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A novel potentiometric approach for detection of beta-adrenergics and beta-adrenolytics in high-performance liquid chromatography

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

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

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Abstract

Potentiometric approach enabling sensitive and reliable detection for a series of 20 autonomic beta-adrenergic ligands with the use of poly(vinyl chloride) (PVC) based liquid membrane electrode coatings in the normal-bore cation exchange HPLC and narrow-bore reversed phase HPLC system is presented. It was found that in both kinds of HPLC modes with a contemporary hybrid polymer-silica packings an application of electrodes containing a tetrakis(*p*-chlorophenyl)borate (TCPB) gives limits of detection below to 8.0×10^{-7} mol l^{-1} (injected concentrations). In case of highly hydrophobic beta-adrenergic drugs the use of binary aqueous mobile phases with high concentrations of acetonitrile (up to 25% v/v) shifting an observable detection limits (DL) down to 2.0×10^{-8} mol l^{-1}, especially for electrodes with addition of trioctylated α-cyclodextrin. The characteristics of developed potentiometric detectors was established by proposed a quantitative structure–potentiometric response relationships (QSPRRs) for a series of diversified beta-adrenergic compounds and for a set of the PVC based electrodes using TCPB alone as well as in combination with trioctylated α-cyclodextrin, dibenzo-18-crown-6, or calix[6]arene hexaethylester as the neutral macrocycle ionophore. A highly significant QSPRRs equations were obtained leading to reasonable prediction of the DL of specified electrodes in terms of the computationally derived set of molecular descriptors of beta-adrenergics and beta-blocking agents and similar amino alcohol type xenobiotics.

Keywords: Liquid membrane electrodes; Electrochemical detection; HPLC; Macrocycles; QSAR; beta-Adrenergic agonists; beta-Adrenergic blockers

1. Introduction

Currently, the doubtless target identification for newly synthesized beta-adrenergics and beta-adrenolytics with regards to their agonistic or antagonistic activity includes mainly a distinct types of classical neurotransmitter-binding trasmembrane G-protein-coupled receptors [1,2]. Most of approved beta-adrenergic drugs are still distributed and administered as a racemic mixture [3] for treatment of hypertension, thyrotoxicosis, angina pectoris, cardiac arrythmics and chronic pulmonary diseases [4,5]. They may provoke severe adverse effects such as chronic heart failure, bronchospasm, reduced renal blood flow or myocardial infarction [3,4]. Certain beta-adrenergic drugs were included in many countries on the recently published lists of abused and doping substances [6]. Their application should be restricted and their residues monitored in biochemical [7] or even environmental media [8].

Present methods for their determination include optical immunobiosensor assay [9], microtiter plate enzyme immunoassay (EIA) kits [10], GC–MS–MS

* Corresponding author.
E-mail address: gbazylak@amb.bydgoszcz.pl (G. Bazylak).

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with pre-derivatization [11,12], direct HPLC with tandem mass spectrometry [11] or HPLC-DAD [11,13]. Electrochemical detection modes are often more selective than EIA kits or UV detection in analyses of multicomponent and/or contaminated samples [14]. In some cases they offer sensitivities comparable with chromatography/MS hyphenated techniques [15], but they may exhibit problems with reproducibility and stability [16]. HPLC methods employing amperometric detection (constant or pulsed modes) were reported for the determination of a limited number of beta-adrenergics in body fluids and tissues [14,15]. Ali Quresi and Eriksson [17] and McGrath et al. [18] 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 beta-adrenergics or beta-adrenolytics includes differential pulse voltammetry [19–21] and potentiometry [22,23]. Electrodes constructed with porous carbon [15,17], Nafion® modified carbon paste [19,20], molecularly imprinted composites [21], polyaniline- [22] or cyclodextrin-poly(vinyl chloride) (PVC) membranes [23] were used.

Potentiometric detection coupled with HPLC and CE can be a versatile low-cost method for the determination of ionizable substances [24–27]. 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 for organic [28,30] ions. Our group used potentiometric detection in HPLC and in CE [31,32] methods. Organic acids of biochemical importance [24,26,33–35], and amines [25] could be determined sensitively with potentiometry, after HPLC separation. Potentiometric discrimination of organic compounds in liquid membrane ion-selective electrodes can be extended by the use of supramolecular systems creating host–guest interactions, or by the formation of self-assembled species [28,36]. The present study describes for the first time the use of potentiometric detection for the HPLC determination of beta-adrenergic drugs. 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 randomly created set of 20 racemic beta-adrenergic drugs (see Fig. 1 for molecular structures) as supplied from different manufacturers was used in all experiments. Hydrochloride salts of tulobuterol, carbuterol, cimaterol, oxprenolol, *p*-oxprenolol, mabuterol, clenbuterol, alprenolol, celiprolol, bufuralol, tertatolol, and propranolol were used. Bisoprolol was supplied as a hemifumarate salt. Terbutaline and salbutamol was purchased as a hemisulfate salt. Carazolol, practolol, tolamolol, bevantolol, and nadolol were obtained in a free base form.

Standard stock solutions of drugs (ca. 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 hexaethylester; the plasticizer bis(2-ethylhexyl)sebacate (DOS), and tetrahydrofurane as the solvent. The high relative molecular mass PVC was obtained from Janssen Chimica (Geel, Belgium). The structures of TCPB, and of the neutral ionophores are presented in Fig. 2.

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 (9 mm diameter) were carefully polished with a 5 μm grid polishing sheet (3M, St. Paul, MN), 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 calix[6]arene hexaethylester electrode (CLX): PVC (29.31%), DOS (63.5%) calix(6)arene hexaethylacetate ester (5.29%) and TCPB (1.89%). Three hundred milligram 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 perm-selectivity. The molar ratio of TCPB/neutral macrocyclic ionophore was 1:1, 0.133:1 and 0.083:1 for the CDX, CRW and CLX electrodes, respectively.

