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Mexiletine-sensitive membrane electrode for medical application

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Abstract

Response characteristics of mexiletine-sensitive membrane electrodes based on crown ether and ion-exchanger were examined in a physiological saline in order to find an electrode suitable for determining concentrations of this drug under physiological conditions. Among various crown ethers screened, 4′,4″(5″)-di-*tert*-butyldicyclohexano-18-crown-6 showed the highest sensitivity to mexiletine in physiological saline containing 0.15 M NaCl and 5 mM 4-(2-hydroxyethyl)-2-piperazineethanesulfonic acid (Hepes)–NaOH (pH 7.4). However, the detection limit of 30 μM was 10 times higher than that of 3 μM observed with the electrode based on an ion-exchanger, sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate. Having high selectivity against inorganic cations such as Na^+ or K^+, the electrode using the ion-exchanger enabled us to determine the level of mexiletine in saliva, the monitoring of which is quite effective for controlling the dose of this drug noninvasively. The mexiletine concentrations determined with the mexiletine electrode compared favourably with those determined by high-performance liquid chromatography which requires an additional procedure to extract mexiletine from saliva.

Keywords: Ion-selective electrode; Mexiletine determination; Sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate; 4′,4″(5″)-Di-tert-butyldicyclohexano-18-crown-6; Clinical analysis; Saliva

1. Introduction

The development and application of ion-selective electrodes continue to be of interest for pharmaceutical analysis because these sensors offer the advantage of simple design and operation, reasonable selectivity, fast response, applicability to colored and turbid solutions and possible interfacing with automated and computerized systems [1–4]. To date, however, few clinical applications of pharmaceutical substance-sensitive electrodes have been published [4,5], and the application of such electrodes has been rather limited to the determination of specific drug contained in tablets or a dissolution test measuring the rate of dissolu-

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tion of drug under defined conditions [1–3]. This is in contrast to the widespread application of inorganic ion-selective electrodes (such as K^+, Na^+, Ca^{2+} or Cl^-) in clinical analysis [5–8]. We are particularly interested in extending the use of ion-selective electrodes for determining drug concentrations in body fluids, and now several electrodes having high sensitivity to specific drugs, such as procainamide [9], salicylate [10], bretylium [11] and disopyramide [12], have been developed. The key to constructing an electrode is to produce a high selectivity membrane against Na^+ or Cl^- present at high levels in body fluids. Such a membrane can be prepared by incorporating a lipophilic ion-exchanger and solvent mediator into a poly(vinyl chloride) (PVC) membrane matrix [4,9,11,12]. However, the potentiometric performance of the electrode of this kind is dependent on the associate formation ability of the analyte drug ions with the ion-exchanger involved. Among different kinds of amines, primary amines generally show the least tendency to form such ion-associates leading to poor response characteristics of the electrode [2,3,13]. In this case, the use of neutral carrier such as crown ether is potentially effective, because some crown ethers are specifically capable of forming host-guest complexes especially with primary amines [3,4,14–18].

Mexiletine, an antiarrhythmic drug, is one of the primary amines that needs monitoring for concentration in body fluids. The chemical structure of mexiletine, together with those of other antiarrhythmic drugs tested in this study, is shown in Fig. 1. So far two types of electrodes, one using crown ether, the other ion-exchanger, have been reported [15,19]. However, the application was limited to the determination of the concentration of mexiletine in tablets [15] and in a dissolution study [19]. In order to apply the mexiletine electrode to clinical analysis, high selectivity against Na^+ is required; however, the selectivity coefficient of the electrode against Na^+ (expressed as the logarithmic value) reported previously was -2.8 [15], and this seemed to be insufficient for clinical application. Thus, we reinvestigated the response characteristics of various mexiletine electrodes based on crown ethers and ion-exchanger extensively in order to find the most suitable electrode for the measurement of this drug under physiological conditions. Among various crown ethers tested, 4′,4″(5″)-di-*tert*-butyldicyclohexano-18-crown-6 (DtB-DC18C6) discriminated mexiletine selectively over various antiarrhythmic drugs. However, the selectivity of this electrode against inorganic cations such as Na^+ or K^+ is worse than that of electrode based on the ion-exchanger, sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (NaHFPB). For reference, the chemical structures of DtB-DC18C6 and NaHFPB are shown in Fig. 2. The lower limit of detection of the electrodes using the crown ether and the ion-exchanger in physiological saline containing 0.15 M NaCl and 5 mM 4 - (2 - hydroxyethyl) - 2 - piperazineethanesulfonic acid (Hepes)–NaOH (pH 7.4) was 30 and 3 μM, respectively. We applied the latter electrode for the determination of mexiletine concentrations in saliva. The results compared favourably with those obtained by high-performance liquid chromatography.

