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Potentiometric detection of exogenic beta-adrenergic substances in liquid chromatography

Grzegorz Bazylak[a,b], Luc J. Nagels[a,*]

[a]*Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp, Belgium*
[b]*Department of Drug Chemistry, Faculty of Pharmacy, Ludwik Rydygier Medical University, Jagiellonska 13, PL-85-067 Bydgoszcz, Poland*

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Abstract

Potentiometric detection with poly(vinyl chloride) (PVC) based liquid membrane electrode coatings is presented for a series of 18 exogenic β-adrenergic substances in cation-exchange-HPLC and RP-HPLC systems. In both types of HPLC modes employing hybrid polymer-silica packings we observed that use of tetrakis(*p*-chlorophenyl)borate (TCPB) containing electrodes yielded limits of detection (DL) down to 10^{-7}–10^{-8} *M* (injected concentrations). The use of eluents with high concentrations of acetonitrile (up to 55%) yielded detection limits down to 10^{-9} *M* (injected concentrations). A quantitative structure-potentiometric response activity relationship (QSAR) was developed for the set of β-adrenergic substances and for a set of PVC-based electrodes using TCPB alone or in admixture with trioctylated α-cyclodextrin, dibenzo-18-crown-6, or calix[6]arene hexaethylester. A multiple linear regression model based on a computationally derived set of 14 molecular descriptors allowed prediction of the detection limits of β-adrenergic substances and other amine substances from their molecular structure.

Keywords: Quantitative structure–activity relationships; Potentiometric detection; Liquid membrane electrodes; β-Adrenergic blockers; β-Adrenergic agonists

1. Introduction

The large class of cationic drugs, which includes the exogenic β-adrenergic substances, represents 70–80% of currently approved medicines [1]. Synthetic phenylethanolamine- and phenyloxypropanolamine-type β-adrenergic substances are a class of sympatholytic drugs possessing activity to G-protein coupled adrenergic receptors [2]. Most such exogenic β-adrenergic substances are still distributed and administered as a racemic mixture [3] for treatment of hypertension, angina pectoris, arrythmia and chronic pulmonary disease [4,5]. They may provoke severe adverse effects such as chronic heart failure, bronchospasm, reduced renal blood flow or myocardial infarction [3,4]. Certain exogenic β-adrenergic substances have been included in many countries in recently published lists of abused and doping substances [6]. Their application should be

*Corresponding author. Present address: Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp, Belgium. Tel.: +32-3-218-0386; fax: +32-3-218-0233.

E-mail address: lnagels@ruca.ua.ac.be (L.J. Nagels).

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restricted and their residues monitored in biochemical [7] or even environmental media [8].

Present methods for their determination include microtiter plate ELISAs [9], GC–MS–MS with prederivatization [10,11], direct HPLC–MS–MS [8,10,11] or HPLC–DAD [10,12]. Electrochemical detection modes are generally more selective than UV detection. In some cases they offer sensitivities comparable with chromatography/MS hyphenated techniques [14], but they may exhibit problems with reproducibility and stability [22]. HPLC methods employing amperometric detection (constant or pulsed modes) were reported for the determination of a limited number of exogenic β-adrenergic substances in body fluids and tissues [13,14]. Quresi and Ericksson [15] and McGrath et al. [16] applied a coulometric detector in ion-pair HPLC systems for the determination of clenbuterol and mabuterol in plasma, and in hair samples. In batch conditions, determination of single β-adrenergic substances includes differential pulse voltammetry [17–19] and potentiometry [20,21]. Electrodes constructed with porous carbon [14,15], polyaniline- [20] or cyclodextrin-PVC membranes [21], Nafion® modified carbon paste [17,18] and molecularly imprinted composites [19] have been used.

Potentiometric detection coupled with HPLC and CE can be a versatile low-cost method for the determination of ionizable substances [23–26]. Although potentiometry is one of the most widely used analytical methods, its application as an HPLC detector is in its infancy. A limited number of applications recently appeared in the literature for inorganic [28,29] and organic [28,30] ions. Our group used potentiometric detection in HPLC and in CE [31,32] methods. Organic acids of biochemical importance [23,25,33–35], and amines [24] could be determined sensitively with potentiometry, after HPLC separation. Potentiometric discrimination of organic compounds in liquid membrane ion-selective electrodes (ISE) can be extended by the use of supramolecular systems creating host–guest interactions, or by the formation of self-assembled species [27,28]. The present study describes for the first time the use of potentiometric detection for the HPLC determination of β-adrenergic substances. A quantitative structure–activity relationship (QSAR) approach is used to predict the potentiometric response of the studied substances on the basis of selected physicochemical properties.