For preparation of the solid-state electrodes, the prepared membrane cocktail was deposited directly on

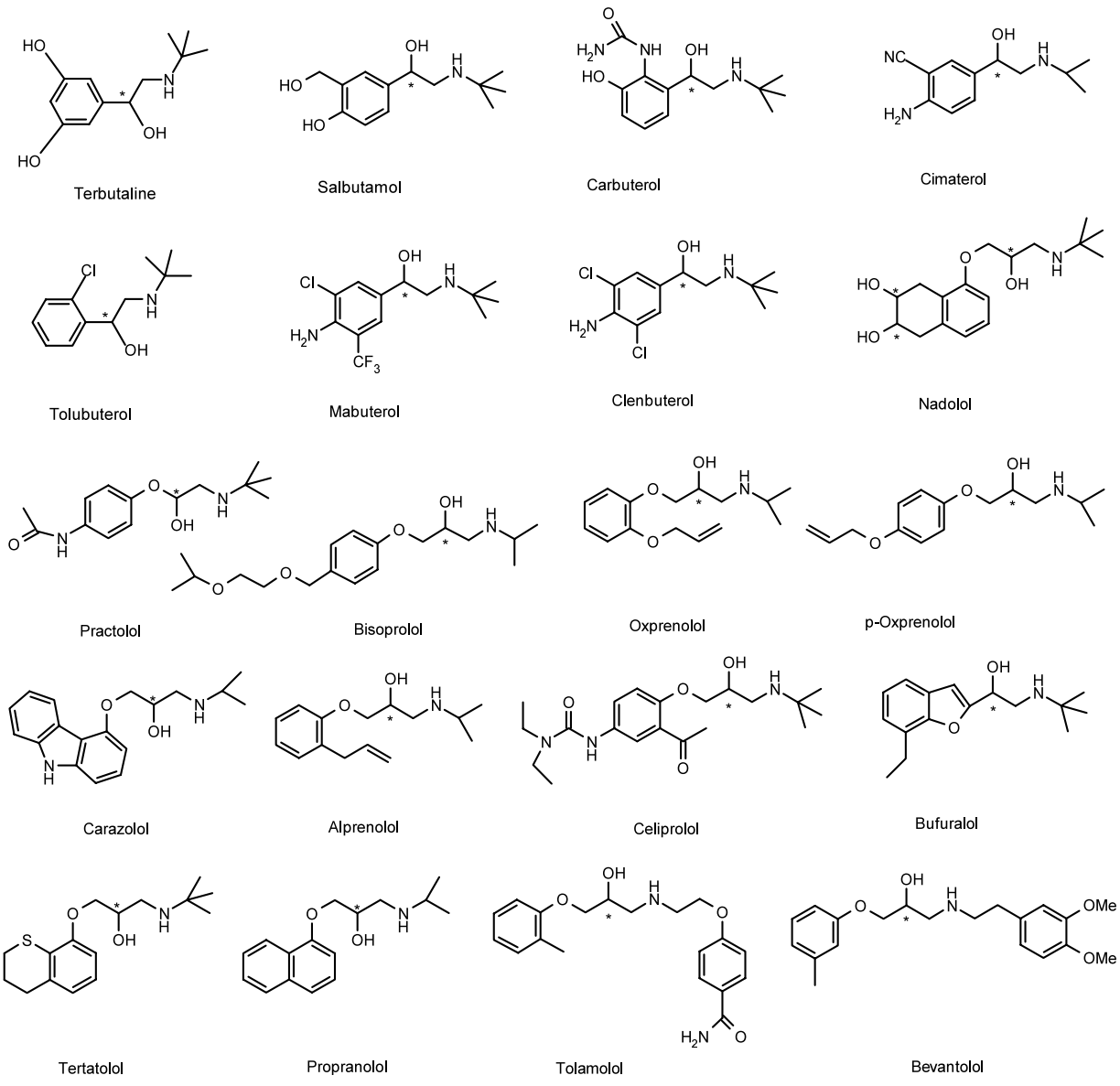

Fig. 1. Structure of 20 beta-adrenergic and beta-adrenolytic drugs used in this studies.

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 ca. 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 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 1.0 ml min^{-1} before starting the HPLC analyses.

2.3. HPLC instrumentation

In all HPLC experiments a isocratic pump P200 and AS100 autosampler (tsp-Thermo Separation Products, Riviera Beach, FL) with a Rheodyne 7010 sample injector (20 μl) (Rheodyne Inc., Cotati, CA) was used. The UV measurements were made with a multiwavelength detector type UV100 (Spectra Physics, Fremont, CA) operated at 230 nm. The DataJet type integrator (Spectra Physics) and data acquisition software PC1000

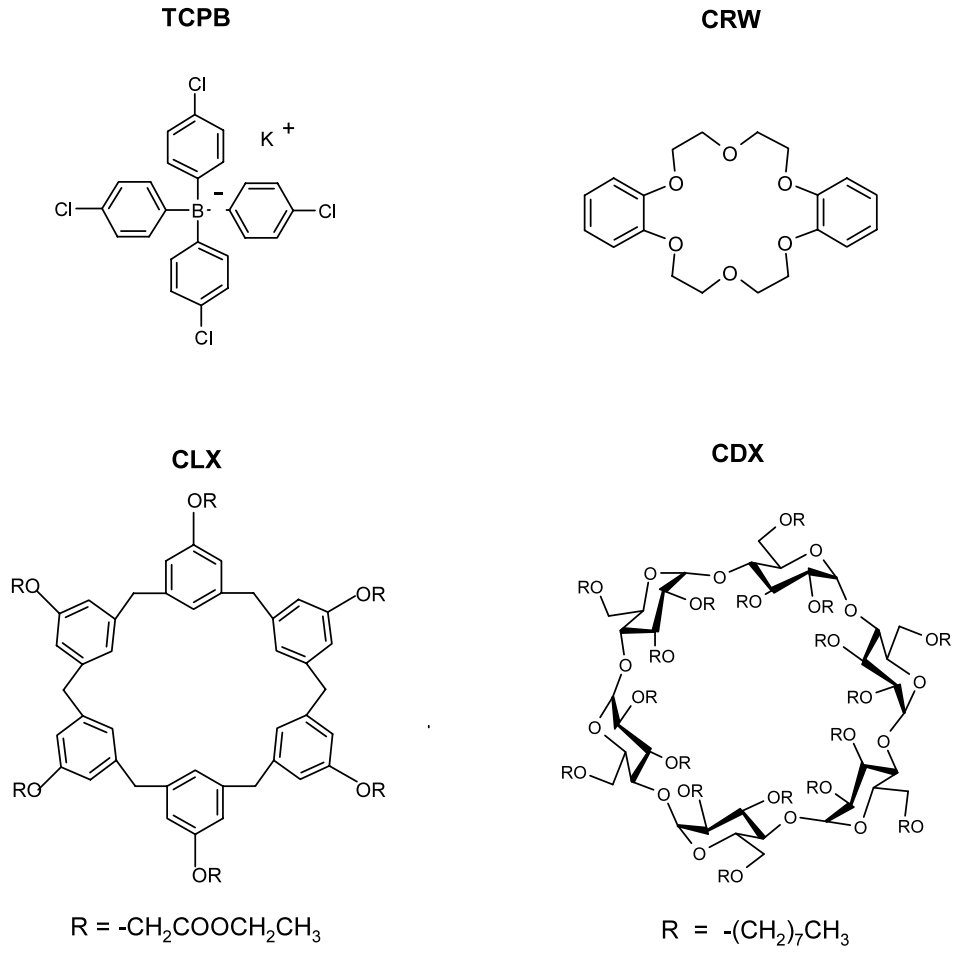

Fig. 2. Structure of cation sensing compounds: TCPB, tetrakis(*p*-chlorophenyl)borate; CRW, dibenzo-18-crown-6; CLX, calix[6]arene hexaethylester; CDX, trioctylated α-cyclodextrin.

