SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
16 (2010)

NEW TYPE OF PVC-MEMBRANE ION-SELECTIVE ELECTRODES AND THEIR APPLICABILITY TO DETERMINE SOME ANTIDEPRESSANT DRUGS

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Received September 30, 2010

The construction and electrochemical characterisation of potentiometric sensors of the PVC-membrane type are described being developed for the determination of selected antidepressants; namely: Olanzapine, Oxazepam, and Lorazepam. The sensing PVC-membranes incorporate the ion-associates of the drug cations with ammonium reineckate as selective materials dispersed in 2-nitrophenyl-octylether (NPOE) or dibutyl sebacate (DBS) as the plasticizers of choice. These ISEs exhibit rapid, stable and nearly Nernstian response over a relatively wide concentration range of 1×10^{-7} mol l^{-1}-0.01 mol l^{-1} for Olanzapine and 1×10^{-6}-0.01 mol l^{-1} for Oxazepam and Lorazepam, in mild acidic solutions with pH 4.5. No interferences caused by either inorganic or organic species were found. The three electrodes developed have been tested for potentiometric titrations of all three drugs, as well as for their direct determination via the respective calibration curves. The individual experiments then resulted in average recovery rates in an interval of

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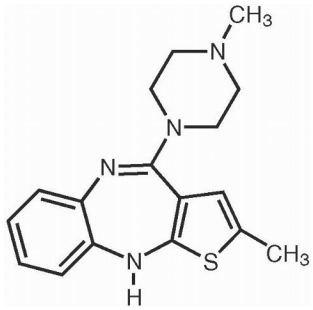
98.4-99.2 %, with the R.S.D. of ca. ±1.5 % (n = 3) for all three analytes of interest. Finally, the method proposed has been applied to the determination of the three drugs in pharmaceutical formulations and urine, when the results obtained were comparable to those obtained with the reference HPLC measurements.

Introduction

Benzodiazepine drugs (BZD) belong to a group of substances that are known for their sedative, hypnotic, tranquillizing, and anticonvulsant properties and are being prescribed worldwide as therapeutics to treat anxiety, sleep disorders, status epileptics, insomnia, frequent nocturnal awakenings, early morning awakenings schizophrenia, and related disorders. In addition, they are used as muscle relaxants for alleviation of panic attacks and as induction agents in anesthesiology [1].

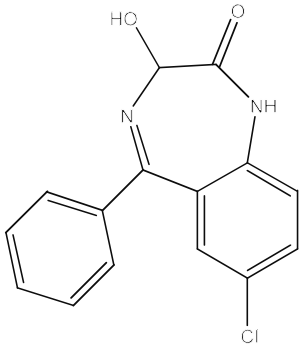
Olanzapine (*Olan* — see Scheme 1) is an antipsychotropic agent that belongs to the thienobenzo-diazepine class, a relatively new benzodiazepine which has been found useful to treat schizophrenia and some other psychosis [2-4]. *Olanzapine* is a crystalline solid substance of yellow colour and with a melting point of 263.2 ºC [5]. Its molecular formula is $C_{17}H_{20}N_4S$, corresponding to a molecular weight of 312.44 g mol^{-1}.



Scheme 1 Chemical formula of *Olanzapine* (2-methyl-4-(4-methyl-1-piperazinyl)-*10H*-thieno[2,3-b][1,5]benzodiazepine)

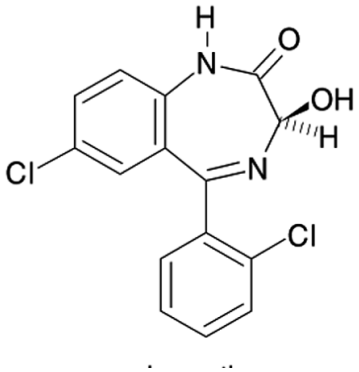
Oxazepam (aka *Oxazem* or *Serax*-tablets, *Oxaz* — see Scheme 2) is an anti-anxiety agent of the benzodiazepine type used primarily for treating mild to moderate anxiety. It is also prescribed as a sedative, hypnotic, anticonvulsant, and muscle relaxant [6-9].