2. Experimental

2.1. Chemicals

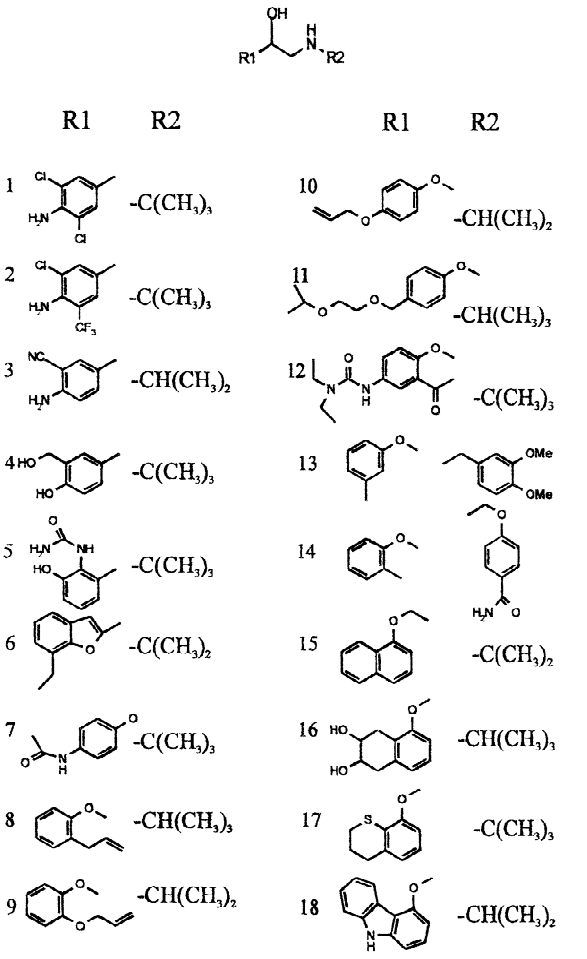
A set of 18 β-adrenergic substances in racemic forms (see Table 1 for molecular structure) was used in all experiments. Hydrochloride salts of carbuterol (Glaxo Research Institute, NC, USA), cimaterol (ICN Biomedicals, Asse-Relegem, Belgium), oxprenolol (Sigma–Aldrich, Bornem, Belgium), *p*-oxprenolol (Ciba-Geigy, Barcelona, Spain), mabuterol (RIVM, Bilthoven, Netherlands), clenbuterol (ICN Biomedicals), alprenolol (Sigma–Aldrich), celiprolol (Selectol-Rorer, Bielefeld, Germany), bufuralol (Hoffman-La Roche, Nutley, NJ, USA), tertatolol (Servier, Neuilly-sur-seine, France) and propranolol (Sigma–Aldrich) were used. Bisoprolol (Tocris Cookson, Bristol, UK) was supplied as a hemifumarate salt. Salbutamol (Sigma–Aldrich) was purchased as a hemisulfate salt. Carazolol (Digen, UK), practolol (Tocris), tolamolol (KarlThomae, Biberach, Germany), bevantolol (UBE Industries, Tokyo, Japan), and nadolol (Sigma–Aldrich) were obtained in a free base form.

Standard stock solutions of β-adrenergic substances (~3 mg per 10 ml) were prepared in the running mobile phases and stored at 4 °C. Working solutions were prepared by appropriate dilution in the fresh mobile phase just before use.

Concentrated reagent grade ortho-phosphoric acid (UCB, Leuven, Belgium), glacial acetic acid (Acros Organics, Geel, Belgium) and HPLC grade acetonitrile (Acros Organics) were used for the preparation of the mobile phase.

All membrane components were of the selectophore quality series available from Fluka (Buchs, Switzerland) and included potassium tetrakis(*p*-chlorophenyl)borate (TCPB); the neutral macrocyclic ionophores hexakis(2,3,6-tri-*O*-octyl)-α-cyclodextrin, dibenzo-18-crown-6 and calix[6]arene hexaethylacetate; the plasticizers bis(1-butylpentyl) adipate (BPA), bis(2-ethylhexyl)sebacate (DOS), and tetrahydrofurane as the solvent. The high relative molecular mass poly(vinyl chloride) (PVC) was

Table 1
Structures of the 18 β-adrenergic compounds



1 clenbuterol, 2 mabuterol, 3 cimaterol, 4 salbutamol, 5 carbuterol, 6 bufuralol, 7 practolol, 8 alprenolol, 9 oxprenolol, 10 p-oxprenolol, 11 bisoprolol, 12 celiprolol, 13 bevantolol, 14 tolamolol, 15, propranolol, 16 nadolol, 17 tertatolol, 18 carazolol

obtained from Janssen Chimica (Geel, Belgium). The structures of TCPB, and of the neutral ionophores are presented in Fig. 1.

2.2. Electrode preparation

The substrate electrodes containing platinum or glassy-carbon (3- or 2-mm diameter) mounted in polyacetate or polyurethane plastic cylindrical bodies

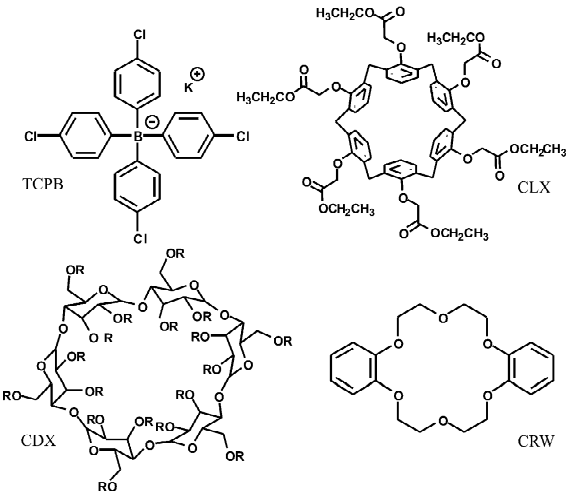


Fig. 1. Structure of the cation sensing compounds (see also Section 2.1).

(9-mm diameter) were carefully polished with a 5-μm grid polishing sheet (3M, St. Paul, MN, USA), cleaned with distilled water and acetone, and dried in air.