2. Experimental

2.1. Reagents

The sources of the reagents were as follows: DtB-DC18C6, dibenzo-15-crown-5 (DB15C5), dibenzo-21-crown-7 (DB21C7), *o*-nitrophenyl octyl ether (NPOE) and tris(2-ethylhexyl) phosphate (TEHP) from Fluka (Buchs, Switzerland); dicyclohexano-18-crown-6 (DC18C6), dicyclohexano-24-crown-8 (DC24C8), dibenzo-18-crown-6 (DB18C6), dibenzo-24-crown-8 (DB24C8) and dibenzo-30-crown-10 (DB30C10) from Aldrich (Milwaukee, WI, USA); NaHFPB, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) and 2-fluoro-2′-nitrodiphenyl ether (FNDPE) from Dojindo Laboratories (Kumamoto, Japan); dioctyl phthalate (DOP) and tricresyl phosphate (TCP) from Tokyo Kasei (Tokyo, Japan); PVC (degree of polymerization, 1020) from Nacalai Tesque (Kyoto, Japan); mexiletine hydrochloride, *N*-acetylprocainamide hydrochloride, bretylium tosylate, disopyramide

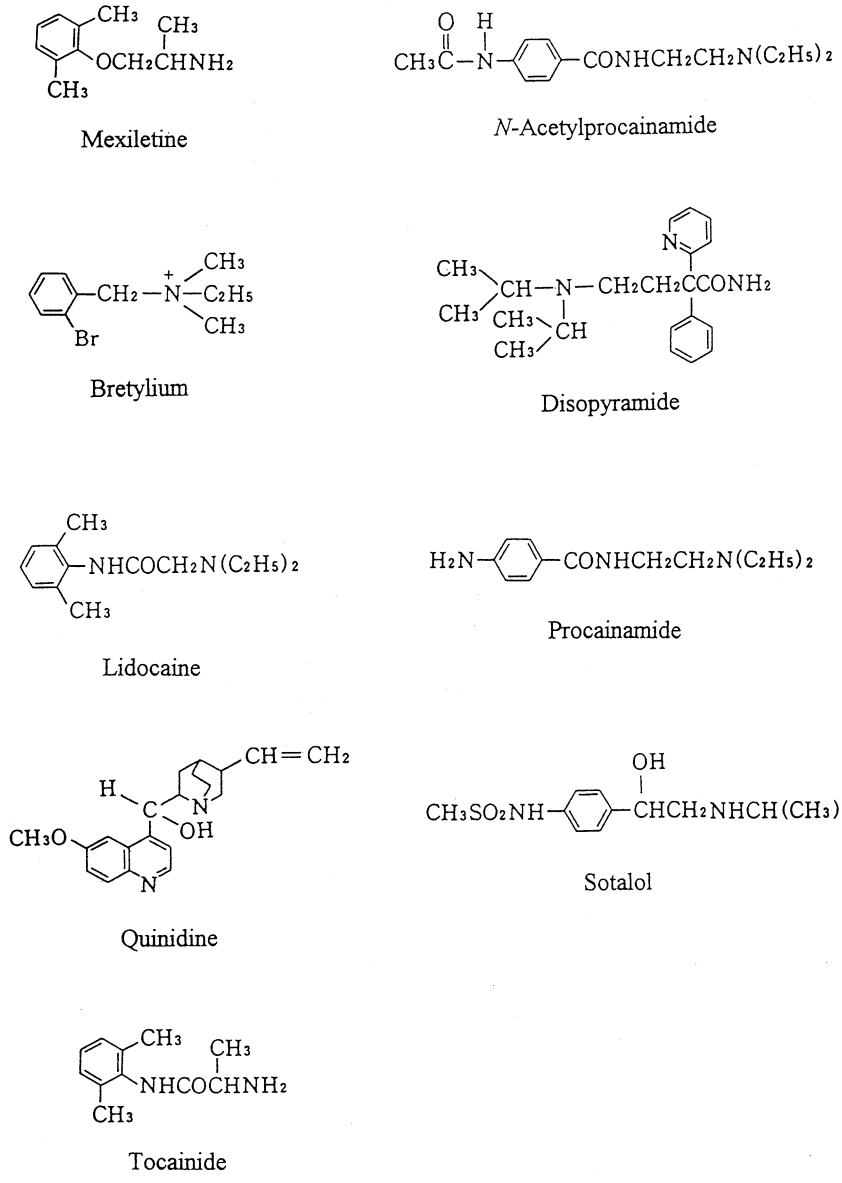

Fig. 1. Chemical structures of mexiletine and antiarrhythmic drugs tested.

phosphate, lidocaine hydrochloride, procainamide hydrochloride, quinidine hydrochloride monohydrate and fluorescamine from Sigma (St. Louis, MO, USA); sotalol hydrochloride from Bristol-Myers Squibb (Tokyo, Japan); tocainide hydrochloride from Astra Hässle (Mölndal, Sweden). All other chemicals used were of analytical reagent grade.

2.2. Electrode system

PVC matrix type ion-selective membranes were prepared according to previously described procedures [4,11,12,18]. For sensor membranes based on crown ether, the components were 1 mg of the crown ether, 60 μl of DOP and 30 mg of PVC. Ionic additive of NaTFPB (0.37 mg; 20 mol%

relative to the crown ether) was further added in the case of sensor membrane containing DtB-DC18C6. For membranes based on ion-exchanger, the components were 0.1 mg of NaHFPB, 60 μl of solvent mediator and 30 mg of PVC. The materials were dissolved in tetrahydrofuran (about 1 ml), poured into a