This drug is frequently encountered in clinical and forensic toxicology, having been featured in an increasing number of misuses and abuses over the past years [10,11]. Finally, it is also used to treat irritable bowel syndrome. *Oxazepam* is a creamy white or pale yellow crystalline substance, odourless, and with melting point of 205 ºC. Its molecular formula is $C_{15}H_{11}ClN_2O_2$ for both possible enantiomers, corresponding to a molecular weight of 284 g mol^{-1}.



Scheme 2 Chemical formula of *Oxazepam* (7-chloro-2,3-dihydro-3-hydroxy-5-phenyl-*1H*-1,4-benzodiazepin-2-one)

Lorazepam (*Lora* — see Scheme 3) is classified pharmacologically as a short acting benzodiazepine and under the trademark *Ativan*® (1 mg or 2 mg of active substance) is a generic representative for the popular anti-anxiety medication, indicated for generalized anxiety disorder (GAD), panic disorder, anxiety associated with depression and insomnia. Also, it is sometimes used as a sleeping agent, anti-convulsant, and for alcohol detoxification [12-15]. *Lorazepam/Ativan*® is a white — or, almost white — microcrystalline powder with a melting point of 245 ºC. Its molecular formula is $C_{15}H_{10}Cl_2N_2O_2$, corresponding to a molecular weight of 321.2 g mol^{-1}.



and enantiomer

Scheme 3 Chemical formula of *Lorazepam* (7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3hydroxy-*1H*-1,4-benzodiazepine-2-one)

In this article, a simple and rapid method for the determination of the three, above described pharmaceuticals has been developed based on the employment of ion-selective electrodes (ISEs) whose PVC-membranes are saturated with the corresponding BZD/reineckate ion-associate and that can be used for indication in direct potentiometry, as well as in potentiometric titrations.

Experimental

Chemicals and Reagents

All reagents and chemicals used throughout the work were of analytical-reagent grade and solutions were prepared with deionised distilled water (DDW). Both polyvinylchloride (PVC) powder and ammonium reineckate (A-RE; purity: 98 %) used as reagent and titrant were obtained from Aldrich. Dibutyl sebathete (DBS; a plasticizer of purity ~ 99 %) was purchased from BDH (British Drug Houses, Pool, England); 2-Nitrophenyl octyl ether (*o*-NPOE; a plasticizer of purity ca. 99 %) from Fluka. Tetrahydrofuran (THF; a solvent with purity of about 99 %) was purchased from Aldrich.

An acetate buffer solution of pH 4.5 was prepared by dissolving 13.6 mg sodium acetate with approx. 5 ml glacial acetic acid in 1000 ml deionised and doubly distilled water. A phosphate buffer solution of pH 4.5 was prepared by mixing the appropriate quantities of 0.1 M H_3PO_4 with 0.2 M Na_2HPO_4 in 500 ml deionised distilled water.

In interference studies, selected inorganic cations, amino acids, and sugars were examined *via* 1×10^{-3} M solutions prepared in acetate buffer (pH 4.5), when containing the following species: Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Cu^{2+}, Zn^{2+}, and diphenhydramine. The remaining solutions of urea, starch, glucose, and maltose were 0.01 mol l^{-1}.

Standard Solutions and Precipitates for Membrane Modification

Standard Solutions of the Drugs: The stock solutions of *Olanzapine*, *Oxazepam*, and *Lorazepam* (or *Olan*, *Oxaz*, and *Lora*, resp.) were prepared as 0.01 mol l^{-1}, by dissolving the proper weight of solid substance into suitable amount of 0.002 M HCl added in droplets under continuous stirring till a complete dissolution of the drug was achieved.

The resultant solution was made up to 100 ml in a measuring flask using de-ionized water. Working diluted standards were then prepared in a series of calibration solutions in the concentration range from 1×10^{-7} up to 0.01 mol l^{-1} for *Olan* and from 1×10^{-6} to 0.01 mol l^{-1} for *Oxaz* or *Lora*; all being made as solutions in acetate buffer (pH 4.5). Both stock solutions and diluted standards were kept in dark and in a refrigerator (at 4 °C).