The composition (weight percentage) of the membrane material deposited on the substrate electrode was as follows: for a borate electrode (BOR): PVC (26.09%), DOS (57.07%) and TCPB (1.68%); for an octylated α-cyclodextrin electrode (CDX): PVC (32.45%), DOS (63.95%), hexakis(2.3.6-tri-*O*-octyl)-α-cyclodextrin (3.08%) and TCPB (0.52%); for a crown ether electrode (CRW): PVC (33.52%), DOS (58.90%), dibenzo-18-crown-6 (6.43%) and TCPB (1.18%); for a calixarene electrode (CLX): PVC (29.31%), DOS (63.5%), and calix[6]arene hexaethylacetate (5.29%) and TCPB (1.89%). A 300-mg sample of each membrane cocktail was dissolved in 3 ml tetrahydrofuran. The TCPB was used as the component of all electrodes in order to enhance membrane conductivity and to obtain permselectivity. The molar ratio of TCPB/neutral macrocyclic ionophore was 1:1, 13.3:100 and 8.3:100 for the CDX, CRW and CLX electrodes, respectively.

For preparation of the solid-state electrodes, the prepared membrane cocktail was deposited directly on the surface of the platinum or glassy-carbon substrate electrode. Three subsequent layers of the membrane cocktail were deposited on the surface of the electrode at an interval of 20 min using a Pasteur

pipette. Each layer was obtained by deposition of ~40 μl of the membrane cocktail. For each deposited layer the tetrahydrofuran was allowed to evaporate under atmospheric conditions for 20 min and finally for at least 2 h. The prepared liquid membrane electrodes were preconditioned by soaking them for 24 h (or 120 h in the case of CDX electrode) in freshly distilled water.

Between experiments, the prepared liquid membrane electrodes were stored in deionized water. Each electrode was equilibrated for 30 min in the wall-jet type potentiometric detector [37] using the required mobile phase at a flow-rate of 1 ml/min before starting the HPLC analyses.

2.3. HPLC equipment

The HPLC system consisted of a P4000 isocratic pump (Thermo Separation Products, FL, USA) and a Valco LC injector fitted with a 10-μl injection loop. Before injection each sample was filtered through a 0.45-μm syringe cellulose acetate filter (Alltech Associates). In some series of HPLC experiments a P200 pump and an AS100 autosampler (Thermo Separation Products) with a Rheodyne injector (20 μl) was used. The UV measurements were made with a multiwavelength detector type UV100 (Spectra Physics, Fremont, CA, USA) operated at 230 nm. The DataJet type integrator (Spectra Physics) and data acquisition software PC1000 (Thermo Separation Products) were applied for monitoring detector signals.

A universal cation-exchange HPLC column (100× 4.6 mm I.D.; Alltech Associates) packed with a 7-μm silica-based support coated with polybutadiene–maleic acid copolymer was used with a precolumn (7.5 mm×4.6 mm I.D.) module. All cation-exchange HPLC separations were performed using a daily prepared mobile phase of acetonitrile–40 m*M* phosphoric acid, pH 2.35 (15:85, v/v), flow-rate 1 ml/min.

In RP-HPLC experiments the XTerra RP_{18} column (50×3.0 mm I.D.) containing 3-(chlorodimethylsilyl)propyl-*N*-dodecylcarbamate bonded amorphous organosilica with spherical particles d_p = 3.5 μm (Waters, Brussels, Belgium), was applied without pre-column. The mobile phase was acetonitrile–20 m*M* phosphoric acid, pH 2.60 (25:75, v/v).

The apparent pH of the mobile phase was controlled using a common HI8314 pH meter (Hanna Instruments, Germany) without any corrections. The mobile phases were filtered through a 0.2-μm cellulose acetate membrane filter (Alltech Associates) and degassed by continuous bubbling of helium through the solution. All experiments were done at the ambient temperature of 20 °C.

The coated-wire solid-state platinum electrodes were placed in the flow-cell of the wall-jet type potentiometric detector [37]. The HPLC column effluent was directed perpendicularly towards the plane of polymeric liquid membrane deposited on the surface of electrode by PEEK tubing (130 μm I.D., Alltech Associates). The distance from the LC tubing-outlet to the membrane was 100 μm. The Schott B3510 potassium chloride saturated calomel electrode (Hofheim, Germany) was applied as a reference electrode. The potential of the working polymeric membrane electrode was determined against the reference electrode using a high impedance amplifier type 87F with internal resistance 10^{13} Ω (Knick, Germany). The electrode signals were amplified ten times with a laboratory-made amplifier and recorded and integrated by a data acquisition system.

2.4. Molecular modeling and statistical calculations

Molecular modeling of β-adrenergic substances and calculations of their molecular descriptors as molecular surface area (S_{grid}), molecular volume (V_M), dipole moment (μ), polarizability (P), refractivity (R) and hydration energy (H_e) were made with HyperChem v.6.0 (Hypercube, Gainesville, FL, USA) software installed on an IBM-compatible PC. These calculations were performed on the in vacuo optimized geometry of the molecular structure of the analytes in neutral form after employing the MM+ molecular mechanics mode with bond dipoles option. The Polak-Ribiere conjugate gradient procedure was used for optimization. The terminating gradient of 0.001 kcal/mol Å was employed in all cases.

The values of *n*-octanol–water partition coefficients ($\log P$) characterizing the lipophilicity of the

analytes were calculated for their neutral forms by using neural network computing software developed by Parham et al. [38]. The number of hydrogen bond acceptors (HBA) and the number of hydrogen bond donors (HBD) as well as their ratio (RATIO) and their difference (Δ) were calculated according to the method of Lipinski et al. [39,40] available on the internet [38]. The values of the topological polar surface areas (TPSA) were calculated by summarizing the respective fragmental constants of the two-dimensional structure of the considered drug according to a procedure proposed by Ertl et al. [41]. The values of the logarithm of the first global (macroscopic) acidic ionization constants pK_a of β-adrenergic substances were calculated with Pallas v.1.1 software (CompuDrug Chemistry, Budapest, Hungary). Statistical calculations for the interpretation of collected experimental data were performed with Statistica v.4.3 (Stat-Soft, Tulsa, OK, USA) software.