flat Petri dish (28 mm diameter), and the solvent was evaporated at room temperature. The resulting membrane was excised and attached to a PVC tube (4 mm o.d., 3 mm i.d.) with tetrahydrofuran adhesive. Each PVC tube was filled with an internal solution of 1 mM mexiletine hydrochloride in 10 mM NaCl and the sensor membrane was conditioned overnight. The electrochemical cell arrangement was Ag, AgCl/internal solution/sensor membrane/sample solution/1 M NH_4NO_3 (salt bridge)/10 mM KCl/Ag, AgCl. The electromotive force (emf) between the silver | silver chloride electrodes was measured using a voltmeter with high input impedance produced by a field-effect transistor operational amplifier (LF356; National Semiconductor, Sunnyvale, CA, USA; input resistance $> 10^{12}$ Ω) and recorded. The detection limit was defined as the intersection of the extrapolated linear regions of the calibration graph [20]. The selectivity coefficients of the electrode, $k_{i,j}^{\mathrm{Pot}}$, were determined by the separate solution method [20] using the respective chlorides, except for disopyramide and bretylium, for which we used phosphate and tosylate, respectively. The values were calculated from the equation,

$$\log k_{i,j}^{\mathrm{Pot}} = (E_j - E_i)/S + \log c_i - \log c_j^{1/zj},$$

where E_i and E_j represent the emf readings measured for mexiletine and the interfering ion, respectively, S is the theoretical slope of the electrode (58.2 mV at 20°C), c_i and c_j are the concentrations of mexiletine and the interfering ion, respectively, and z_j is the charge of the interfering ion. Test reagents were dissolved in a buffer comprising 0.5 M tris(hydroxymethyl) aminomethane–HCl (pH 7.4) at a concentration of 10 mM, except for quinidine, which was adjusted to 5 mM because of low solubility in the buffer.

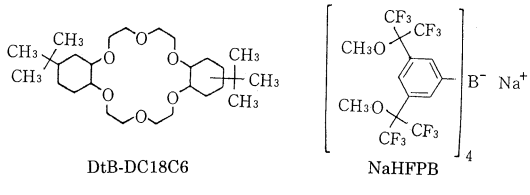

Fig. 2. Chemical structures of DtB-DC18C6 and NaHFPB.