Standard of ammonium reineckate; i.e., ammonium tetrathiocyanato-diammine chromate(III), $NH_4[Cr^{III}(SCN)_4(NH_3)_2]\cdot H_2O$; (A-RE): A 0.01 M standard solution was prepared by dissolving 2.6300 g in 100 ml of deionised distilled water.

Preparation of the BZD / Reineckate Ion-Associates: Each BZD / RE ion-pair

complex was prepared by mixing the equal volumes of 0.01 M A-RE with 0.01 M *Olan, Oxaz* or *Lora*; when the latter being added drop-wise under continuous stirring. The resultant precipitates were left in contact with their native liquor overnight to ensure the complete coagulation, then filtered off through a *Whatman*® filter paper No. 42, washed thoroughly and repeatedly with distilled water, left to dry at room temperature for at least 24 hours, and finally ground to fine powder.

Apparatus and Other Equipment

All potentiometric measurements were performed at 25±2 °C using a pH meter (model H-8417, Hanna; for further specification, see Ref. [16]) with pH sensitivity of ± 0.05 pH units with Drug-PVC matrix membrane sensors in conjunction with a double-junction Ag/AgCl reference electrode (model 900200, Orion) containing a solution of (10 %, w/w) KNO_3 in the outer compartment. The combined glass electrode (model F-22E, Horiba [16]) was used for all the pH measurements after calibration with the use of a set of commercially available buffers.

The electrochemical system used can be represented as follows: Ag|AgCl |KCl (saturated, ca. 3 mol l^{-1})||Salt bridge||Sample, it means: Reference electrode half-cell|Membrane|Filling solution|Ag/AgCl|Ion-selective electrode half-cell; where the filling solution is a mixture of 0.01 M solution for each of investigated drugs and KCl.

Procedures and Samples

Membrane Preparation: A 10 mg portion of drug/reineckate ion-pair complex was thoroughly mixed in a glass Petri dish (5 cm in diameter) with 190 mg of PVC powder and 350 mg of 2-nitrophenyl octylether (NPOE) or dibutyl sebacate (DBS).

The resultant cocktail was dissolved in 5 ml THF, covered with a filter paper and left overnight to evaporate to dryness at ambient temperature. Using this procedure, a semi-transparent and plastic-like membrane with a thickness 0.1 mm could be obtained. Then, the parts of the membrane material were cut using a cork borer and, when plasticizing with THF, an exchangeable PVC-tip was clipped onto the end of the electrode glass body. The tip was left to stand for 24 hours to allow complete evaporation of THF and, finally, the fabricated electrode was soaked into 0.01 M solution of the corresponding drug for 24 hours before use (and also being stored in the same solution when not in use).

The constructed sensor was washed with deionised distilled water and blotted with tissue paper between measurements. The effect of membrane

composition was studied for Olanzapine-modified sensor which was plasticized with NPOE — by weighing three different amounts of ionophore (5 mg, 10 mg, or 15 mg, respectively), and mixing with 190 mg PVC + 350 mg NPOE + 5 ml THF in a glass mortar. To obtain homogeneous and uniform thickness of the mixture [17], the membranes were left to dry free (not less than 24 hours).

Measurement and Construction of Calibration Curves: 10 ml aliquots of each sample solutions — in acetate buffer of pH 4.5 — having concentrations of 1×10^{-7}-0.01 M for *Olan* or 1×10^{-6}-0.01 M for *Oxaz* and *Lora* were transferred into 50ml beakers. The potential in mV of each sample solution was directly measured using the respective (drug/reineckate ion-pair modified) ISEs based on DBS or NPOE as plasticizer as mentioned in the previous section.

The solutions were stirred and the potential readings were recorded from the low to the high concentration after stabilization to ±0.3 mV [18]. The potentials were plotted as a function of –log (drug concentration). These graphs were used for the subsequent determination of unknown concentrations of corresponding drug. The theoretical (aka "lower") detection limit was determined at the point of intersection of the extrapolated linear segments of the corresponding drug calibration curve.