(tsp-Thermo Separation Products) were applied for monitoring detector signals.

A universal cation exchange HPLC column (100 × 4.6 mm i.d.) (Alltech Associates, Lokeren, Belgium) packed with a 7 μm silica-based support coated with polybutadiene–maleic acid copolymer was used with a precolumn (7.5 × 4.6 mm i.d.) module. All normal-bore cation exchange HPLC separations were performed using a daily prepared mobile phase of acetonitrile—40 mM phosphoric acid (15:85, v/v), pH* 2.35, flow rate 1.0 ml min^{-1}.

In narrow-bore RP-HPLC experiments the XTerra® RP18 column (50 × 3.0 mm i.d.) containing 3-(chlorodimethylsilyl)propyl-*N*-dodecylcarbamate bonded amorphous organosilica with 3.5 μm spherical particles (Waters, Brussels, Belgium), was applied without precolumn. The mobile phase was acetonitrile—20 mM phosphoric acid (25:75, v/v), pH* 2.60 and flow rate 1.0 ml min^{-1}.

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 HPLC 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 beta-adrenergic drugs and calculations of their molecular descriptors as dipole moment (μ), polarizability (P_o), and hydration energy (H_e) were made with HyperChem v.6.0 (Hypercube, Inc., Gainesville, FL) 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. A terminating gradient of 0.001 kcal mol^{-1} $Å^{-1}$ 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) was calculated according to the method of Lipinski et al. [39] available on the internet [38]. The values of the logarithm of the first global (macroscopic) acidic ionization constants pK_a of investigated drugs 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, Inc., Tulsa, OK) software.

3. Results and discussion

3.1. HPLC separation and detection

The construction details and setup of potentiometric detector applied in this study was described in earlier publications from the group [24–26,31–35]. In the present experiments, 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 [24]. Their response depends on the distribution coefficient of the analyte cation in the aqueous buffer/membrane system [24]. If substances are added to the membrane which strongly complex 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

Table 1
Retention parameters of beta-adrenergic and beta-adrenolytic drugs in applied HPLC systems

Compound	Cation exchange HPLC [a]			Reversed phase HPLC [b]		
	t_R	k	α	t_R	k	α
beta-Adrenergics						
Terbutaline	2.85	1.588	7.818	0.29	0.261	13.311
Salbutamol	3.00	1.725	7.197	0.38	0.652	5.329
Carbuterol	4.48	3.069	4.046	0.71	2.085	1.666
Cimaterol	5.40	3.908	3.177	1.89	7.211	2.075
Tulobuterol	8.07	6.339	1.958	0.60	1.608	2.161
Mabuterol	13.66	11.402	1.088	1.72	6.471	1.862
Clenbuterol	14.77	12.416	–	1.03	3.475	–
beta-Adrenolytics						
Nadolol	6.00	4.456	2.786	0.45	0.956	3.634
Practolol	6.30	4.721	2.629	1.87	7.128	2.051
Bisoprolol	11.80	9.705	1.279	1.40	5.081	1.462
Oxprenolol	12.60	10.447	1.188	1.38	4.988	1.435
p-Oxprenolol	13.50	11.271	1.106	1.70	6.382	1.836
Carazolol	13.80	11.534	1.076	4.10	16.826	4.842
Alprenolol	15.50	13.062	1.052	3.10	12.474	3.589
Celiprolol	18.20	15.559	1.253	1.25	4.436	1.276
Bufuralol	20.20	17.338	1.396	4.26	17.498	5.035
Tertatolol	25.30	21.978	1.770	3.31	13.396	3.855
Propranolol	45.00	39.902	3.213	3.16	12.735	3.664
Tolamolol	47.00	41.686	3.345	1.42	5.176	1.489
Bevantolol	58.10	51.760	4.169	4.96	20.558	5.916

Injected volume 20 μl; $n = 4$. *Symbols:* t_R, net retention time; k, capacity factor; α, selectivity related to clenbuterol peak.
[a] Injected concentrations 2.0×10^{-4} mol l^{-1}, $t_o = 1.10$ min.
[b] Injected concentrations 2.0×10^{-5} mol l^{-1}, $t_o = 0.23$ min.

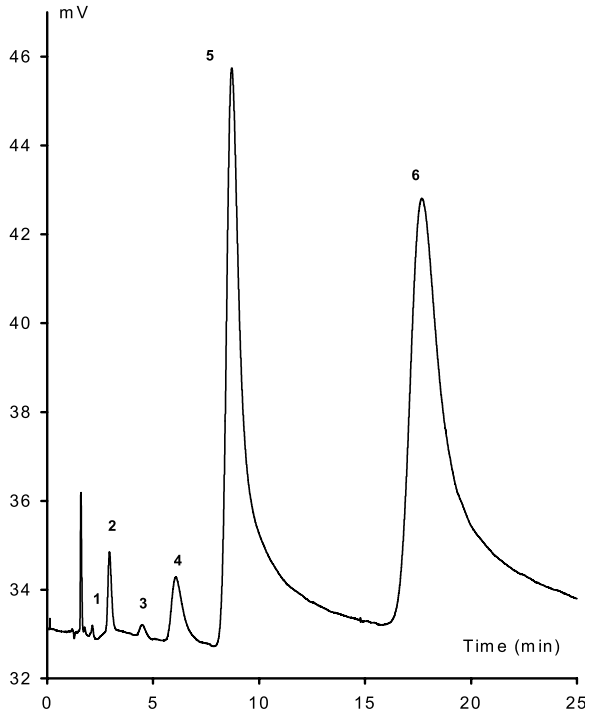

Fig. 3. Isocratic separation of six beta-adrenergics in the normal-bore cation exchange HPLC with potentiometric detection using CDX liquid membrane electrode (Pt, 2 mm i.d.). Peaks (injected concentrations): (1) salbutamol (2.7×10^{-4} mol l^{-1}); (2) terbutaline (4.71×10^{-4} mol l^{-1}); (3) carbuterol (5.58×10^{-4} mol l^{-1}); (4) cimaterol (2.15×10^{-4} mol l^{-1}); (5) tulobuterol (0.98×10^{-4} mol l^{-1}); (6) clenbuterol (1.05×10^{-4} mol l^{-1}). Injected volume: 20 μl with autosampler. Column: Alltech universal cation exchange, 100×4.6 mm i.d. ($d_p = 7$ μm) with guard-column. Mobile phase: acetonitrile—40 mM phosphoric acid (15:85, v/v), pH* 2.35. Flow rate: 1.0 ml min^{-1}.