2.3. Collection of saliva

Saliva secreted in buccal cavity (defined as mixed saliva or whole saliva) was collected for 5 min by means of continuous mouth and tongue movement [21,22]. Pre-saliva (i.e. residual saliva in buccal cavity) was discarded before the periodical saliva collection. After stimulation, the salivated fluid (i.e. mixed saliva) accumulated in the mouth cavity was expectorated into a beaker, transferred to a plastic tube and centrifuged at 1200 × g for 10 min to remove the mucosal tissue debris. The saliva supernatant obtained was frozen until use.

2.4. Assay procedure

A typical mexiletine assay in saliva proceeded as follows. The electrodes were placed in 100 μl of saliva and constantly stirred with a bar. This electrode system, including the reference electrode [23], is compact. Therefore, volumes as low as 100 μl can be assayed Samples containing mexiletine were prepared by adding mexiletine hydrochloride to saliva. Between measurements, the electrode was soaked in distilled water and wiped. The electrode was stored in 1 mM mexiletine hydrochloride containing 10 mM NaCl when not in use. All measurements were performed at room temperature (about 20°C).

2.5. High-performance liquid chromatography

Mexiletine concentrations in the saliva samples were also determined by means of high-performance liquid chromatography [21,24]. The saliva samples were prepared as follows. A 50 μl aliquot of saliva was rendered alkaline by adding 10 N NaOH (40 μl) and the total volume was made up to 0.5 ml with distilled water. Thereafter, 3 ml of

diethyl ether was added, the mixture was vortex-mixed, and the ethereal extract was evaporated to dryness after addition of 50 μl of a 0.1 M solution of hydrochloric acid in acetone. The residue was reconstituted in 50 μl of a borate buffer (0.02 M $Na_2B_4O_7$/HCl, pH 7.2) by vortex mixing, and then 50 μl of a fluorescamine solution in acetone (0.25 mg ml^{-1}) was added, the resulting solution being mixed vigorously. A 50 μl volume of mobile phase for chromatography (acetonitrile: water: 1-pentane sulfonic acid (PIC B-5; Waters-Millipore, Milford, MA, USA): acetic acid (65: 33.6: 0.4: 1 v/v%)) was then added and 75 μl of the resulting solution was injected into a conventional high-performance liquid chromatography system (Waters-Millipore, Milford, MA, USA) consisting of a pump (Model 600-E), a fluorescence detector (Model 470), an automatic sample injector (Model 700-S WISP) and a chromatogram data calculator (Model 730 Data-Module). The excitation and emission wavelengths were set at 390 and 475 nm, respectively. Separation was performed on a stainless-steel column (μBondapak C18, 30 cm × 3.9 mm i.d.; Waters-Millipore, Milford, MA, USA) packed with ODS (10 μm particle size) using the mobile phase described above at a flow rate of 1.0 ml min^{-1}.

3. Results and discussion

3.1. Response characteristics of the electrodes

The use of crown ethers as carriers for mexiletine-sensitive electrode is based on the fact that these compounds have strong affinity with primary amines forming host-guest complexes [25,26]. The size of the cavities provided by the crown ether is of importance and better electrode performance is to be expected for those carriers with cavity size sterically matching the amine molecules to be sensed. Thus, various crown ethers were used to examine the response to mexiletine (Fig. 3). Calibration plots were obtained by measuring known amounts of mexiletine hydrochloride added to a physiological saline containing 0.15 M NaCl and 5 mM Hepes–NaOH (pH 7.4) and plotting the concentrations against

