The Membrane Sensors Sensitive to Fluoxetine - Optimization of the Analytical Parameters

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Abstract: *Background*: The analytical characteristics of electrodes containing liquid PVC membrane depend on the purity of the electroactive material and the applied membrane solvents.

Methods: Fluoxetine - one H-bond donor, formed ion-associated complexes with tetraphenylborate, tetrakis(4-chlorophenyl)borate and dipicrylamine. To confirm the quality and purity obtained complexes the elemental and TLC analysis were performed. The construction and general characteristics of fluoxetine ion-selective plastic membrane sensors, based on the use of a fluoxetine – tetrakis(4-chlorophenyl)borate ion-pair complex as electroactive material with 2-nitrophenyloctyl ether, bis(2-ethylhexyl)sebacate, bis(2-butylpentyl)adipate and 1-isopropyl-4-nitrobenzene as solvent mediators.

Results: The electrode containing Fl-ClTPB-NPhOE (fluoxetine-tetrakis(4-chlorophenyl)borate-2-nitrophenyloctyl ether showed a linear response to fluoxetine at concentration ranges of 30.93 – 2.16 $g \cdot L^{-1}$ with a super Nernstian cationic slope 60.19 $mV \cdot decade^{-1}$.

Conclusions: This Fl-ClTPB-NPhOE sensor was used for the potentiometric determination of the content of fluoxetine hydrochloride in pure form and pharmaceutical formula.

Keywords: Ion-selective electrode, potentiometric titration, drug analysis, fluoxetine hydrochloride.

1. INTRODUCTION

Simple analytical tools such an ion-selective electrodes offer a direct measurement of active substances even in colored or turbid solution without previous extraction. Low cost, simple service, fast response time, selectivity and adequate detection limit are indisputable advantages of ion-selective electrodes [1]. In recent years an increased interest in the use electrochemical sensors in pharmaceutical analysis has been observed. Especial attention in drug analysis is paid on sensors based on plasticized polymeric PVC membranes. The most frequently used as solvent mediators to obtain different sensors are dioctyl phthalate [2-7], nitrobenzene and benzyl acetate [2,7-16], dibutyl phthalate [5,8-17], nitrophenyloctyl ether [3,11,13,15-20], bis(2-ethylhexyl)sebacate [4,5,20,21]. Much less frequently do researchers use less common membrane-solvents like di- or tri- butyl phosphate [17] and 1-isopropyl-4-nitrobenzene [20].

In spite of successful progress in development of highly selective electrodes for various ions, there has not been any report on how to decide about choice plasticizer (which is mostly determined by availability and popularity of the selected solvent).

A new look at quality of electroactive materials, solvent mediators and their parameters is included in this paper. In the experimental part, there were prepared sensitive electrodes for fluoxetine (Figure **1**) an antidepressant drug that works by inhibiting the uptake of serotonin by neurons in the brain.

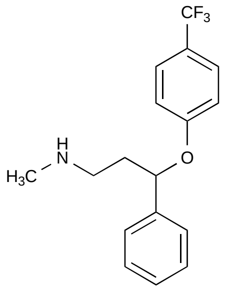

Figure 1: Fluoxetine, Prozac; IUPAC name: N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine.

Electroactive materials were three ion-association complexes fluoxetine with: tetraphenylborate [22,23], its chlorine derivative and dipicrylamine. The usefulness of four plasticizers: 2-nitrophenyloctyl ether, 1-isopropyl-4-nitrobenzene, bis(2-ethylhexyl)sebacate and bis(1-butylpenthyl)adipate was analyzed and compared.

2. METHODS

2.1. Reagent and Materials

Fluoxetine hydrochloride (Fl), sodium tetraphenylborate (TPB), potassium tetrakis(4-chlorophenyl)borate

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(ClTPB), 2-nitrophenyloctyl ether (NPhOE), bis(2-ethylhexyl)sebacate (b(EH)S), bis(1-butylpentyl)adipate (b(BP)A), 1-isopropyl-4-nitrobenzene (IPNB), high-molecular weight polyvinylchloride (PVC) and substances used to selectivity test were obtained from Sigma-Aldrich (Poznań, Poland); tetrahydrofuran (THF) from Romil Chemicals LTD (United Kingdom). Dipicrylamine (hexanitrodiphenylamine; DPA) and the others reagents were produced by POCh (Gliwice, Poland), and all of them were of analytical reagent grade. *Bioxetin* tablets (20 mg; Sanofi, Poland) were bought in local store.