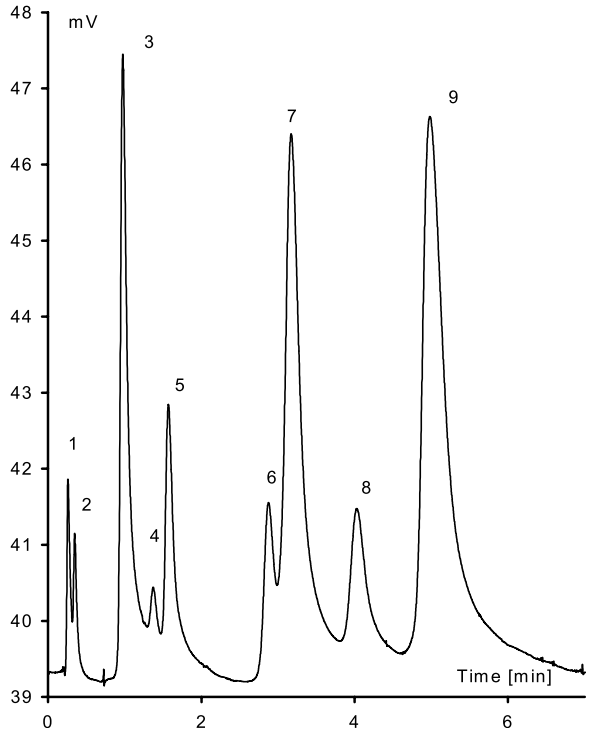

Fig. 4. Isocratic separation of (1) sabutamol, (2) nadolol, (3) clenbuterol, (4) bisoprolol, (5) practolol, (6) alprenolol, (7) propranolol (8) carazolol, and (9) bufuralol in narrow-bore RP-HPLC with potentiometric detection using TCPB-based liquid membrane electrode. Injected volume: 20 μl; injected concentrations: 1.0×10^{-6} mol l^{-1}. Mobile phase: acetonitrile—20 mM phosphoric acid (25:75, v/v), pH* 2.60. Column: XTerra® RP18, (50×3.0 mm i.d., $d_p = 3.5$ μm), without pre-column. Flow rate: 1.0 ml min^{-1}. Chromatogram corrected for baseline drift.

macrocyclic ionophores added: see Fig. 2 for their structures.

In Table 1, the retention data of the 20 beta-adrenergic drugs in the normal-bore cation exchange HPLC system, and in the narrow-bore reversed phase HPLC system were summarized. Fig. 3 shows a representative chromatogram obtained for the separation of six beta-adrenergics on a normal-bore cation exchange HPLC system, and detection with a liquid membrane electrode containing trioctylated α-cyclodextrin as an ionophore. Terbutaline, carbuterol, cimaterol and salbutamol have low responses (high detection limits (DL), see also Table 2) as compared to the two other compounds. Fig. 4 shows potentiometric detection of nine beta-adrenergic drugs in narow-bore RP-HPLC. Measured values of potentiometric DL are quite comparable in both types of HPLC systems used. However, the narrow-bore reversed phase HPLC system offers spectacular reduction of total analysis time in comparison to the normal-bore cation exchange HPLC system: see Figs. 3 and 4, and also Table 1. This was especially the case for clenbuterol, propranolol, and bufuralol. These results indicated that potentiometric detection offers comparable detectability in both fast eluting and more dispersive HPLC systems. Hence, it is noticeable that performance of HPLC column would not be diminished with the sensitivity of developed potentiometric detectors by effect of peak capacity [40].

Table 2 contains DL for the four types of electrodes which were used in this study, and for a UV detector. TCPB containing electrodes (no addition of neutral macrocyclic ionophores) give more than fivefold improvement in limits of detection for tulobuterol, mabuterol, bufuralol, alprenolol and tertatolol in comparison with UV detection. This clearly shows that the sensitivity of potentiometric detection can be competitive to 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 [25,41,42]. From the results shown in Table 2, one can also conclude that the use of trioctylated α-cyclodextrin as the ionophore in liquid membrane electrodes generally yields the most sensitive potentiometric detection of the beta-adrenergic drugs. DL in the 10^{-7} mol l^{-1} range (injected concentration) were obtained for clenbuterol, tulobuterol, alprenolol, oxprenolol, *p*-oxprenolol, propranolol, tertatolol and

Table 2
DL values of beta-adrenergic and beta-adrenolytic drugs in cation exchange HPLC/UV and cation exchange HPLC/potentiometric detectors as expressed by injected concentrations (mol l^{-1})