Studies on pH-Effect and Response Time: The effect of pH of the test solution on the potential values of the electrode system in solutions of different concentrations of pharmaceutical compounds was tested using two concentrations of each drug ion (1×10^{-3} and 1×10^{-4} M) over a range of pH (2-11) and recording the potential readings of the studied sensors by immersing the Ross combination glass electrode, corresponding drug-sensor and a double junction Ag/AgCl reference electrode in 50 ml beakers containing 25 ml aliquots of 1×10^{-3} and/or 1×10^{-4} M drug-aqueous solutions. The pH of each solution was gradually changed by adding small aliquots of dilute sodium hydroxide and/or hydrochloric acid solutions. The mV vs. pH profile of each drug concentration was plotted for each electrode system. The potential readings that were insensitive to pH changes were obtained from the mV–pH plots. The dynamic response time of the ISEs could also be characterised by the fact that the respective measurements required to reach a steady-state potential within ±0.3 mV only, even if the analysed concentrations differed also in one order.

Determination of Sensor Selectivity: The influence of some inorganic cations, sugars and amino acids, used as additives or binders, on the response of the electrodes towards their respective drugs was tested. The separate solution method [19], which requires two potential measurements: first, the potential is measured in a solution containing a known activity of the ion for which the electrode is selective, second, the potential is measured in a solution containing the interfering

ion, was used to determine the selectivity coefficient value, $K_{d,i}$ — see later in Table III. Separate drug primary ion (d) and interfering secondary ion (i) solutions having equal concentrations were prepared. Their potentials E_d and E_I were measured using prepared ISEs in the previously mentioned cell. Selectivity coefficients were calculated using either one of the following equations

$$\log K_{d,I} = \frac{E_I - E_d}{S} \tag{1}$$

$$\log K_{d,I} = \frac{E_I - E_d}{S} + \left(1 - \frac{z_d}{z_i}\right) \log d \tag{2}$$

where E_I and E_d are the electrode potentials of 0.01 M solution of each of the investigated drug and interfering cation, I, respectively. Equation (1) is used for mono-valent secondary ions whereas Eq. (2) is used for divalent or higher ones. z_d , z_i are the charges on the primary or secondary ions, and S is the slope of calibration curve for the primary ion.

The individual interfering inorganic ions and organic species used in the studies on the selectivity of the different membranes were already specified above. For the proper measurements, the respective drug solution was transferred into a 50 ml beaker containing 9.0 ml acetate buffer (pH 4.5). The drug sensor in conjunction with a double junction Ag/AgCl reference electrode was immersed into the solution and the potential read (in mV). Then, the solution with interfering species was transferred into a 50 ml beaker containing 9.0 ml the same buffer and the potential change was again recorded (as E_I). Selectivity coefficients were calculated as shown before.

Potentiometric Titrations. The individual aliquots of 0.01 M BZD were transferred into 50 ml titration cell and diluted to 10 ml with the acetate buffer of pH 4.5. The resulting solutions were stirred and titrated against 0.01 M A-RE according to the corresponding ion-exchanger used in constructing the electrode. The proper titration curves, i.e., the electrode potential vs. the titrant volume (*E*-vs.-*V*) plots and their end-points were evaluated conventionally.

Pharmaceutical Samples of the Drugs Studied. Five tablets of the drug formulations were weighted and finely powdered in a small mortar. An accurately weighted portion of powdered drug, equivalent to 1.562 mg *Olan*, 1.433 mg *Oxaz*, or 1.606 mg *Lora*, was transferred into 5ml measuring flask and dissolved in the minimum volume of de-ionized distilled water and few drops of 0.002 M HCl. The solution was filtered into a 50ml calibrated flask, then diluted to the mark with the acetate buffer of pH 4.5 and shaken well.

A series of 10, 15, and 20 ml aliquots of each drug solution were transferred into 25ml volumetric flasks and made up to the mark with acetate buffer. Thus, solutions having concentrations of 12.5, 18.75 and 25 μg ml^{-1} *Olan*, 11.46, 17.2 and 22.92 μg ml^{-1} *Oxaz*, or 12.85, 19.30 and 29.70 μg ml^{-1} *Lora* were made and the potentials of the resultant drug solutions could be directly measured at the corresponding ISE.