2.2. Preparation of Fluoxetine Ion – Pair Complexes

To obtain Fl complexes, equal volume and 10^{-2} mol·L^{-1}concentrations of Fl and counter ion water solutions were mixed: TPB - water solution or ClTPB-solution in ethyl alcohol (400 g·L^{-1}) or DPA – later dissolved in warm (about 50°C) 1% Na_2CO_3 solution. The crystalline precipitates (whitish Fl-TPB and Fl-ClTPB; orange-red Fl-DPA) formed after cooling or evaporation of the ethyl alcohol, were filtered using a sintered glass crucible, washed with water to remove any non-complexed material, dried at room temperature, and finely ground to a powder.

2.3. Preparation of the Membranes

In the first step, PVC 170 mg was precisely dissolved in 6 ml THF. Next, as plasticizing solvent mediator 360 mg of NPhOE or b(EH)S or IPNB or b(BP)A was added and carefully mixed. Solid content 20 mg of the ion-pair complex (Fl-TPB or Fl-ClTPB or Fl-DPA) was added to previously obtained solution. Then, after ingredients were thoroughly dissolved, homogeneous mixtures were poured into a glass ring (diameter 3.1 cm) and covered with a sheet of filter paper; next glass plate was placed over it. The membrane formed as the THF was evaporated slowly at room temperature. The thickness of all obtained membranes was about 0.2 mm.

2.4. Construction of the Electrode

To prepare an electrode according to procedures described previously [24], a membrane disc of 8 mm diameter was cut from obtained film and glued (PVC in THF mixture) onto the front and body of PVC electrode containing an inner Ag/AgCl junction. A 10^{-3} mol·L^{-1} solution Fl in ionic strength NaCl (10^{-3} mol·L^{-1}) solution was used as the inner electrolyte. The membrane electrodes were conditioned overnight in a solution of the same composition as the internal filling solution and stored in the same solution before use.

2.5. Construction of the Calibration Graphs

Multifunctional apparatus Elmetron CX (measurement accuracy ±0.1 mV) was used for measuring the electrode potentials. Electromotive force (e.m.f) measurements were performed using the cell assembly: Ag/AgCl│ Fl (10^{-3} mol·L^{-1}) in NaCl (10^{-3} mol·L^{-1}) solution│PVC membrane│test solution│SCE. All the measurements were carried out at room temperature 21±2°C. A magnetic stirrer was used; however, the readings of the potential value were taken after the stirrer had been switched off. The performance of the obtained electrodes was investigated by measuring e.m.f. values of different Fl concentration within the range 10^{-2} - 10^{-7} mol·L^{-1}.

2.6. Potentiometric Titration of Fluoxetine Hydrochloride

Potentiometric titration method by means of the standard TPB solution (10^{-2} mol·L^{-1} standardized as previously described [25] was used for determining Fl. Samples of solution containing 3.09 ppm of Fl or accurately weight 17.5 mg powdered *Bioxetin* tablets (in NaCl 10^{-3} mol·L^{-1}) were titrated. End point was determined by a sudden rapid change in electrode potential.

3. RESULTS AND DISCUSSION

The purity of ion pair as electroactive material ensures correct ion exchange between the solution and the membrane of electrode. It is conditioned by a change in diffusion potential resulting from the processes taking place in the membrane and in the layer adherent to solution. The ratio of fluoxetine and counter ions forming a complex is 1:1. It possible because Fl is one H-bond donor and three examined counter ions are one H-bond acceptors. To exclude mistakes arising from badly prepared ion-pair, analysis of its composition was performed. The results of the elemental analysis agree well with the molar ratio of the reacting substances (Fl-TPB – 2.51% nitrogen amount in complex, Fl-ClTPB – 1.70%, Fl-DPA – 14.68%). To confirm the purity of the complexes TLC analysis was performed. RP18-TLC plates with methanol-0.09 phosphate buffer (pH 1.98) 60:40 (*v:v*) as the mobile phase and detection UV at 254 nm were used [26]. Chromatograms of ion-pairs do not show any free spots of Fl compared to the pure substance chromatogram.