Compound	UV230 [a]		TCPB [b]		CDX [c]		CRW [d]		CLX [e]	
beta-Adrenergics										
Terbutaline	1.1×10^{-7}	(6.95)	1.9×10^{-5}	(4.72)	3.5×10^{-5}	(4.46)	4.6×10^{-5}	(4.33)	8.2×10^{-8}	(7.08)
Salbutamol	2.6×10^{-8}	(7.58)	3.5×10^{-5}	(4.46)	3.7×10^{-5}	(4.43)	2.5×10^{-5}	(4.6)	2.9×10^{-5}	(4.53)
Carbuterol	1.7×10^{-7}	(6.76)	8.5×10^{-5}	(4.07)	7.7×10^{-5}	(4.11)	1.9×10^{-4}	(3.72)	2.4×10^{-4}	(3.62)
Cimaterol	3.0×10^{-7}	(6.52)	2.5×10^{-5}	(4.60)	4.8×10^{-6}	(5.32)	2.7×10^{-5}	(4.57)	3.6×10^{-5}	(4.44)
Tulobuterol	1.3×10^{-7}	(6.89)	7.3×10^{-7}	(6.13)	2.3×10^{-8}	(7.63)	2.1×10^{-6}	(5.67)	5.6×10^{-6}	(5.25)
Mabuterol	2.9×10^{-6}	(5.53)	2.2×10^{-7}	(6.65)	5.6×10^{-8}	(7.25)	3.2×10^{-6}	(5.49)	3.4×10^{-5}	(4.47)
Clenbuterol	2.6×10^{-7}	(6.58)	6.7×10^{-7}	(6.17)	1.7×10^{-7}	(6.96)	3.4×10^{-6}	(5.47)	1.7×10^{-6}	(5.77)
beta-Adrenolytics										
Nadolol	2.3×10^{-7}	(6.64)	5.9×10^{-5}	(4.23)	2.1×10^{-5}	(4.67)	6.2×10^{-5}	(4.21)	1.0×10^{-4}	(4.00)
Practolol	1.7×10^{-7}	(6.77)	5.4×10^{-5}	(4.27)	6.2×10^{-5}	(4.21)	1.1×10^{-4}	(3.96)	1.9×10^{-4}	(3.72)
Bisoprolol	3.5×10^{-7}	(6.45)	1.3×10^{-5}	(4.88)	3.7×10^{-7}	(5.43)	2.6×10^{-6}	(5.58)	2.7×10^{-6}	(5.57)
Oxprenolol	1.0×10^{-6}	(6.00)	1.7×10^{-6}	(5.77	7.7×10^{-6}	(6.11)	4.5×10^{-6}	(5.34)	5.8×10^{-7}	(6.23)
p-Oxprenolol	0.7×10^{-6}	(6.15)	1.6×10^{-6}	(5.79)	7.5×10^{-7}	(6.12)	7.8×10^{-6}	(5.11)	3.5×10^{-7}	(6.45)
Carazolol	4.4×10^{-7}	(6.36)	6.0×10^{-7}	(6.22)	2.0×10^{-7}	(6.69)	3.0×10^{-6}	(5.52)	2.1×10^{-6}	(5.68)
Alprenolol	2.2×10^{-6}	(5.66)	6.7×10^{-7}	(6.17)	3.6×10^{-7}	(6.44)	4.8×10^{-6}	(5.32)	3.0×10^{-7}	(6.52)
Celiprolol	1.6×10^{-7}	(6.79)	1.5×10^{-5}	(6.77)	1.6×10^{-5}	(4.79)	5.0×10^{-5}	(4.30)	6.8×10^{-5}	(4.16)
Bufuralol	3.5×10^{-7}	(6.45)	2.8×10^{-7}	(6.55)	2.2×10^{-8}	(7.66)	3.9×10^{-6}	(5.41)	1.5×10^{-6}	(5.82)
Tertatolol	1.2×10^{-6}	(5.92)	3.5×10^{-7}	(6.45)	1.2×10^{-7}	(6.92)	3.7×10^{-6}	(5.43)	1.8×10^{-6}	(5.74)
Propranolol	2.7×10^{-8}	(7.56)	2.1×10^{-7}	(6.67)	1.0×10^{-7}	(7.00)	2.8×10^{-7}	(6.55)	5.3×10^{-7}	(6.27)
Tolamolol	1.3×10^{-6}	(5.89)	1.4×10^{-5}	(4.85)	1.3×10^{-6}	(5.88)	1.5×10^{-6}	(5.82)	2.0×10^{-5}	(4.69)
Bevantolol	1.3×10^{-6}	(5.88)	2.2×10^{-6}	(5.66)	1.0×10^{-6}	(6.00)	1.1×10^{-6}	(5.96)	1.6×10^{-6}	(5.79)

In brackets the values of negative logarithms of detection limit ($-\log$ DL) are given.
[a] Spectrophotometric detection at 230 nm.
[b] Electrode with TCPB.
[c] Electrode with trioctylated α-cyclodextrin.
[d] Electrode with dibenzo-18-crown-6.
[e] Electrode with calix[6]arene hexaethylester.

carazolol. In case of tulobuterol, mabuterol and bufuralol DL were in 10^{-8} mol l^{-1} range. Crown ether-, or calix[6]arene-based electrodes offer no advantage over electrodes without added ionophore, except in a few specific cases as terbutaline.

In potentiometric detector, the set of diversified physicochemical interactions of components of PVC liquid membrane, which acting as the 'selector element' of working electrode, with the all ions present in the mobile phase are transformed into the potential difference ΔE between an working (indicator) electrode and a reference electrode [24]. DL listed in Table 2 were determined for each analyte on detector signal-to-noise ($4\sigma_{noise}$) ratio of three ($S/N = 3$) [43], and calculated as the means from results of four subsequent measurements with relative standard deviation not exceeding 8.0%. Thus, in dynamic HPLC conditions DL of potentiometric detector employing given liquid membrane electrode can be more generally specified as the change in its potential difference ΔE_{dl} (in mV) which exceeding three times the stabilized output noise ($4\sigma_{noise}$) of this electrode. High sensitivity of the flow-independent potentiometric detector means a large detector response, i.e. generated potential difference ΔE, expressed by peak height or peak area, for a given amount of organic analyte ion (e.g. injected concentration) [44].

The substantial difference in meaning of DL values determined in static (batch) and HPLC conditions was shown in Fig. 5. For this explanation the calibration graph of the CDX-based liquid membrane electrode used in cation exchange HPLC conditions for sensing of bufuralol was presented. A linear supernerstian response of this electrode could be observed in the concentration range 10^{-2} to 10^{-4} mol l^{-1} with slope 61.0 mV per concentration decade of analyte ion and DL at 6.3×10^{-5} mol l^{-1} (point A in Fig. 5), which was calculated according to IUPAC recommendations for the static potentiometric measurements as the crossing point of tangent drawn to the growing linear part of the calibration curve and abscissa [45,46]. However, DL observed for bufuralol in mentioned cation exchange HPLC conditions was shifted to the value of 2.0×10^{-8} mol l^{-1} (point B in Fig. 5). In fact the horizontal part of calibration graph indicate a slight positive slope [46] in the concentration range 10^{-5} to 10^{-8} mol l^{-1} (cf. Fig. 5). Considering the Nicolsky–Eiseman equation for the response of ion-sensitive electrodes [24,46–48] it should

be pointed out that in case of monovalent analyte ion the observed DL of potentiometric detector in the dynamic HPLC conditions could be related with minimal activity of analyte ion $a_i^{\lim}$ at the top of the Gaussian concentration band which passes through the detector. These relations can be presented with following logarithmic formula:

$$\log \mathrm{DL} = \log a_i^{\lim} = 0.0169\Delta E_{\mathrm{dl}} + \log \sum_{1}^{n} K_{i,k}^{\mathrm{pot}} a_k \qquad (1)$$

In Eq. (1) the $K_{i,k}^{\mathrm{pot}}$ means the selectivity coefficient describing the response of electrode for the each eluent ion k versus analyte ion i, the a_k means activity of the each eluent ion and the ΔE_{dl} (in mV) is observed total response of electrode corresponding with DL obtained in HPLC which equals three times the width of the stabilized electrode noise ($4\sigma_{\mathrm{noise}}$). Eq. (1) was developed with assumption that $a_i^{\lim}$ is much less compared to the term $K_{i,k}^{\mathrm{pot}} a_k$ and temperature of measurements was maintained at 20 °C.