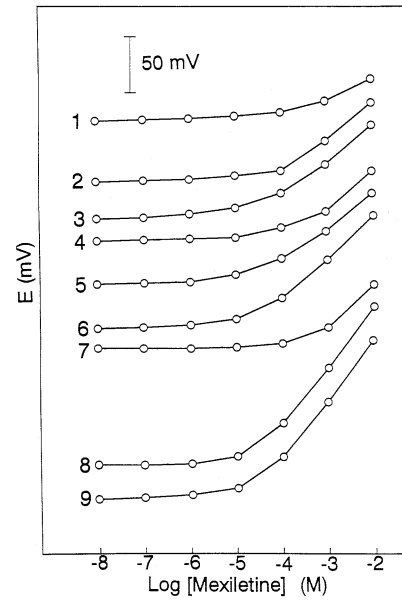

Fig. 3. Comparison of the response of electrodes based on crown ethers to mexiletine in 0.15 M NaCl and 5 mM Hepes–NaOH (pH 7.4). DB15C5 (1), DB18C6 (2), DB21C7 (3), DB24C8 (4), DB30C10 (5), DC18C6 (6), DC24C8 (7), DtB-DC18C6 (8) and DtB-DC18C6 + NaTFPB (20 mol% relative to DtB-DC18C6) (9). DOP was used as solvent mediator.

the corresponding emf values obtained. Among various dibenzo and dicyclohexano-analogs of crown ether (DB15C5, DB18C6, DB21C7, DB24C8, DB30C10, DC18C6 and DC24C8), DC18C6 afforded the lowest detection limit. We further examined DtB-DC18C6 having more increased lipophilicity than DC18C6, and this gave the best response to mexiletine among the crown ethers examined in this study. The slope and the detection limit were 54.1 mV per concentration decade and 30 μM, respectively. Further addition of lipophilic anionic salt such as NaTFPB (20 mol% relative to the crown ether) increased slightly the slope of the electrode response (54.8 mV per concentration decade), but the detection limit was not improved apparently. Effect of solvent mediators was also examined on DtB-DC18C6; however, no significant change in the electrode response was observed with the use of NPOE and bis(2-ethylhexyl) sebacate which were also widely used as solvent mediators for neutral carrier-based membrane electrode [27,28].

Then, we examined the response characteristics of the mexiletine electrode based on the ion-exchanger NaHFPB. This ion-exchanger possesses a strongly lipophilic character and high stability [29], being quite effective for constructing many drug sensitive-electrodes [4,9,11,12]. We examined the effect of solvent mediators (Fig. 4), since these affect largely the response characteristics of the electrodes of this type [4]. Among five typical solvent mediators tested (DOP, TCP, TEHP, FNDPE and NPOE), both FNDPE and NPOE afforded higher sensitivity to mexiletine, though the response characteristics of NPOE were slightly superior to those of FNDPE. The slope and the detection limit of the electrode made from NaHFPB and NPOE were 58.8 mV concentration decade and 3 μM, respectively. It should be emphasized that the detection limit obtained by this electrode was 10 times lower than the best case of crown ether using DtB-DC18C6.

To compare differences in the response characteristics of the two electrodes based on crown ether and ion-exchanger in more detail, we measured the selectivity coefficients of electrodes made from the combinations of (1) DtB-DC18C6, NaTFPB and DOP and (2) NaHFPB and NPOE

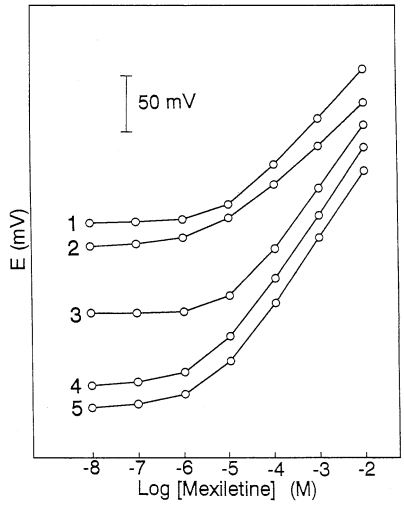

Fig. 4. Effect of solvent mediators on the response of electrode based on the ion-exchanger NaHFPB to mexiletine in 0.15 M NaCl and 5 mM Hepes–NaOH (pH 7.4). DOP (1), TCP (2), TEHP (3), FNDPE (4) and NPOE (5).

