion pair (c). Different magnifications used: 186× (a), 341× (b) and 625× (c). The white line in each picture represents a distance of 100 μm.

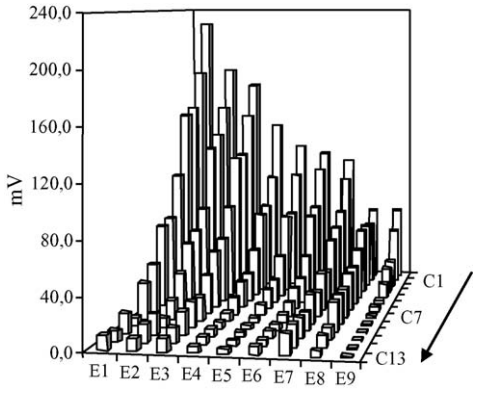

Fig. 2. Block diagram of the FIA responses of eight drugs and five biogenic amines (first set of compounds) on all nine tested electrodes. The *X*-axis represents the different electrodes with E1 (PF(CW)), E2 (MM(CW)), E3 (NaY(CW)), E4 (BOR(IS)), E5 (CC(IS)), E6 (BOR(CW)), E7 (CRW(CW)), E8 (CLX(CW)) and E9 (PMZ(IS)). The *Z*-axis represents the different products ranked from highest to lowest response (C1 → C13) on an E6 electrode, with C1 (bromhexine), C2 (promazine), C3 (cocaine), C4 (clenbuterol), C5 (hexylamine), C6 (heroin), C7 (atropin), C8 (codeine), C9 (cadaverine), C10 (spermine), C11 (adrenaline), C12 (dopamine) and C13 (noradrenaline). The *Y*-axis represents the responses in mV.

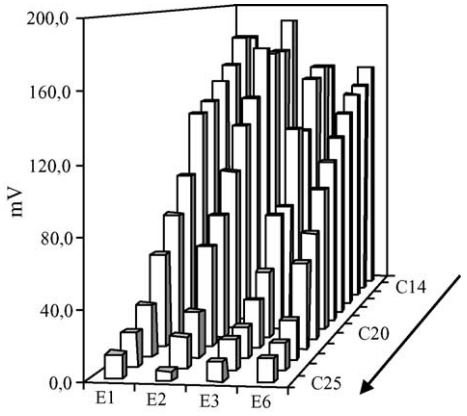

Fig. 3. Block diagram of the responses of 12 extra drugs (second set of compounds) on four tested electrodes. The *X*-axis represents the different electrodes with E1 (PF(CW)), E2 (MM(CW)), E3 (NaY(CW)), E6 (BOR(CW)). The *Z*-axis represents the different products ranked from highest to lowest response (C14 → C25) on an E6 electrode, with C14 (trimipramine), C15 (drofenine), C16 (promethazine), C17 (fluphenazine), C18 (diphenylpyraline), C19 (chlorpheniramine), C20 (pheniramine), C21 (ritodrine), C22 (ephedrine), C23 (etilefrine), C24 (salbutamol) and C25 (procaterol). The *Y*-axis represents the responses in mV.

3.2. Response behavior of the nine electrode types

The nine types of electrodes were used in an FIA setup (see Section 2). Responses provoked by a first set composed of eight drugs and five biogenic amines, injected in equimolar concentrations (1×10^{-4} M, dissolved in the eluent), are shown in a block diagram in Fig. 2.

Three electrodes were used of each electrode type. The FIA measurements were performed in standardized conditions. Electrodes were first equilibrated in the FIA system for 1 h with the running eluent. The measurements of the drug samples were done using six consecutive injections of each drug.

A second set of 12 basic drugs was injected on an FIA system containing PF(CW), MM(CW), NaY(CW) and BOR(CW) electrodes. These responses were measured in an identical manner as the ones described above for the first set, and are shown in Fig. 3.