3. Results and discussion

3.1. HPLC separation and detection

The potentiometric detector set-up used in this study was described in earlier publications from our group [23–25,31–35]. In the present study, it was equipped with liquid membrane type coated wire electrodes based on PVC. These materials develop surface potentials as analyte cations can penetrate the membrane matrix, whereas anions are excluded. Their response depends on the distribution coefficient of the analyte cation in the aqueous buffer–membrane system [23]. If substances are added to the membrane which strongly complexes the analyte ion (ionophores), the response and the selectivity will be enhanced. We tested one membrane without added ionophore, and three membranes with different neutral macrocyclic ionophores added (see Fig. 1 for their structures). The electrodes were used in a cation-exchange HPLC system (see Experimental section), and in a reversed-phase HPLC system. Fig. 2 shows a representative chromatogram obtained for the separation of six β-adrenergic substances on a reversed-phase HPLC system, and detection with a TCPB liquid membrane electrode (no ionophore added). All compounds were injected in 10^{-6} *M* concentrations. Measured values of potentiometric detection limits are quite comparable in both types of HPLC systems used. However, the reversed-phase HPLC system offers spectacular reduction of total analysis time in comparison to the cation-exchange HPLC system. This was especially the case for clenbuterol, propranolol, and bufuralol.

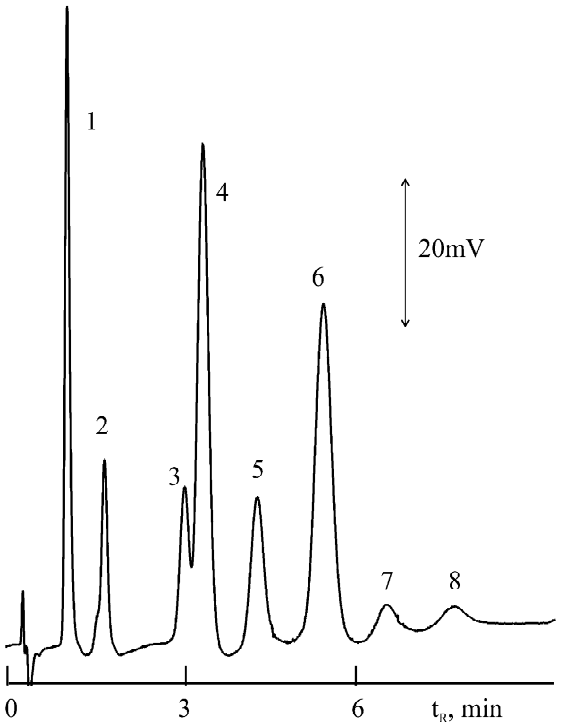


Fig. 2. Isocratic separation of a mixture of six β-adrenoceptors in RP-HPLC with potentiometric detection using a TCPB liquid membrane-based electrode. Peaks: (1) clenbuterol, (2) practolol, (3) alprenolol, (4) propranolol, (5) carazolol, (6) bevantolol, (7) unknown, (8) unknown. Injected volume 20 μl, injected concentration 10^{-6} *M*. Mobile phase: 20 m*M* phosphoric acid–acetonitrile (75:25, v/v). Column: XTerra RP_{18} without pre-column, 50 mm×3.0 mm I.D., d_p=3.5 μm, flow-rate 1 ml/min.

Table 2 shows detection limits for the four types of electrodes which were used in this study, and for a UV detector. The detection limit (DL) was determined experimentally as the injected concentration which yields a peak height of three times the random baseline noise band ($4\sigma_{noise}$). This definition of the detection limit is used in chromatographic conditions. People working with potentiometry usually

Table 2
Detection limit (DL) values of β-adrenergic drugs in cation-exchange HPLC as expressed by injected molar concentrations: comparison of UV and potentiometric detection

Compound	UV_{230} [a]	TCPB [b]	CDX [c]	CRW [d]	CLX [e]
β-Adrenergics					
Salbutamol	2.6×10^{-8}	3.5×10^{-5}	3.7×10^{-5}	2.5×10^{-5}	2.9×10^{-5}
Carbuterol	1.7×10^{-7}	8.5×10^{-5}	7.7×10^{-5}	1.9×10^{-4}	2.4×10^{-4}
Cimaterol	3.0×10^{-7}	2.5×10^{-5}	4.8×10^{-6}	2.7×10^{-5}	3.6×10^{-5}
Mabuterol	2.9×10^{-6}	2.2×10^{-7}	5.6×10^{-8}	3.2×10^{-6}	3.4×10^{-5}
Clenbuterol	2.6×10^{-7}	6.7×10^{-7}	1.7×10^{-7}	3.4×10^{-6}	1.7×10^{-6}
β-Adrenolytics					
Nadolol	2.3×10^{-7}	5.9×10^{-5}	2.1×10^{-5}	6.2×10^{-5}	1.0×10^{-4}
Practolol	1.7×10^{-7}	5.4×10^{-5}	6.2×10^{-5}	1.1×10^{-4}	1.9×10^{-4}
Bisoprolol	3.5×10^{-7}	1.3×10^{-5}	3.7×10^{-7}	2.6×10^{-6}	2.7×10^{-6}
Oxprenolol	1.0×10^{-6}	1.7×10^{-6}	7.7×10^{-6}	4.5×10^{-6}	5.8×10^{-7}
p-Oxprenolol	0.7×10^{-6}	1.6×10^{-6}	7.5×10^{-7}	7.8×10^{-6}	3.5×10^{-7}
Carazolol	4.4×10^{-7}	6.0×10^{-7}	2.0×10^{-7}	3.0×10^{-6}	2.1×10^{-6}
Alprenolol	2.2×10^{-6}	6.7×10^{-7}	3.6×10^{-7}	4.8×10^{-6}	3.0×10^{-7}
Celiprolol	1.6×10^{-7}	1.5×10^{-5}	1.6×10^{-5}	5.0×10^{-5}	6.8×10^{-5}
Bufuralol	3.5×10^{-7}	2.8×10^{-7}	2.2×10^{-8}	3.9×10^{-6}	1.5×10^{-6}
Tertatolol	1.2×10^{-6}	3.5×10^{-7}	1.2×10^{-7}	3.7×10^{-6}	1.8×10^{-6}
Propranolol	2.7×10^{-8}	2.1×10^{-7}	1.0×10^{-7}	2.8×10^{-7}	5.3×10^{-7}
Tolamolol	1.3×10^{-6}	1.4×10^{-5}	1.3×10^{-6}	1.5×10^{-6}	2.0×10^{-5}
Bevantolol	1.3×10^{-6}	2.2×10^{-6}	1.0×10^{-6}	1.1×10^{-6}	1.6×10^{-6}