The membrane electrode lifetimes were studied quantitatively in acetonitrile/dilute acid eluent conditions in a former study [25]. The activity of a TCPB based electrode reduces to 79% of its initial activity (measured peak area) after continuous use in acetonitrile—5 mM nitric acid (5:95, v/v), pH* 2.3, mobile phase during 51 days. In the present study, acetonitrile concentrations exceeding 20% were often used. Fig. 6 shows the effect of acetonitrile concentration in mobile phase on the response of CDX-based liquid membrane electrode used for detection of clenbuterol in isocratic narrow-bore RP-HPLC system. The potentiometric response significantly increased with increasing concentration of acetonitrile [49]. In these conditions, the electrode activity is lost after 3 days of continuous use. Remarkable DL are noted however in such eluents with high acetonitrile content (> 50%): injected concentrations down to 2.0×10^{-9} mol l^{-1} were routinely obtained for e.g. clenbuterol [42]. The effect was also clearly noted for other classes of compounds presently under study in our group, i.e. ethanolamines (set of 11 compounds) [41], and linear chain aliphatic amines [25]. This results suggest that further improvement in performance of potentiometric detection in HPLC could be expected by using fast gradient elution. The positive effect of organic modifiers like acetonitrile on DL will be investigated in our another study [42].

The liquid membrane electrodes used need some preconditioning treatment. Fig. 7 shows the change in response of the trioctylated α-cyclodextrin containing liquid membrane electrode CDX as a function of time. The electrode was kept in deionized water, and placed in the detector at specified time intervals. Stabilized response and 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 micrometer [50]. This water inclusion process may be rather slow. The PVC/weakening agent (DOS) 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 [51,52].

Human saliva samples were analyzed by direct injection on the HPLC similar to formerly described in Fig. 4 (no sample clean-up). Spiking with clenbuterol and cimaterol down to a 2.0×10^{-6} mol l^{-1} concentration yielded interference-free chromatographic peaks only for clenbuterol. Contrary, application of UV detection was clearly inferior in this respect as in the latter mode, spiking to a 2.0×10^{-4} mol l^{-1} concentration was needed to obtain a reasonably interference-free clenbuterol peak but in case of cimaterol sensitivity of this detection can be acceptable. These both mentioned results were illustrated in Fig. 8.

3.2. Structure–potentiometric response relationships

Potentiometric sensors for batch (static) 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 determination of other classes of compounds such as aliphatic amines [25] and aliphatic aminoalcohols [41]. 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 towards different analytes are quite diffuse in the literature [28,46]. Mostly, only qualitative arguments are given to relate the selectivity coefficients to the chemo-physical properties of the analytes [24,28,36].

QSAR are used often in pharmacochemical screening methods [53]. The solubility of drugs in bio-membranes is an important factor which has to be estimated on the basis of molecular descriptors [54,55]. The distribution properties of our analytes over the (water-based) eluent phase and the liquid membrane determine the sensitivity of the potentiometric sensor [24]. It seems quite logical then, that the pharmacochemical methodology can be applied also to the structure–activity (response, or DL) relationship for potentiometric sensing. As far as we know, this approach has never been used 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 far as no strong

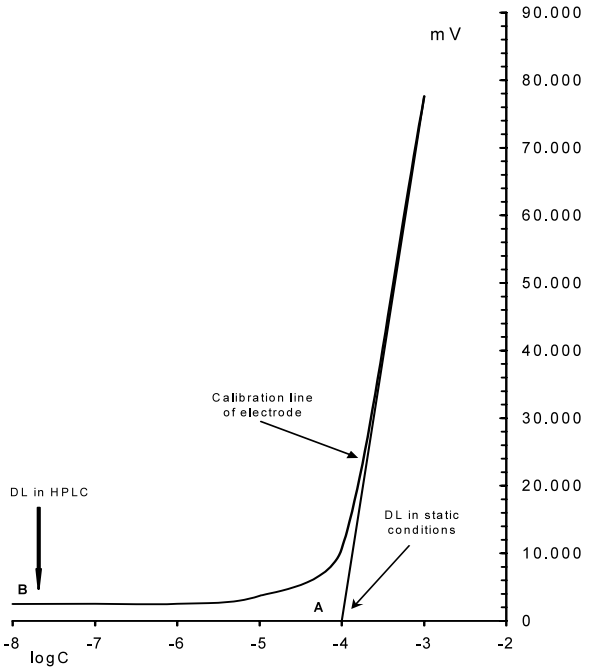

Fig. 5. Calibration graph for CDX-based liquid membrane electrode used in detection of bufuralol in cation exchange HPLC system as described in Fig. 3. The arrows indicate position of DL values determined, respectively, in static (batch) and dynamic (HPLC) conditions.

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.

Eight molecular descriptors were calculated for the 20 investigated drugs here. The set of molecular descriptors differing in their 'dimensionality' consisted of pK_a, log P, log D, μ, P_o, H_e, (one-dimensional, 1D descriptors) and HBA, HBD (2D descriptors). These molecular descriptors were chosen because they are used in pharmacochemical screening of beta-adrenergics, beta-adrenolytics and other drugs candidates for preliminary evaluation of properties such as intestinal adsorption, blood–brain barrier penetration, and other membrane transport related processes [4,39,53–57]. A limited set of calculated molecular descriptors is given in Table 3.