In the block diagrams of Figs. 2 and 3, the response order of a standard BOR(CW) electrode was taken to rank the analytes from highest to lowest response. We preferred to plot the response in mV for the different substances, instead of using $k_{i,j}^{\text{pot}}$ values for all compounds referring to one arbitrarily chosen (i) compound. In FIA measurements, $k_{i,j}^{\text{pot}}$ values are related to the measured responses (mV at plateau value) given in Fig. 2, by the following equation:

$$\frac{E_i - E_j}{S} = -\log k_{i,j}^{\text{pot}} \quad (1)$$

This is the rearranged Nicolsky equation [23]. E_i is the signal provoked by compound i, and E_j the signal provoked by compound j in equimolar concentrations. S is the slope of the calibration curve. The E_j values measured in FIA are shown in Figs. 2 and 3. This way of working is comparable to the use of the "separate solution method" (SSM) in batch techniques. Several conclusions can be drawn from a closer examination of the data shown in Figs. 2 and 3, as will be done in the next paragraphs.

3.3. Selectivity behavior of the different electrode types

3.3.1. Log P dependence of the response

The responses (mV at plateau value) were plot versus the calculated log P values of the injected compounds. The log P values of the first set of 13 compounds varied between −1.54 (noradrenaline) and 4.36 (promazine) (see Section 2). The R values (non-parametric Kendall's tau-b correlation coefficients) obtained for the first set of compounds determined with nine different electrodes (see also Fig. 2) are given in Table 1.

The responses of the second set of compounds (see also Fig. 3) were also plot versus the calculated log P values of the injected compounds. The log P values of the 12 investigated products in this second set varied between −0.71 (procaterol) and 5.55 (promethazine) (see Section 2). The R values obtained for this second set for the four different electrodes are given in

Table 1
R values (non-parametric Kendall's tau-b correlation coefficients) of responses plot against log P for the 1st set of compounds and for nine different electrodes (see Fig. 2)

Electrode type	R values
PF(CW)	0.813*
MM(CW)	0.795*
NaY(CW)	0.769*
BOR(IS)	0.872*
CC(IS)	0.744*
BOR(CW)	0.769*
CRW(CW)	0.795*
CAL(CW)	0.718*
PMZ(IS)	0.744*

* Correlation is significant at the 0.01 level (two-tailed).

Table 2

	Electrode type			
	PF(CW)	MM(CW)	NaY(CW)	BOR(CW)
R values (Fig. 2)	0.818*	0.788*	0.848*	0.848*
R values (Figs. 2 and 3)	0.765*	0.783*	0.751*	0.778*

The top row shows the R values of responses plot against log P for the second set of compounds and for 4 different electrodes (see Fig. 3). The bottom row shows the R values of the responses plot against log P, for all 25 compounds investigated, for the four different electrodes used in Fig. 3.

* Correlation is significant at the 0.01 level (two-tailed).

the top row of Table 2. The bottom row of Table 2 shows the R values of the responses for all 25 compounds investigated, plot against log P on these four electrodes.

Fig. 4 shows the log P dependence for the most sensitive electrode type, the PF(CW) electrode, for all 25 injected compounds. The standard deviations of the six repeated measurements are indicated.

It is clear that, for all electrode types used, there is a high correlation between the response provoked by the compounds (in mV), and the calculated log P values. This means that neither the anionic nor the cationic component of the ionically conducting membranes has much influence on the selectivity of the membrane response. Even membranes of the "ion-pair" type with the analyte cation being one part of the ion-pair, are not found to be selective to the countercation, but clearly show the log P dependence. Such selectivities are however claimed by most authors who developed such electrodes, and this is misleading. The "possible interferents" tested, are always much more hydrophilic than the target analyte, evidently leading to unfavorable selectivity coefficients! Ion-pair membranes with inner solution are mostly used in the literature [18,19,24,25]. In the present study, they showed no selectivity or sensitivity advantage.