[a] Spectrophotometric detection at 230 nm.
[b] Electrode with tetrakis(*p*-chlorophenyl)borate.
[c] Electrode with trioctylated α-cyclodextrin.
[d] Electrode with dibenzo-18-crown-6.
[e] Electrode with calix[6]arene hexaethylacetate.

take another definition of the detection limit. For a more thorough discussion of this, and for a discussion of calibration curves obtained with potentiometric detection, the reader is referred to another paper from our group, i.e. Ref. [35] and articles cited therein. The detection limits were measured using the cation-exchange chromatographic system. TCPB containing electrodes (no addition of neutral macrocyclic ionophores) give more than five-fold improvement in limits of detection for mabuterol, bufuralol, alprenolol and tertatolol in comparison with UV detection. This clearly shows that the sensitivity of potentiometric detection can be competitive with UV detection even for strongly UV absorbing compounds. In contrast to a UV detector, the potentiometric detector will also detect non-UV absorbing amine contaminations sensitively [24,42]. From the results shown in Table 2, one can also conclude that the use of octylated α-cyclodextrin as the ionophore in liquid membrane electrodes generally yields the most sensitive potentiometric detection of the β-adrenergic substances. Crown ether-, or calixarene-based electrodes offer no advantage over electrodes without added ionophore, except in a few specific cases. DL values in the 10^{-7} *M* range were obtained (injected concentration). When measured with an XTerra reversed-phase column, the detection limits are generally lower, down to the 10^{-8} *M* range.

The membrane electrode lifetimes were studied quantitatively in acetonitrile–dilute acid eluent conditions in a previous study [24]. The activity of a TCPB-based electrode reduces to 79% of its initial activity (measured peak area) after continuous use in acetonitrile–5 m*M* HNO_3 (5:95, v/v) during 51 days. In the present study, acetonitrile concentrations exceeding 50% were often used. In these conditions, the electrode activity is lost after 3 days of continu-

ous use. Remarkable detection limits are noted however in such eluents with high acetonitrile content: injected concentrations down to 10^{-9} *M* were routinely obtained for, for example, clenbuterol. The effect was also clearly noted for other classes of compounds presently under study in our group, i.e. ethanolamines (set of 11 compounds) [42], and linear chain aliphatic amines [24]. The positive effect of organic modifiers like acetonitrile on detection limits becomes clear when Fig. 3 is considered: increasing the AcN concentration from 5 up to 25% increased the peak area by a factor of 12 for clenbuterol at injected concentrations of 10^{-8} *M*. The effect of gradient elution using increasing acetonitrile concentrations has not been studied in the present paper.

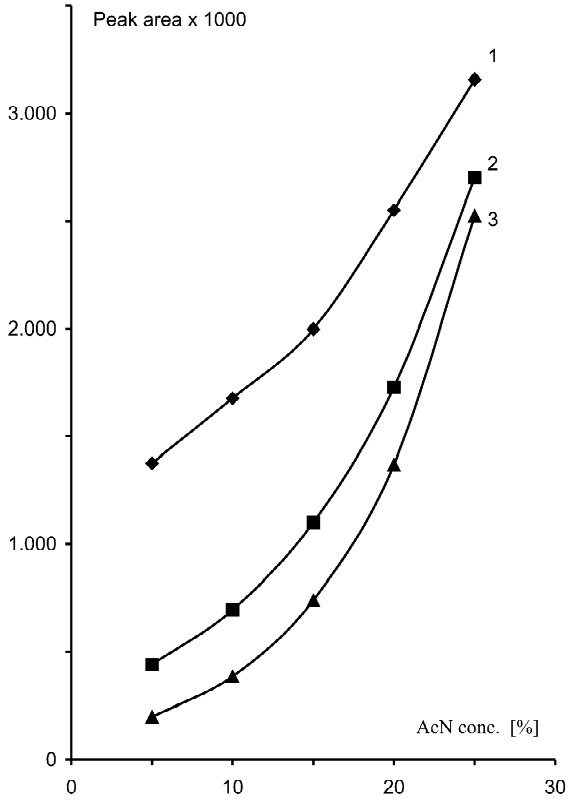


Fig. 3. Effect of acetonitrile (AcN) concentration in the HPLC mobile phase on the response of a CDX-based liquid membrane electrode used for the detection of clenbuterol in an RP-HPLC system as described in Fig. 2. The injected concentrations were 1.2×10^{-6} *M* (curve 1), 1.2×10^{-7} *M* (curve 2) and 1.2×10^{-8} *M* (curve 3).