Four different solvent mediators of the membrane composition were employed to evaluate their effect on the response characteristics of electrodes sensitive to Fl. Plasticizers were selected from a group marked by different partition coefficient n-octan-1-ol/water XlogP. For NPhOE it is equal 5.1; for IPNB 3.5, for b(EH)S 9.0 and for b(BP)A 6.8.

Membranes composed of all used solvent mediators and ion-complexes Fl-TPB or Fl-DPA were not flexible and extensible after THF evaporation. On the membrane surfaces there were drops of extruded plasticizers. The introduction additional halogen atoms into the functional group of electroactive substance raised the melting points of compounds. Therefore, plasticizer did not crystallize in the membrane phase and membrane was useful. Besides, additional halides increased the lipophilicity of the membrane compounds and consequently also the sensor membrane lipophilicity. For this reason only membrane with Fl-ClTPB complex were employed to obtain electrodes sensitive to Fl. Electrodes were denoted by the solvents mediator: NPhOE – I, b(EH)S – II, b(BP)A – III, IPNB – IV.

The working characteristics for the investigated sensors I – IV were assessed on the basis of calibration curves obtained by measuring of the e.m.f. values of the set of Fl solutions respectively, in concentration intervals of 10^{-2} – 10^{-7}mol·L^{-1} concentrations.

The tested electrode contained Fl-ClTPB ion pair complex with NPhOE had super Nernstian cationic response (above 60 mV·decade^{-1}), the one with b(BP)A (50.7 mV·decade^{-1}) had under Nernstian response to Fl activity in different concentration ranges. Slope characteristics of the electrodes, detection limits, linear ranges and potential drift also depend on the properties of the solvent mediator. The response times of the electrodes were tested for 10^{-2} – 10^{-7} mol·L^{-1} Fl solutions, respectively. The sequence of measurements was low to high concentrations. Those electrodes exhibited a fast dynamic response of about 5s for the higher concentrations. Below 10^{-4} mol·L^{-1}, the response time was expectedly sluggish, about 15 – 20 s, which is typical for ion-selective electrodes containing bulky PVC membranes of similar thickness (about 0.2 mm). Maximum variability response ±6 mV below 10^{-5} mol·L^{-1} was observed for electrodes containing NPhOE, b(EH)S or IPNB and below 10^{-4}mol·L^{-1} for b(BP)A. However, there were differences between the linearity ranges and response slopes for different electrodes. This implies that the different solvent mediators used do significantly influence the response characteristics of electrodes.

The chemical structure solvent mediators used to prepare membrane sensors sensitive to Fl shows no dependence between XlogP solvent mediators and analytical parameters of obtained sensors. Different value XlogP (9.0 and 6.8 respectively) and long and branched alkyl chain describes both b(EH)S and b(BP)A solvents. Analytical parameters of the obtained sensors with membranes containing these solvents indicate the benefit of b(EH)S. Similarly, NPhOE and IPNB show a slight difference in the XlogP (5.1 and 3.5, respectively); NPhOE has long alkyl chain, and for this solvent better analytical parameter were obtained. There is a lot of difference in chemical structure of NPhOE in comparison to b(BP)A, but their XlogP values are slightly different (5.1 and 6.8, respectively). For sensors containing membranes with the listed solvents completely different analytical parameters were obtained. The better results were obtained for electrodes with NPhOE as plasticizer.

The effect of pH on the potential readings of the electrodes Fl-ClTPB I – IV was checked by recording the e.m.f. of 10^{-3} mol·L^{-1} Fl with various pH values. They were obtained by the addition of small volumes of HCl and/or NaOH solution of 10^{-1} or 1.0 mol·L^{-1} each to the basic solution of Fl, respectively. For electrodes sensitive to Fl the potential did not fluctuate by more than about ±1 mV in the pH range 5.0–6.5 for all obtained electrodes.