Can the high correlation of the response of equimolar injections of the drugs to log P be in accordance with earlier theories on the selectivity of potentiometric membranes? Eisenman [26] gave the following equation, relating $k_{i,j}^{\mathrm{pot}}$ values to distribution coefficients of compounds i and j over the membrane/water system:

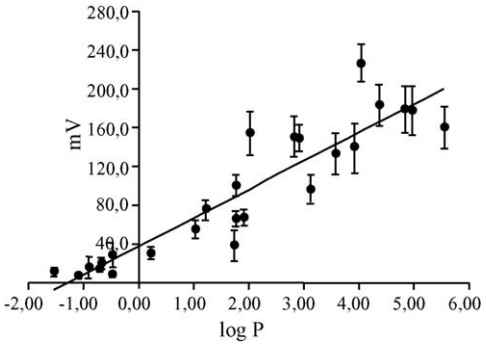

Fig. 4. The log P dependence for the electrode type PF(CW) for all 25 injected compounds. The standard deviations of the six repeated measurements are indicated. Log P values calculated with http://www.logp.com.

$$k_{i,j}^{\mathrm{pot}} = \frac{U_j K_j}{U_i K_i} \tag{2}$$

U_j and U_i are the respective mobilities of ions j and i in the membrane phase. If these mobilities are comparable, the distribution coefficient K of the ions between the water analyte solution and the membrane is the selectivity determining component. P is a distribution coefficient of our compounds over a water/n-octanol system. This system is different from a water/potentiometric membrane system. The (non-faradaic) potentiometric response provoked by the analyte cations is mainly due to a change in Donnan potential at the membrane–analyte solution interface. This change in Donnan potential depends on the nature of the analyte cation penetrating the membrane. It is related to the difference in Gibbs free energy of the analyte cation between the analyte solution and the membrane (ΔG_{tr} where tr stands for transfer from solution to membrane). In a former publication on the subject [27], we used the following equation to discuss the terms which could determine this Gibbs free energy difference:

$$\Delta G_{\mathrm{tr}} = \Delta G_{\mathrm{hydr}} - (\Delta G_{\mathrm{solv}} + \Delta G_{\mathrm{ex}} + \Delta G_{\mathrm{complex}}) \tag{3}$$

with ΔG_{hydr} representing the Gibbs free energy of hydration of the analyte ion in the water phase. $\Delta G_{\mathrm{solv}} + \Delta G_{\mathrm{ex}} + \Delta G_{\mathrm{complex}}$ are the summed contributions of the analyte ion's free energy in the membrane phase, i.e. respectively solvation energy, ion–ion interaction energy (with the lipophilic anion), and eventually complex formation energy with a neutral ionophore. An analoguous equation is used by physico-chemists to explain equilibria of the exchange of ions in ion-exchangers [28]. In the latter case, the interest is more in the bulk cation-exchange phenomena, rather than in the potential-generating phenomena at the surface. Potentiometric membranes and ion-exchangers are comparable systems, and as well the thermodynamics (equilibrium constants, selectivity) as the kinetics (rates of exchange of ions) have been more thoroughly investigated for ion-exchangers. For ion-exchangers, only ΔG_{hydr}, ΔG_{solv}, and ΔG_{ex} are used to explain the ion-exchange equilibria where they are named, respectively, ΔG_{is}, ΔG_{im}, and ΔG_{ii} [28]. With is = counter-ion and solvent interaction, im = counter-ion and resin matrix interaction, and ii = counter-ion and fixed ion interaction. For ion-exchange materials, the extent and rate of ion exchange is important. For potentiometric membranes, both the surface potential formation, and the bulk ion-exchange phenomena have to be carefully studied. The first phenomenon is a rapid one, the second is very slow (see further in this text for a discussion of the ion-exchange behavior of our electrode membranes).

All our tested membranes' responses correlate highly to log P. P being a distribution coefficient for a water–n-octanol system, it can only depend on Gibbs free energy factors such as ΔG_{hydr}, and ΔG_{solv} (ion–ion interactions are not present in the water–octanol system). This is an indication that ion–ion interactions of the nine membranes with the set of compounds were either low, or highly correlated. Given the fact that the inorganic ion-exchange particles doped membranes had far better

Table 3
Inter-electrode matrix (non-parametric Kendall's tau-b correlation coefficients) of the responses of the 13 products (first set of products, see Fig. 2)