Biological Fluids (Drugs in Urine): Solutions of 5×10^{-3} M *Olan* (31.24 μg ml^{-1}), *Oxaz* (28.66 μg ml^{-1}) or *Lora* (32.12 μg ml^{-1}), were prepared by dissolving the appropriate amount of pure substances — specifically: 1.562 mg, 1.433 mg, and 1.606 mg — in 50 ml in the order and using solvent as described above. Aliquots of 10, 15, and 20 ml (pipetted from the respective drug solutions) were transferred into 25ml measuring flasks; each containing 5 ml urine, and filled up to the mark with solvent. The actual electrode potentials of the individual solutions were measured in the same way as above.

Results and Discussion

Principles of the Electrode Functioning

According to the literature (see, e.g., Ref. [20]), the respective drug moiety can be protonised and such a cation can readily react with the anionic form of reineckate, following the scheme(s)

$$2\,Olan + H^+ + [Cr(SCN)_4(NH_3)_2]^- \rightarrow \{H^+–(Olan)_2;[Cr(SCN)_4(NH_3)_2]^-\} \quad (3)$$

$$Oxaz + H^+ + [Cr(SCN)_4(NH_3)_2]^- \rightarrow \{H^+–Oxaz;[Cr(SCN)_4(NH_3)_2]^-\} \quad (4)$$

$$Lora + H^+ + [Cr(SCN)_4(NH_3)_2]^- \rightarrow \{H^+–Lora;[Cr(SCN)_4(NH_3)_2]^-\} \quad (5)$$

where H^+-$(OLAN)_2$ or H^+-*OXAZ* and H^+-*LORA*, respectively, denote the protonated form of the respective drug and the compounds on the right-hand side the resultant ion-associates.

The Operability of the Three Electrodes Studied

Olan-, *Oxaz*-, and *Lora*-ISEs with the electroactive material chosen, i.e., H^+-DRUG / reineckate were constructed using PVC as a matrix and NPOE or DBS as the plasticizers. The ISEs were investigated in order to compare their electrode performance and the results are summarized in Tables I and II. The corresponding

calibration graphs, given in Figs 1, 2 and 3, indicate that for all the electrodes tested, having nearly Nernstian cationic response slopes (26.12 and 24.22 mV decade^{-1} with a limit of detection 1.2×10^{-7} and 0.9×10^{-7} M for *Olan*-ISE plasticized with NPOE and DBS, respectively, and 52.10- 54.30 mV decade^{-1} for both *Oxaz*-ISE and *Lora*-ISE. These slopes also confirm the pathways of the

Table I Potentiometric responces characteristics for *Olan-RE*/membrane ISE with NPOE or DBS as plasticizer

Parameter evaluated	Olanzapine-RE sensor	
	NPOE	DBS
Concentration range, mol l^{-1}	10^{-7}-10^{-2}	10^{-7}-10^{-2}
Slope, mV decade^{-1}	26.12	24.22
Intercept	162.8	167.7
R.S.D, %	0.62	0.77
Limit of detection, mol l^{-1}	3.2×10^{-7}	4.2×10^{-7}
Correlation coefficient (r^2)	0.9939	0.9933
Actual pH (range)	4.5	4.5
Response time, s	20	20
Life span, weeks	4	4

Table II Potentiometric responces characteristics for *Oxaz*- and *Lora*-membrane ISEs with NPOE or DBS as plasticizer

Parameter evaluated	Oxa-RE sensor		Lora-RE sensor	
	NPOE	DBS	NPOE	DBS
Concentration range, mol l^{-1}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}
Slope, mV decade^{-1}	54.30	52.10	55.01	54.43
Intercept	354.8	355.4	343.9	354.5
R.S.D, %	4.6×10^{-6}	5.3×10^{-6}	3.7×10^{-6}	4.8×10^{-6}
Limit of detection, mol l^{-1}	0.56	1.20	0.74	1.30
Correlation coefficient (r^2)	0.9989	0.9978	0.9979	0.9978
Actual pH (range)	4.5	4.5	4.5	4.5
Response time, s	30	30	40	40
Life span, weeks	40336	40336	40336	40336

above-described reactions (1) and (2), and the stoichiometry of the corresponding ion-pairs formed.