In a previous paper from our group [25], the detector was used successfully with a gradient of sodium hydroxide.

The liquid membrane electrodes used need some preconditioning treatment. Fig. 4 shows the change in response of an octylated α-cyclodextrin containing liquid membrane electrode as a function of time. The electrode was kept in deionized water, and placed in the detector at specified time intervals. Maximum sensitivity was obtained after 6 days. This is probably due to molecular rearrangements taking place inside the membrane phase during contact with the water phase. According to Harrison et al., PVC membranes include microdroplets or clusters of water varying in diameter from 16 nm to several μm [43]. This water inclusion process may be rather slow. The PVC–weakening agent mixture used in liquid membranes is in a ''rubbery'' state: it has a glass transition temperature, T_g, which is lower than the ambient temperature. This state has much slower molecular dynamics than the liquid state.

Human saliva samples were analyzed by direct injection on the HPLC system described in Fig. 2 (no sample clean-up) (Fig. 5). Spiking with clenbuterol down to a 1.0×10^{-6} *M* concentration yielded interference-free chromatographic peaks for clenbuterol (Fig. 5A, tracing 3). Application of UV detection was clearly inferior in this respect as in the latter

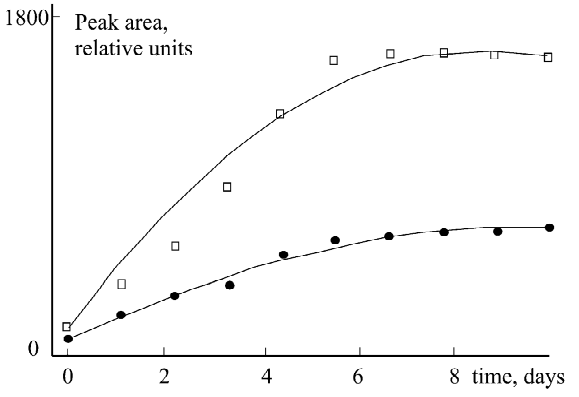


Fig. 4. Time related changes in response of a CDX-based liquid membrane electrode used in the cation-exchange HPLC conditions (see Section 2.3) for clenbuterol (upper curve) and oxprenolol (lower curve). In between two determinations, the electrode was stored in deionized water.

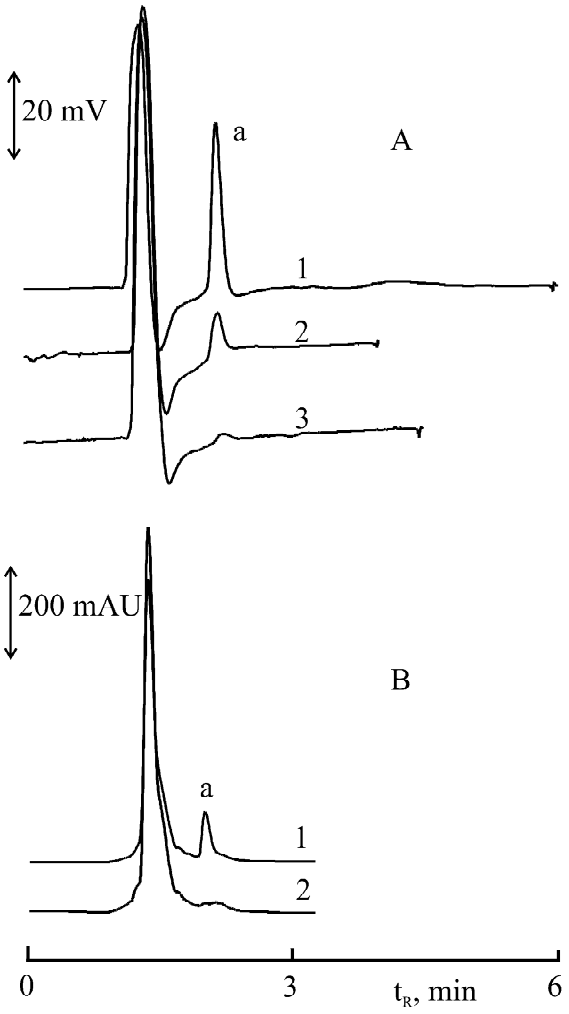


Fig. 5. Comparison of direct determination of clenbuterol (a) in spiked human saliva samples at concentrations 2.0×10^{-4} *M* (tracing 1), 2.0×10^{-5} *M* (tracing 2), and 2.0×10^{-6} *M* (tracing 3) by RP-HPLC (Hypersil C_{18}ec, 125×4.6 mm, AcN-1 m*M* H_3PO_4 (52:48, v/v) at 0.5 ml/min, 10-μl injections) with potentiometric detection using a TCPB-based liquid membrane electrode (A) and using UV detection at 230 nm (B).

mode, spiking to a 1.0×10^{-4} *M* concentration was needed to obtain a reasonably interference-free clenbuterol peak (Fig. 5B, tracing 1).

3.2. Structure–potentiometric response relationships

Quantitative structure–activity relationships (QSAR) are used often in pharmacochemical screening methods [44–46]. The solubility of drugs in bio-membranes is an important factor which has to be estimated on the basis of molecular descriptors [47]. The distribution properties of our analytes over the (water-based) eluent phase and the liquid membrane determine the sensitivity of the potentiometric sensor [23]. It seems quite logical then, that the pharmacochemical methodology can be applied also to the structure–activity (response, or detection limit) relationship for potentiometric sensing. As far as we know, this approach has never been described in the literature for predicting the sensitivity or selectivity of a potentiometric membrane towards analyte ions. This approach can be only valid of course as long as no strong and very selective ionophore–analyte interactions are used in the membranes. The latter situation is mostly valid however for potentiometric sensors used in batch determinations, as high selectivity is wanted in such cases. In our HPLC systems however, extreme selectivities will be mostly unwanted.