The influence of pharmaceutical substances on the response characteristics of the development electrodes was investigated. Potentiometric selectivity coefficients were performed by standard separate solution method (SSM) [27] and calculated from the equation

$$\log K = (E_j - E_i)/S$$

where: E represents the e.m.f. measured for the primary ion (i), and the interfering ion (j), respectively, and S is the slope of the corresponding Fl electrode.

Interfering substances were selected on the basis of pharmacological properties, chemical structures and their relative impact on the selectivity of the electrodes. For the Fl sensitive electrodes anxiolytics, antipsychotics and inert fillers were chosen. The selectivity coefficients values are summarized in Table **1**. Chlorpromazine hydrochloride and promazine hydrochloride may cause harmful interference with

Table 1: Response Characteristics and Potentiometric Selectivity Coefficient ($\log K_{ij}^{pot}$) of Obtained Fl Sensitive Sensors (n=9)

parameter	(I) NPhOE	(II) b(EH)S	(III) b(BP)A	(IV) IPNB
Slope (mV·decade^{-1})	60.19	58.55	50.79	51.85
Intercept (mV)	235.72	248.31	166.87	231.64
Linear range (mol·L^{-1})	10^{-2} - 7×10^{-6}	10^{-2} - 5×10^{-5}	10^{-2}-4×10^{-4}	10^{-2} - 6×10^{-5}
Detection limit (mol·L^{-1})	2×10^{-7}	6.5×10^{-6}	6×10^{-5}	4.12×10^{-6}
Correlation coefficient in linear range	0.9987	0.9952	0.9903	0,9951
Potential drift (±mV)	2	2.5	4	2.5
Response time (s)	5	5	10	10
Working pH range	4.43 -5.45 6.5 - 7.05	2.45 –3.40 5.35 - 6.5	2.5 - 3.5 5.0 - 6.05	3.05 - 4.00 4.43 - 5.35
Life time (weeks)	9	6	4	6
Interfering substance	potentiometric selectivity coefficient			
chlorpromazine hydrochloride	+0.4555	+0.4622	+0.5061	+0.7401
promazine hydrochloride	+0.4778	+0.8957	+0.9535	+0.7750
chlordiazepoxide hydrochloride	-0.2135	-0.2307	-0.0974	-0.1915
diazepam	-0.1693	-0.2471	-0.0846	-0.1752
benzoate sodium	-0.1659	-0.2450	-0.1347	-0.1794
acetate sodium	-0.2018	-0.2541	-0.1558	-0.1944
lactose	-0.1960	-0.2379	-0.1178	-0.1828
glucose	-0.1900	-0.2369	-0.0962	-0.1635

determination of the potential of the electrodes sensitive to Fl of a comparative degree. Fortunately, these substances are seldom formulated in combination with Fl but they can be found in biological fluids. When determining Fl in body fluids, the effect of interfering ions on the used Fl electrode should be taken into account.

Because of stability the potential indication and electrode life time, three obtained electrodes sensitive to Fl were used as indicator electrodes to determination Fl in bulk substance. The latter contained NPhOE (I), b(EH)S) (II) and IPNB (IV). Next only one was used as indicator electrode to determination Fl in Bioxetin tablets.

Titration permits to carry out the measurements with considerably greater precision than that of the direct method. An essential advantage of substance determination is a possibility to use titrating solution, the titre of which can be determined by independent method, with no necessity to use the reference solutions. The potentiometric titrations of Fl were carried out with standard solution of 10^{-2} mol·L^{-1} TPB. The potential breaks of about 50 mV at the points corresponding to 1:1 Fl:TPB reaction were obtained. Gran's method (V_0 + V·$10^{E/S}$) [28] was used to determine the equivalence points. Figure **2** is an example of the obtained titration curve and Gran's curve of Fl solution using Fl-ClTPB-NPhOE sensor. Collective results given in Table **2** reveal that the proposed method shows a high degree of accuracy.