which afforded the best response in their groups. The result is shown in Fig. 5. The electrode based on the crown ether showed the highest selectivity against mexiletine among various antiarrhythmic drugs. This electrode responded to primary amines such as mexiletine and tocainide more strongly than quaternary ammonium ion such as bretylium, showing that the crown ether acts as a carrier for primary amine, in accordance with previous results [3,14–18]. Although the present electrode based on DtB-DC18C6 showed somewhat higher selectivity against Na^+ ($\log k_{i,j}^{Pot} = -3.0$) than the mexiletine electrode based on dinaphthyl-23-crown-7 ($\log k_{i,j}^{Pot} = -2.8$) [15], its sensitivity in physiological saline was remarkably worse than that of the electrode containing the ion-exchanger NaHFPB as investigated above. Indeed, the electrode based on NaHFPB showed good selectivity against Na^+ ($\log k_{i,j}^{Pot} = -3.7$) as shown in Fig. 5. The electrode containing NaHFPB, however, suffered marked interference from many lipophilic antiarrhythmic drugs. The interference by lipophilic amines is characteristic of an ion-selective electrode prepared with an ion-exchanger as in the present case [4,9,11,12]; however, such interference could be excluded in many cases of clinical analysis, since these lipophilic amines are usually not present in normal biological fluids and only inorganic ions are noticeable. Thus, we applied the mexiletine electrode based on the ion-exchanger NaHFPB for clinical analysis.

3.2. Application of the electrode to clinical analysis

We are particularly interested in the application of the electrode to drug monitoring in body fluids. For this purpose, the electrode containing NaHFPB combined with an appropriate solvent mediator such as FNDPE has already been used for monitoring procainamide [9], bretylium [11] and disopyramide levels [12] in serum samples as mentioned in the introduction. The clinical range of mexiletine in serum required for antiarrhythmic therapy is shown to be 0.7–2 μg ml^{-1} (4–11 μM) [30]. However, this concentration range was near to the detection limit measured in a physiological

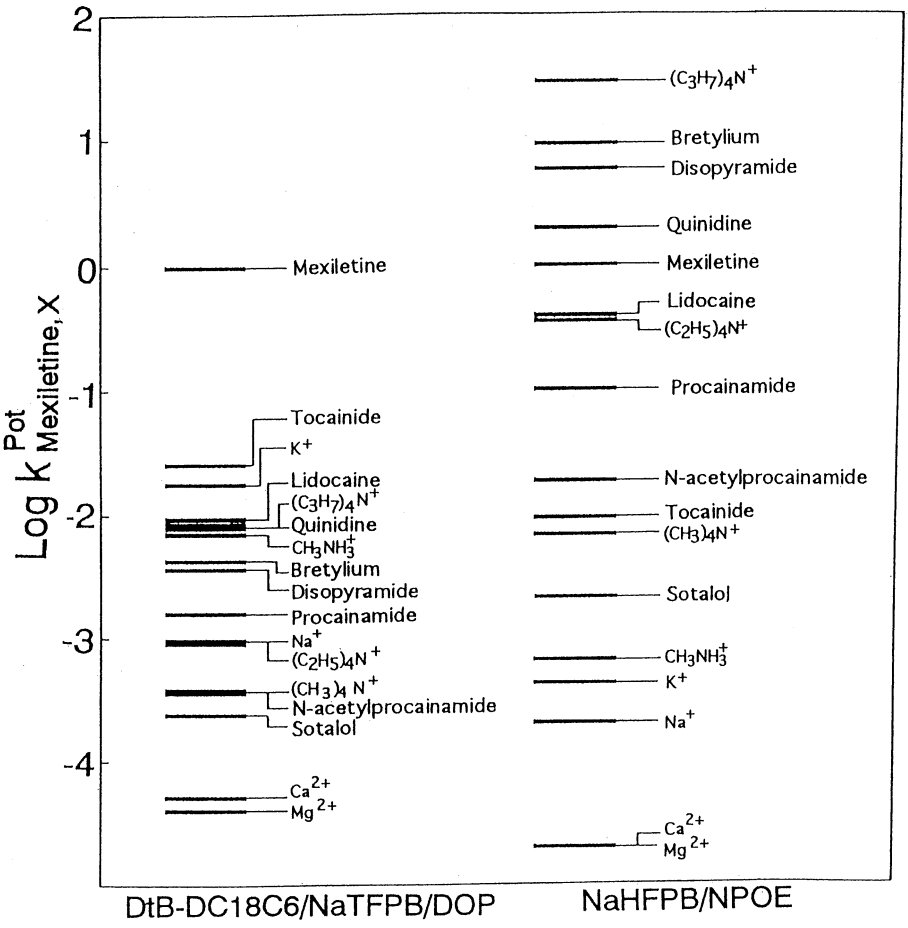

Fig. 5. Selectivity coefficients of the electrodes made from crown ether (DtB-DC18C6/NaTFPB/DOP) and ion-exchanger (NaHFPB/NPOE).

saline and the sensitivity of the present electrode was insufficient to determine mexiletine levels in serum samples.