	Electrode type								
	PF(CW)	MM(CW)	NaY(CW)	BOR(IS)	CC(IS)	BOR(CW)	CRW(CW)	CLX(CW)	PMZ(IS)
PMZ(IS)	0.897*	0.846*	0.923*	0.872*	0.795*	0.923*	0.795*	0.821*	1
CLX(CW)	0.769*	0.769*	0.846*	0.795*	0.667*	0.744*	0.769*	1	
CRW(CW)	0.897*	0.897*	0.821*	0.923*	0.744*	0.872*	1		
BOR(CW)	0.974*	0.923*	0.846*	0.897*	0.872*	1			
CC(IS)	0.846*	0.846*	0.769*	0.769*	1				
BOR(IS)	0.923*	0.923*	0.897*	1					
NaY(CW)	0.872*	0.872*	1						
MM(CW)	0.974*	1							
Slopes	1	0.814	0.732	0.534	0.504	0.494	0.390	0.227	0.235

The bottom row shows the slope of the straight line of the comparison of each electrode with the best responding electrode, i.e. PF(CW).
* Correlation is significant at the 0.01 level (two-tailed).

sensitivities than the, e.g. borate doped membranes, we believe ion-ion interactions play an important role. This means that the ΔG_{ex} ion–ion interaction term in Eq. (3) is important, but highly correlated for all electrodes tested.

3.3.2. Correlations of the responses for all electrode types

For the nine electrode types tested with the 13 compounds of the first set (see Fig. 2), an inter-electrode correlation matrix was calculated. For the 36 possible membrane pairs, the correlation of the responses for the 13 compounds were calculated. The *R* values (non-parametric Kendall's tau-b correlation coefficients) varied between 0.66 and 0.97 (see Table 3). This table also gives the slope of the straight line for each membrane electrode, when compared with the best responding one, i.e. PF(CW): see row "slope". The latter values give a quantitative idea of the differences in response, or the differences in sensitivity (see discussion in next paragraph).

No subgroups could be clearly identified by a clustering method based on the linear correlation coefficients (*R* values) from Table 3. A hierarchical cluster analysis (HCA) based on these *R* values as the similarity measure, and using a single linkage (nearest neighbour) for amalgamation, yielded no clear subsets. This means that the selectivities of all electrode systems were highly correlated. Even the correlation between the standard BOR(CW) electrode and an electrode containing a neutral crown-, or a calixarene ionophore, was high, with *R* values being, respectively, 0.87 and 0.74. Close inspection of the CRW and CLX electrode responses for each individual compound used in this study also showed no significant selectivity differences. This means that the two receptors (ionophores) used in this study had no effect on the response provoked by any of the compounds studied. A good overview on what was known in 1998 on the effect of neutral ionophores on the response behavior of more hydrophilic basic drugs is given by Buhlmann et al. [1]. This overview gives indications that of the three types of neutral ionophores which are much studied, i.e. calixarenes, crown ethers and cyclodextrins, cyclodextrins may have the greatest effects for basic drugs. This cross-correlation examination also again confirms that ion-pair type electrodes have no special selectivity towards the drug which is used as their cationic constituent. Although the selectivities of the nine electrodes tested were identical, there were striking differences in sensitivity.