A total of 14 molecular descriptors were calculated for the 18 β-adrenergic substances. The set of molecular descriptors differing in their ''dimensionality'' consisted of pK_a, log *P*, log *D*, μ, *P*, *R*, H_e, (one-dimensional, 1D descriptors), HBA, HBD, RATIO, Δ (2D descriptors) and S_{grid}, V_M, TPSA (3D descriptors). These molecular descriptors were chosen because they are used in pharmacochemical screening of β-adrenergic substances and other drugs candidates [47] for preliminary evaluation of properties such as intestinal adsorption, blood–brain barrier penetration, and other membrane transport related processes [48–57]. A limited set of calculated molecular descriptors is given in Table 3.

Inspection of the intercorrelation matrix of the 14 molecular descriptors indicated that most of these parameters were non-correlated ($r<0.70$). A highly positive intercorrelation is exhibited only between calculated values of polarizability and refractivity ($r=0.99$). A partial positive correlation ($r=0.88$) was revealed between the values of the logarithm of the partition coefficient of the neutral form of β-adrenergic substances in *n*-octanol–water systems (log *P*) and the values of the logarithm of the partition coefficient of these drugs in *n*-octanol–aqueous buffer (pH 7.4) phase (log *D*) calculated with the following formula [58]:

$$\log D = \log P - \log[1 + 10^{(pK_a - 7.4)}] \quad (1)$$

Table 3
Characteristic molecular descriptors of the β-adrenergic compounds

Compound	pK_a	Log P	Log D	HBA	HBD	μ (D)	P (Å^3)	H_e kcal/mol
Clenbuterol	9.72	2.91	0.58	5	3	3.02	27.2	−2.89
Clenbuterol	9.72	+2.91	0.58	5	3	3.02	27.2	−2.89
Mabuterol	8.16	1.72	0.89	7	3	1.22	22.7	−9.08
Cimaterol	9.78	0.62	−1.76	3	3	4.90	24.2	−12.7
Salbutamol	9.07	−0.69	−2.36	4	4	2.58	21.0	−15.2
Carbuterol	9.93	−1.72	−4.25	6	5	3.65	27.3	−12.9
Bufuralol	9.20	3.24	1.43	3	2	1.47	30.4	−3.31
Practolol	9.44	1.03	−1.01	5	3	3.42	29.1	−9.31
Alprenolol	9.59	2.81	0.62	3	2	2.08	29.3	−5.47
Oxprenolol	9.50	2.07	−0.03	4	2	0.97	30.0	−8.20
p-Oxprenolol	10.01	2.20	−0.48	4	2	1.28	30.0	−9.52
Bisoprolol	9.57	2.04	−0.13	5	2	1.98	36.3	−6.45
Celiprolol	9.66	1.39	−0.87	7	3	6.02	40.9	−1.43
Bevantolol	8.43	2.65	1.58	5	2	1.23	38.6	−10.0
Tolamolol	7.94	1.81	1.15	6	3	3.35	36.2	2.98
Propranolol	9.53	2.96	0.82	3	2	1.16	32.0	−6.02
Nadolol	9.67	0.98	−1.29	5	4	1.23	33.7	−12.0
Tertatolol	10.42	3.36	0.33	4	2	0.69	33.6	−4.07
Carazolol	9.52	2.77	0.64	3	3	2.46	34.3	−6.92

A multivariate statistical analysis was made in which the 14 molecular descriptors were correlated with the logarithm of the detection limit ($\log DL$) values taken from Table 2 which were measured in the cation-exchange HPLC mode. Detection limits are injected concentrations for which a signal-to-noise ratio (S/N) of 3 is reached [22]. In pharmacochemical QSAR studies, concentrations are also used for which the measured activity of the drug exceeds a specified value [59], and the logarithm of this concentration is taken.

In the case of the PVC electrode containing only lipophilic cation-exchanger TCPB, the following statistically significant structure–potentiometric response relationship was obtained:

$$\log DL = -6.4594 - 0.6657 \log P + 0.0703P \quad (2)$$

$$n = 18; r = 0.9023; \mathrm{SE} = 0.4207; F = 32.87$$

Eq. (2) indicates that the DL of β-adrenergic substances on TCPB-based electrodes is mainly dependent of their lipophilicity expressed by $\log P$ values, and on their polarizability P. The contribution of the $\log P$ factor and of the polarizability P in Eq. (2) is of comparable significance. The importance of $\log P$ values is not surprising, as it is known that the response of potentiometric electrodes often increases with increasing lipophilicity of the analyte ions. For a homologous series of linear chain aliphatic amines (C1–C6), a perfectly linear relationship was found between $\log P$ and the detection limits on a TCPB-based electrode (results not shown). Eq. (2) shows however that distribution of a cationic drug between a water phase and a potentiometric membrane cannot be predicted by $\log P$ values alone. Besides solubility in a lipophilic environment, ion–ion interactions of the analytes with the TCPB anion will play an important role. According to Eq. (2), lipophilic ions with low polarizability (''hard'' ions) are likely candidates for sensitive detection at a non-selective TCPB-based electrode.