Recently, increased attention has been placed on the use of saliva samples in place of blood samples for therapeutic drug monitoring in view of the advantage of noninvasive sample collection procedures [21,22,31,32]. Mexiletine concentrations in saliva are reported to be significantly higher than those in serum (by the factor of 3–8) [21,22]. Thus, the therapeutic concentration range becomes higher in saliva samples, exceeding 10 μM, and much easier monitoring of this drug by the present electrode was expected. We measured the calibration graph of mexiletine in saliva and compared it with that in a physiological saline. As shown in Fig. 6, similar calibration graphs between saliva and physiological saline were observed. This is because saliva also contained high concentrations of inorganic ions such as Na^+ and K^+ at levels of 10–20 mM and a neutral pH of around 6.8–7.2 [21,32], similar to the situation in physiological saline. The slope and the detection limit in saliva were 57.3 mV per concentration decade and 4 μM, respectively. It should be emphasized, however, that the determination of the mexiletine concentration down to 1 μM was still easier with an appropriate calibration as shown in Fig. 6. The sensitivity of the electrode was adequate for measuring therapeutic mexiletine levels

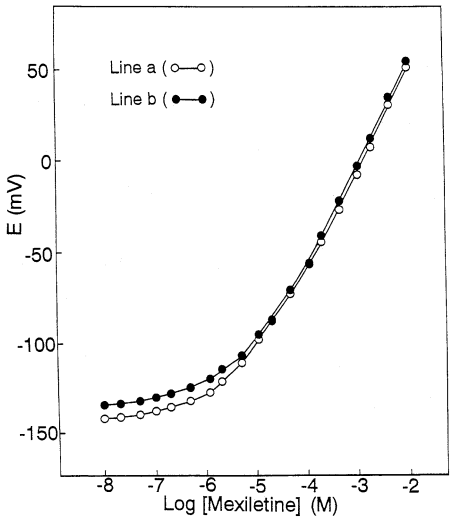

Fig. 6. Comparison of the electrode response to mexiletine in 0.15 M NaCl and 5 mM Hepes–NaOH (pH 7.4) (○) and saliva (●).

in saliva. The response time of the electrode (90% final signal) was below 10 s when the concentration of mexiletine was changed from 2 to 20 μM. We determined the mexiletine concentrations in saliva samples using the calibration graph (Fig. 6, line b) and compared the results with those determined by high-performance liquid chromatography [21,22]. Fig. 7 shows a good correlation over the mexiletine concentration range of 1–40 μM.

As already mentioned by others, the use of an ion-selective electrode has inherent advantages over various other analytical methods, because it requires no special sample pretreatment, the analysis time is shorter, and the necessary equipment is inexpensive. This method will provide a new means of estimating mexiletine levels in saliva samples.

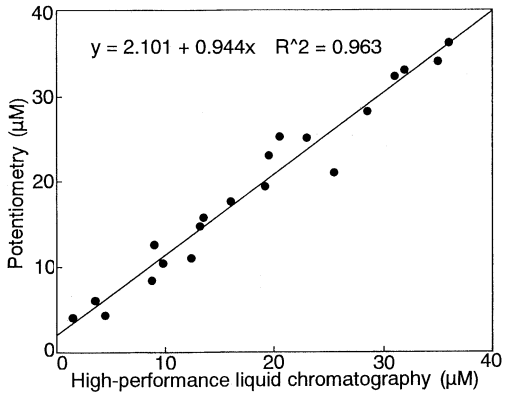

Fig. 7. Correlation of mexiletine concentrations in 20 saliva samples determined by potentiometry using a mexiletine electrode and by high-performance liquid chromatography.

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