3.4. Sensitivity behavior of the different electrode types in FIA, and in HPLC conditions

3.4.1. In FIA conditions

From the data from Fig. 2, and from Table 3 ("slopes" row), we can rank the sensors (decreasing sensitivity) as PF(CW) > MM(CW) > NaY(CW) > BOR(IS) > CC(IS) > BOR (CW) > CRW(CW) > PMZ(IS) > CLX(CW). The "slopes" in the bottom row in Table 3 relate the responses of the different membranes for the 13 compounds of our first set. These slopes are a measure of the sensitivity of the electrodes versus the sensitivity of the PF(CW) electrode (the most sensitive one). For several analytes and electrodes, we also measured this sensitivity via classical calibration curves ($-\log c$ versus potential plots). Typically, we measured a slope of the calibration graph of 56 mV (linear part of the graph). For the sensitively responding electrode/analyte combinations such as, e.g. PF(CW)/bromhexine, the calculated detection limit (calculated from the calibration graph, as specified by IUPAC [29]) for bromhexine was 9.3×10^{-8} M. For the electrode with the lowest sensitivity, e.g. the PMZ(IS) electrode, we measured a detection limit for bromhexine of 7.8×10^{-6} M. This corresponds to an increase in detection limit with a factor 100, when going from the most sensitive electrodes to the least sensitive ones. For ion-pair borate-based electrodes (with cocaine and promazine as countercation), the sensitivity tended to be dependent on the log *P* of these countercations. This was clearly not the case for ion-pair PMA-based electrodes: the PF(CW) electrode (with the lipophilic countercation fluphenazine) was the most sensitive of the whole series. The reason of this different behavior of a mineral (PMA) based electrode versus a borate based electrode is not yet clear, and needs further investigation.

Fig. 5 shows the dependence of the detection limit (measured via calibration graphs) on log *P* of the analyte ion, for electrode type PF(CW). We choose compounds drofenine, diphenylpyraline, chlorpheniramine, ritodrine, cadaverine and adrenaline as

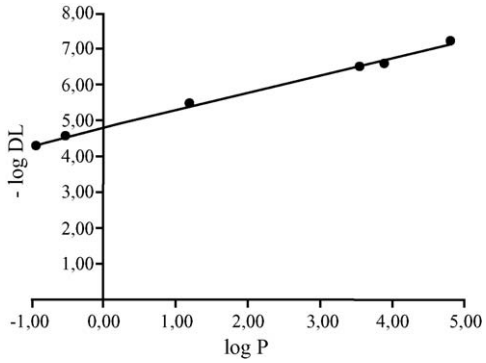

Fig. 5. The dependence of the experimentally determined detection limit DL on log *P*, for electrode type PF(CW). The analytes are, from lowest to highest log *P*: adrenaline, cadaverine, ritodrine, chlorpheniramine, diphenylpyraline and drofenine.

their responses correlated well with their log *P* values (data from Fig. 4).

It is clear from Fig. 5 that an increase of log *P* with 2 units, roughly decreases the detection limit with a factor 10. Or given by the equation of the linear correlation:

$$-\log \mathrm{DL} = 0.489 \log P + 4.74 \quad (4)$$

This equation is very helpfull to estimate the sensitivity with which a basic drug can be measured on an electrode.

3.4.2. In HPLC conditions

Our group uses potentiometric detection in HPLC systems [20,22,30]. When one of the most sensitive electrodes from the above series is applied for the HPLC determination of pharmaceutical drugs, chromatograms as shown in Fig. 6 are obtained. The trends observed in FIA are of course comparable to the ones observed in HPLC. The tracing from Fig. 6 shows a chromatogram with compounds having high log *P* values: bromhexine, fluphenazine, and drofenine. The detection limits, calculated in HPLC conditions were, respectively (3, 4.1 and 2.5) $\times 10^{-7}$ M (injected concentrations). These detection limits are calculated in the characteristic "chromatography" way, i.e.

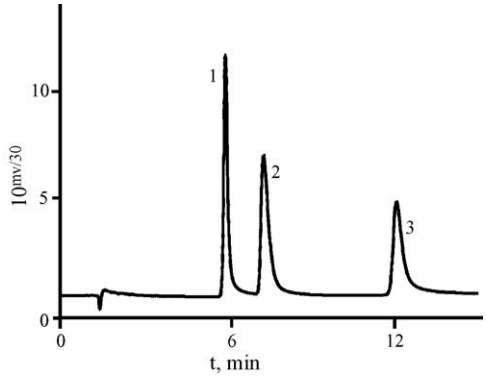

Fig. 6. HPLC determination of compounds with high log *P* on PF(CW). Bromhexine (1), fluphenazine (2), drofenine (3), injected in 2.5×10^{-5} M concentrations on a Nucleodur CN column (Macherey–Nagel), 150 mm × 4.6 mm, 5 mM H_3PO_4, 20% (v/v) CH_3CN, pH 2.67, 1 mL min^{-1}, 20 μL injection.