An analogous statistically significant relationship was obtained for PVC-based electrodes containing octylated α-cyclodextrin as the macrocyclic ionophore:

$$\log DL = -7.0190 - 0.7967 \log P + 0.0815P \quad (3)$$

$$n = 18; r = 0.9249; \mathrm{SE} = 0.4331; F = 44.42$$

Eq. (3) gives higher values of the regression coefficients as compared to the previously discussed Eq. (2). This indicates that the impact of hydro-

phobic interactions on the response of the described electrode was enlarged by the addition of octylated α-cyclodextrin. Indeed, improved DL values were observed for more hydrophobic β-adrenergic substances on the electrodes with octylated α-cyclodextrin ionophores as compared to TCPB-based electrodes (Table 2). This result is not surprising as it is well known that native and modified cyclodextrins prefer hydrophobic interactions inside their cavity [21].

For the PVC-based electrodes containing dibenzo-18-crown-6, and for that containing calix[6]arene hexaethylacetate as the macrocyclic ionophore, the three term relationships (4) and (5) were established, respectively:

$$\log DL = -5.7398 - 0.2597 \log D - 0.0262H_e + 0.1558\mu \quad (4)$$

$n = 18$; $r = 0.8524$; SE = 0.4176; $F = 12.40$

$$\log DL = -7.5563 - 0.1415 \log P + 0.1211\mu - 0.4943HBD + 0.2039HBA \quad (5)$$

$n = 18$; $r = 0.9081$; SE = 0.4540; $F = 15.29$

The equations become more complex, as the interactions of amines with the dibenzo-18-crown-6 and with the calixarene ionophore are quite specific as was pointed out recently by Brouwer et al. [60,61].

Potentiometric sensors for batch systems are always designed for one specific ion, which is not interesting for chromatographic situations. From the data given in Table 2, it is clear that all electrodes used in the present study are not highly selective towards the investigated analytes. Moreover, these electrodes can also be used for the determination of other classes of compounds such as aliphatic amines [24] and aliphatic alcoholamines (to be published). Therefore, a good method for predicting the response behavior of these electrodes versus ionizable organic substances would be very welcome. Approaches for prediction of the response behavior of non-selective potentiometric sensors are quite diffuse in the literature [28]. Mostly, only qualitative arguments are given to describe the ranking of the selectivity coefficients [23,27,28,36]. The situation is different of course if the liquid membrane electrodes are designed for one specific anion or cation, using highly selective complexing ionophores. The above described multiple correlation models can be very useful in predicting the response behavior of the β-adrenergic substances, and of other organic cationic substances in potentiometric sensing using non-selective liquid membrane electrodes. Especially for non-selective electrodes such as the TCPB-based electrode and the octylated α-cyclodextrin containing electrode, the relation between log *DL* values and the lipophilicity (log *P*) plus polarizability (*P*)—as expressed by Eqs. (2) and (3)—is very promising. We applied this equation to two other classes of organic cationic compounds, i.e. aliphatic amines (six compounds) and aminoalcohols (11 compounds). These classes of compounds can also be very sensitively detected in HPLC systems equipped with potentiometric detection [24,42]. Both classes have much lower log *P* values (variation from, for example, −2 to +0.4 for the aminoalcohols) than the here described β-adrenergic substances. Surprisingly, they showed detection limits (on TCPB-based electrodes) over a range which is comparable to the range of the detection limits of the β-adrenergic substances studied (10^{-6}–10^{-8} *M* concentrations injected). These classes have much lower polarizabilities than the β-adrenergic substances. Application of Eq. (2) to these compounds showed that this equation also yields well correlated results for these two classes of totally different chemical nature. For the 11 investigated aliphatic aminoalcohols, a correlation coefficient $r = 0.78$ was found [42] in case of the TCPB-based electrode used in the described cation-exchange HPLC conditions:

$$\log DL = -7.7019 - 0.9567 \log P + 0.1311P_0 \quad (6)$$

$n = 11$; $r = 0.7778$; SE = 0.4782; $F = 6.12$

For linear chain aliphatic amines (from methylamine to octylamine), the equation yields a correlation coefficient $r = 0.95$:

$$\log DL = 18.4296 + 13.4712 \log P - 3.6659P_0 \quad (7)$$

$n = 7$; $r = 0.9461$; SE = 0.2047; $F = 17.08$

However, when an octylated α-cyclodextrin based PVC-electrode was applied, the relationship leads to a further improvement in correlation coefficient up to $r = 0.99$:

$$\log DL = 10.9752 + 7.8199 \log P - 2.2920 P_0 \quad (8)$$

$n = 7$; $r = 0.9946$; SE = 0.1134; $F = 182.63$

Thus, for CDX-based electrodes, the significance of hydrophobic interactions increased as indicated by the enlarged value of the individual regression coefficient of the $\log P$ variable in Eq. (8).

3.3. Conclusions

Potentiometry is able to determine β-adrenergic substances sensitively in HPLC systems. The use of octylated α-cyclodextrin as the macrocyclic ionophore yields the lowest detection limits, down to 10^{-7}–10^{-8} M (injected concentrations) for many compounds. Detection limits in the low pg range could be attained (injected concentrations down to 10^{-9} M) for very lipophilic compounds, and using high acetonitrile concentrations in the eluent. Non-UV absorbing amines (i.e. lysosomotropic amines) can also be directly detected. A QSAR approach was used to predict the sensitivity of potentiometric electrodes for the investigated products. On non-selective liquid membrane electrodes, detection limits of β-adrenergic substances and amines were found to decrease with increasing lipophilicity ($\log P$) and decreasing polarizability (P). New electrode materials are now under study for prolonged use in eluents with high organic modifier content, and with better adhesion characteristics. The materials will be used in miniaturized systems of analysis.

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