Inspection of the intercorrelation matrix of the molecular descriptors indicated that most of these parameters were non-correlated ($r < 0.75$). A partial positive correlation ($r = 0.90$) was revealed between the values of the logarithm of the partition coefficient of the neutral form of beta-adrenergic drugs 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 [56]:

$$\log D = \log P - \log[1 + 10^{(pK_a - 7.4)}] \quad (2)$$

A multivariate statistical analysis was made in which the eight molecular descriptors were correlated with the logarithm of the DL (log DL) values taken from Table 3 which were measured in the normal-bore cation exchange HPLC mode. DL are injected concentrations for which a signal to noise ratio (S/N) of three is reached [24–26,31–35]. In pharmacochemical QSAR studies, concentrations are also used for which the measured activity of the drug exceeds a specified value [53,57], 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:

$$\log \mathrm{DL} = -6.1807 - 0.6619 \log P + 0.0622 P_o$$
$$n = 20;\ r = 0.8991;\ \mathrm{SE} = 0.4150;\ F = 35.87;\ P < 0.0001 \quad (3)$$

Eq. (3) indicates that the DL of beta-adrenergics and beta-adrenolytics on TCPB based electrodes is mainly dependent on their lipophilicity expressed by log P values, and on their polarizability P_o. The contribution of the log P factor and of the polarizability P_o in Eq. (2) is of comparable significance. The importance of log P values is not surprising, as it is known that the response

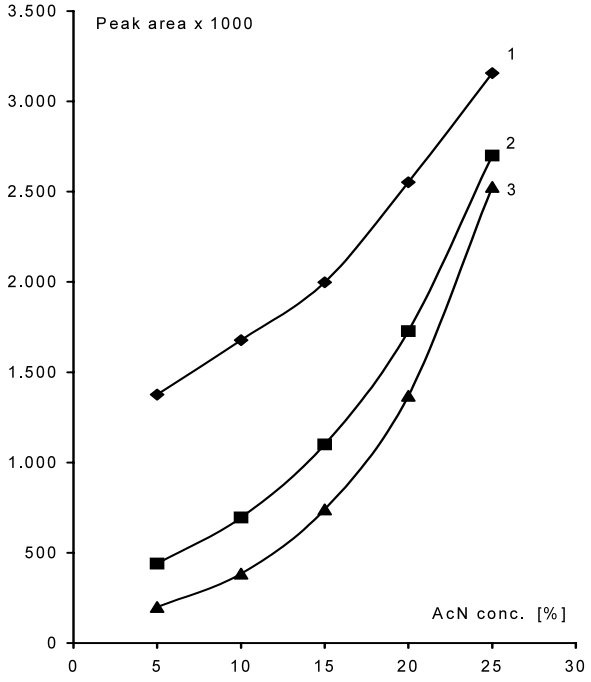

Fig. 6. Effect of acetonitrile (AcN) concentration in HPLC mobile phase on the response of CDX-based liquid membrane electrode used for detection of clenbuterol in RP-HPLC system as described in Fig. 4. The injected concentrations were 1.2×10^{-6} mol l^{-1} (line 1), 1.2×10^{-7} mol l^{-1} (line 2), and 1.2×10^{-8} mol l^{-1} (line 3).

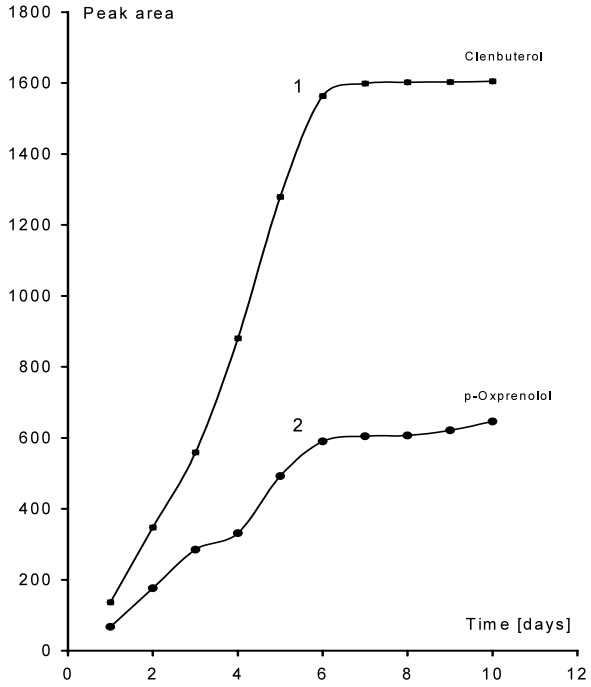

Fig. 7. Time related variation in response of a CDX-based liquid membrane electrode used in cation exchange HPLC conditions (compare Fig. 3) for clenbuterol (line 1) and *p*-oxprenolol (line 2). In between two subsequent determinations, the electrode was stored in deionized water.

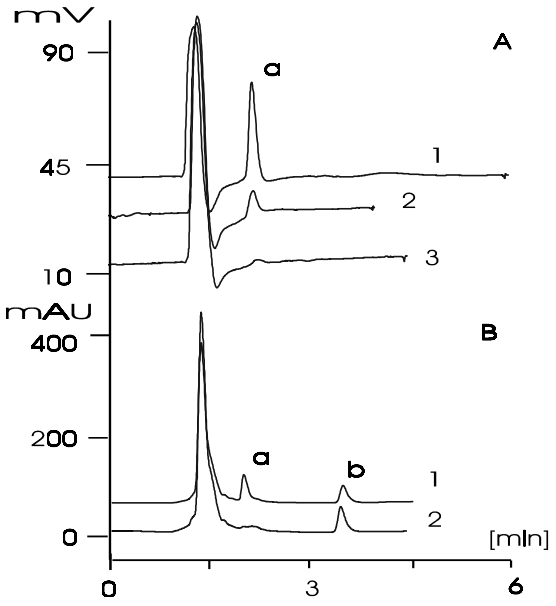

Fig. 8. Comparison of direct determination of clenbuterol (peak a) and cimaterol (peak b) in spiked human saliva samples at concentration 2.0×10^{-4} mol l^{-1} (line 1), 2.0×10^{-5} mol l^{-1} (line 2), and 2.0×10^{-6} mol l^{-1} (line 3) by normal-bore RP-HPLC (Hypersil C18ec, 125 × 4.6 mm i.d., acetonitrile—1 mM phosphoric acid (52:48, v/v) at 0.5 ml min^{-1}, 10 μl injection) with potentiometric detection using TCPB-based liquid membrane electrode (panel A) and using UV detection at 230 nm (panel B).

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 DL on a TCPB based electrode [25,41]. Eq. (3) shows however that distribution of a cationic drug between a water phase and a potentiometric membrane can not 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. (3), 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 trioctylated α-cyclodextrin as the macrocyclic ionophore:

$$\log \mathrm{DL} = -6.8532 - 0.8441 \log P + 0.0789 P_o$$
$$n = 20;\ r = 0.9052;\ \mathrm{SE} = 0.5106;\ F = 38.55;\ P < 0.0001 \quad (4)$$

Eq. (4) gives higher values of the regression coefficients as compared to the previously discussed Eq. (3). This indicates that the impact of hydrophobic interactions on the response of the described electrode was enlarged by the addition of trioctylated α-cyclodextrin. Indeed, improved DL values were observed for more hydrophobic beta-adrenergics and beta-adrenolytics on the electrodes with trioctylated α-cyclodextrin ionophores as compared to TCPB based electrodes (see Table 2). This result is not surprising as it is well known that native and modified cyclodextrins prefer hydrophobic interactions inside their cavity [23,36,58].