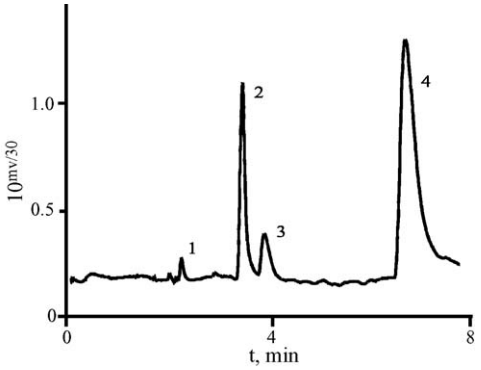

Fig. 7. HPLC determination of compounds with low log *P* on BOR(CW). Adrenaline (1), etilefrine (2), salbutamol (3), ephedrine (4), all 0.22×10^{-3} M injected concentartion, Synergy Hydro-RP column (Phenomenex), 250 mm × 4.6 mm, 1 mM H_3PO_4, 10% (v/v) CH_3CN, pH 3.08, 1 mL min^{-1}, 9 μL injection.

using a signal/noise ratio of 3. Fig. 7 shows a chromatogram with low log *P* compounds adrenaline, etilefrine, salbutamol, and ephedrine. The experimentally measured detection limits here are a factor 10^2 (adrenaline) to 10 (ephedrine) higher than the ones measured for the three lipophilic compounds above. This trend corresponds with the data measured in FIA, which were summarized in Eq. (4).

3.5. Ion-exchange behavior of the electrodes in FIA and HPLC, and long-term stability

In FIA or HPLC conditions, the countercation present in the membrane is exchanged with the cation used in the eluent. Fig. 8 shows the disappearance of methylred from a TCPB/methylred ion-pair electrode in FIA (and HPLC) conditions.

In Fig. 8, the disappearance of the color is given as a function of time, at a flow-rate of 1 mL min^{-1}. At 2 mL min^{-1}, the kinetics of the disappearance of methylred was completely comparable. Methylred disappears in 8 h time at both flow-rates. The color change of the electrode can be observed easily by visual inspection. The independence of the flow-rate indicates that diffusion velocities in the membrane phase are rate determining, rather than diffusion phenomena in the diffusion layer (see [28]

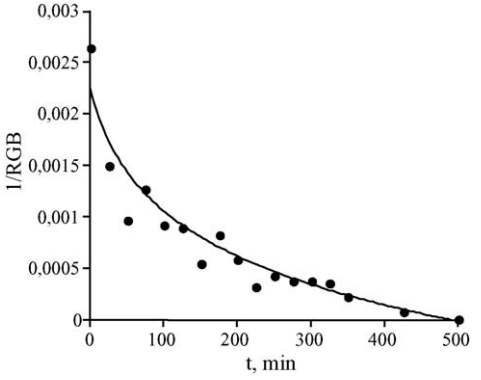

Fig. 8. The disappearance of methylred from a TCPB/methylred ion-pair electrode in FIA (and HPLC) conditions. The eluent used was 1 mM HNO_3, 10% (v/v) CH_3CN, pH 3.02, 1 mL min^{-1}.

for a thorough discussion of this topic for the behavior of ion-exchange materials in this respect). After 8 h, a TCPB/methylred ion-pair electrode exchanges its methylred, and becomes a standard BOR(CW) electrode in hydrodynamic conditions. The sensitivity of the electrode increases in this period of time. This was repeatedly investigated. The polyionic inorganic anion PMA showed a much lower rate of exchange of the countercation fluphenazine in FIA conditions. This very sensitive electrode kept its characteristics for at least 14 days of daily use in the chromatographic conditions of Fig. 6.

4. Conclusions

Nine different ISE's were tested using 25 different basic drugs and biogenic amines. The selectivity behavior of all electrodes was completely comparable. The electrodes' responses towards the compounds were highly correlated to the compounds' log *P* values. A quasi linear relation between log DL and log *P* values could be used as a rule of thumb. Electrodes doped with mineral polyanionic particles clearly showed superior sensitivity over classical borate based electrodes.

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