4. CONCLUSIONS

Electroactive material of liquid heterogenic membranes for ISEs should be clear without additional ions (being determined and counter-ions). Free additional ions in membrane give an additional flow of ions in the layer adjacent to the membrane. This additional flow of ions changes analytical parameters of electrodes: the range of measurable detection limit, the slope response, repeatability of measurements or selectivity factors. In the study were conducted experiments at determining the purity obtained ion-associative connections. The quality of these compounds was carried out using elemental analysis and TLC. The paper also draws attention on the

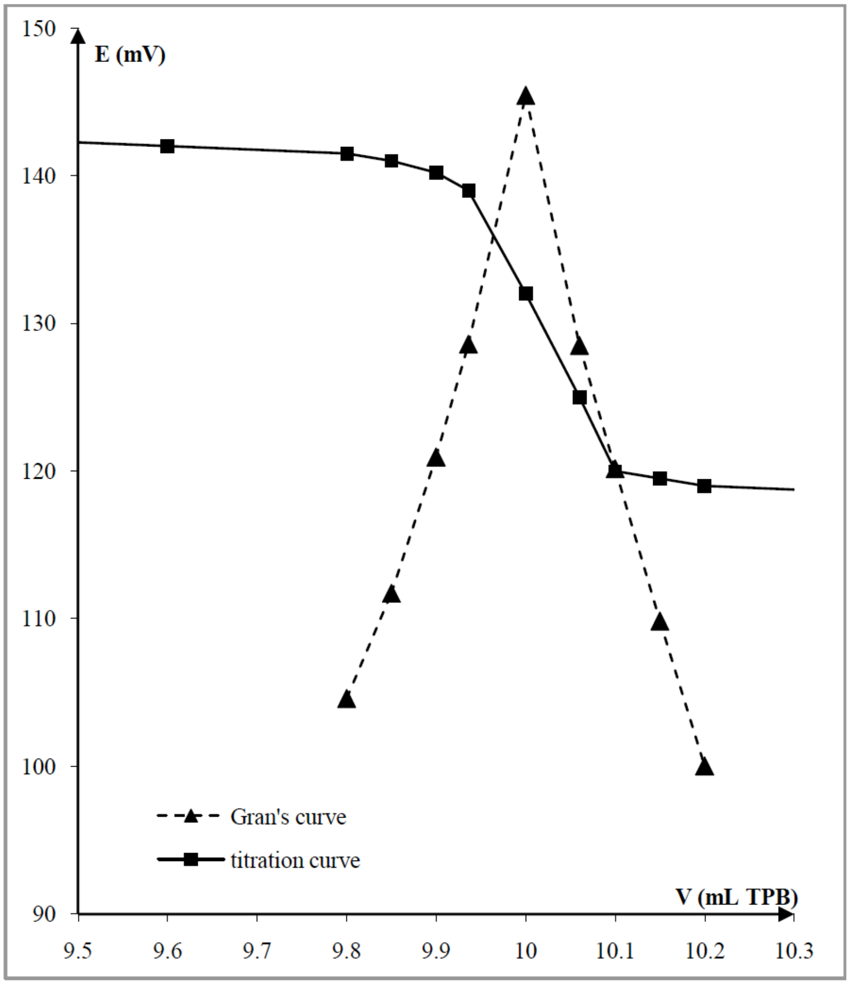

Figure 2: Potentiometric titration and Gran's curve of Fl determination using sensor Fl-ClTPB-NPhOE.

Table 2: Test of Precision Potentiometric Titration Methods Using Three Fl Sensors (n=5)

parameter	Electrode I		Electrode II	Electrode IV
	substance	tablets	substance	substance
$\bar{X}$ (%)	100.11	100.17	95.27	100.03
S	0.19	0.51	9.58	0.74
$\bar{S}$	0.07	0.26	3.62	0.28
RSD (%)	0.19	0.125	10.05	0.741
μ (%)	100.07 – 100.15	100.07 – 100.27	93.46 – 97.08	99.88 – 100.17

selection of solvent membranes for the construction of heterogeneous liquid membranes ISEs. A knowledge of chemical structure and chemical parameters of solvents is necessary to obtain sensors showing a wide linear range, low detection limit and satisfactorily selectivity for Fl with long shelf life. Heterogeneous membranes obtained by the described procedure was used successfully in the construction of sensors which are sensitive to Fl and their original application.

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