For the PVC based electrodes containing dibenzo-18-crown-6, and for the one containing calix(6)arene hexaethylester as the macrocyclic ionophore the three and four term relationships Eqs. (5) and (6) were established, respectively:

$$\log \mathrm{DL} = -5.8694 - 0.2342 \log D - 0.0422 H_e + 0.1688 \mu$$
$$n = 20;\ r = 0.8564;\ \mathrm{SE} = 0.4054;\ F = 14.67;\ P < 0.0007 \quad (5)$$

$$\log \mathrm{DL} = -6.4593 - 0.2690 \log P + 0.1849 \mu - 0.0249 \mathrm{HBD} + 0.2534 \mathrm{HBA}$$
$$n = 20;\ r = 0.8568;\ \mathrm{SE} = 0.4272;\ F = 15.03;\ P < 0.0090 \quad (6)$$

The equations become more complex, as the interactions of amines with the crown ether and with the calixarene ionophore are quite specific as was pointed out recently by Spencer et al. [59] and Fraternali and Wipff [60].

The above described multiple correlation models can be very useful in predicting the response behavior of the beta-adrenergic drugs, and of other organic cationic substances in potentiometric sensing using non-selective liquid membrane electrodes. Especially for non-selective

Table 3
Calculated molecular descriptors of beta-adrenergic and beta-adrenolytic drugs

Compound	pK_a	log P	log D	HBA	HBD	μ (D)	P_o (Å^3)	H_e(kcal mol^{-1})
beta-Adrenergics								
Terbutaline	8.80	0.77	−0.64	4	4	1.815	24.71	−17.17
Salbutamol	9.07	−0.69	−2.36	4	4	2.584	21.04	−15.29
Carbuterol	9.93	−1.72	−4.25	6	5	3.659	27.34	−12.92
Cimaterol	9.78	0.62	−1.76	3	3	4.903	24.22	−12.71
Tulobuterol	9.24	2.59	0.74	3	2	1.486	25.36	−4.22
Mabuterol	8.16	1.72	0.89	7	3	1.227	22.77	−9.08
Clenbuterol	9.72	2.91	0.58	5	3	3.026	27.29	−2.89
beta-Adrenolytics								
Nadolol	9.67	0.98	−1.29	5	4	1.23	33.74	−12.07
Practolol	9.44	1.03	−1.01	5	3	3.428	29.18	−9.31
Bisoprolol	9.57	2.04	−0.13	5	2	1.982	36.35	−6.45
Oxprenolol	9.50	2.07	−0.03	4	2	0.974	30.02	−8.20
p-Oxprenolol	10.08	2.20	−0.48	4	2	1.283	30.02	−9.25
Carazolol	9.52	2.77	0.64	3	3	2.463	34.31	−6.92
Alprenolol	9.59	2.81	0.62	3	2	2.081	29.38	−5.47
Celiprolol	9.66	1.39	−0.87	7	3	6.026	40.99	−1.43
Bufuralol	9.20	3.24	1.43	3	2	1.472	30.44	−3.31
Tertatolol	10.42	3.36	0.33	4	2	0.694	33.63	−4.07
Propranolol	9.53	2.96	0.82	3	2	1.168	32.09	−6.02
Tolamolol	7.94	1.81	1.15	6	3	3.356	36.29	2.98
Bevantolol	8.43	2.65	1.58	5	2	1.239	38.67	−10.06

Symbols: pK_a, first global acidic ionization constant; log P, partition coefficient in n-octanol–water system; log D, apparent partition coefficient between n-octanol and buffer pH 7.4; HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; μ, dipole moment; P_o, polarizability; H_e, hydration energy.

electrodes such as the TCPB-based electrode and the trioctylated α-cyclodextrin containing electrode, the relation between log DL values and the lipophilicity (log P) plus polarizability (P_o)—as expressed by Eqs. (3) and (4)—is very promising. We applied this equation to two other classes of organic cationic compounds, i.e. aliphatic amines (seven compounds) and aminoalcohols (11 compounds). These classes of compounds can also be very sensitively detected in diversified HPLC systems equipped with potentiometric detection [25,41]. Both classes have much lower log P values (around zero, or negative) than the here described beta-adrenergics and beta-adrenolytics. Surprisingly, they showed DL (on TCPB-based electrodes) over a range which is comparable to the range of the DL of the beta-adrenergic drugs studied. These classes have much lower polarizabilities P_o (in range from 3.96 to 20.40 Å^3) to than the beta-adrenergic agonists and beta-blockers. Application of Eq. (3) 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 in case of the TCPB-based electrode used in described here cation exchange HPLC conditions:

$$\log \mathrm{DL} = -7.7019 - 0.9567 \log P + 0.1311 P_o$$

$$n = 11;\ r = 0.7778;\ \mathrm{SE} = 0.4782;\ F = 6.12;\ P < 0.0240 \quad (7)$$

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

$$\log \mathrm{DL} = 18.4296 + 13.4712 \log P - 0.6659 P_o$$

$$n = 7;\ r = 0.9461;\ \mathrm{SE} = 0.2047;\ F = 17.08;\ P < 0.0109 \quad (8)$$

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

$$\log \mathrm{DL} = 10.9752 + 7.8199 \log P - 2.2920 P_o$$

$$n = 7;\ r = 0.9946;\ \mathrm{SE} = 0.1134;\ F = 182.63;\ P < 0.0001 \quad (9)$$

Thus, during penetration of CDX-based electrode by aliphatic amines the significance of hydrophobic interactions increased as indicate the enlarged value of individual regression coefficient of log P variable in Eq. (9).

3.3. Concluding remarks

Potentiometry is able to determine beta-adrenergics and beta-blockers sensitively in HPLC systems. Using trioctylated α-cyclodextrin based electrodes and narowbore reversed phase HPLC, DL in the low pg range were attained (injected concentrations down to 2.0×10^{-8} mol l^{-1}). The detection system is very selective when applied to rich protein extracellular fluids as saliva

which secretion is elicited after single administration of autonomic agonists [61]. Metabolic breakdown products (i.e. lysosomotropic amines) are also detected sensitively by developed potentiometric procedure. A QSAR approach allows prediction of the DL of beta-adrenergic drugs and aliphatic amines on basis of their lipophilicity (log P) and polarizability (P_o). More efficient electrode materials are now under consideration for prolonged use in mobile phases with high organic modifier content, and with better adhesion characteristics to platinum or glassy-carbon surfaces. Such materials will be used in miniaturized systems of analysis.

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