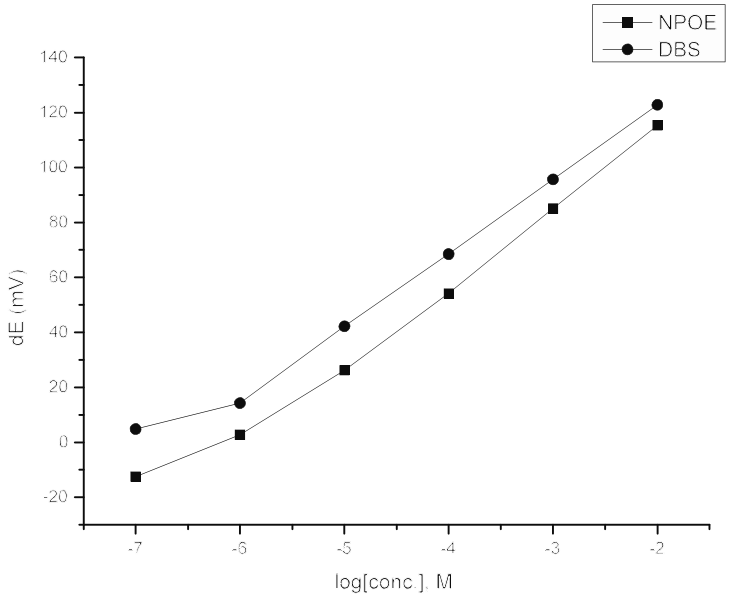


Fig. 1 Calibration curve of *Olan*-membrane ISEs with NPOE or DBD as plasticizer

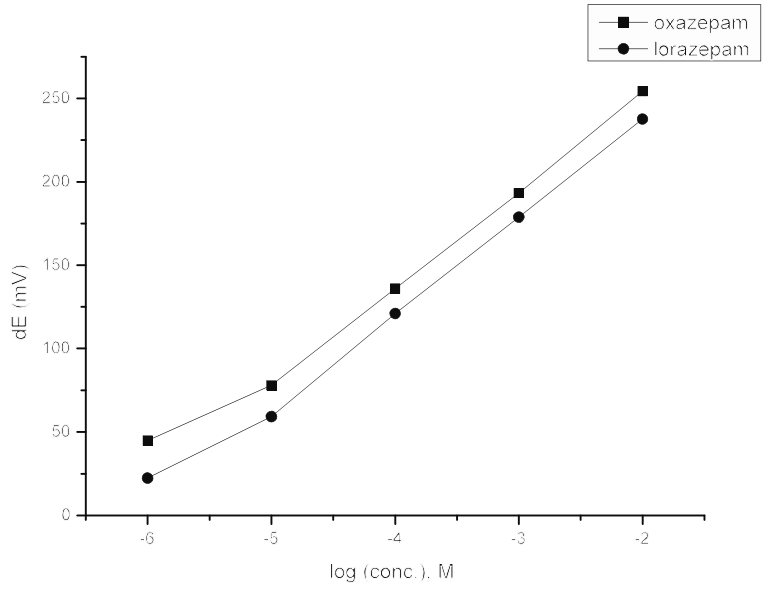


Fig. 2 Calibration curve of *Oxaz*- and *Lora*-membrane ISEs with NPOE as plasticizer

The linearity range extended down to 1×10^{-7} mol l^{-1} for *Olan* and 1×10^{-6} mol l^{-1} for *Oxaz* or *Lora.* Also, the detection limits that were estimated according to a widely adopted criterion "three sigma" [21,22] could be evaluated from the calibration curves. Although the three different electrodes exhibited certain differences between linearity ranges and response slopes, the resultant nuances were found insignificant.

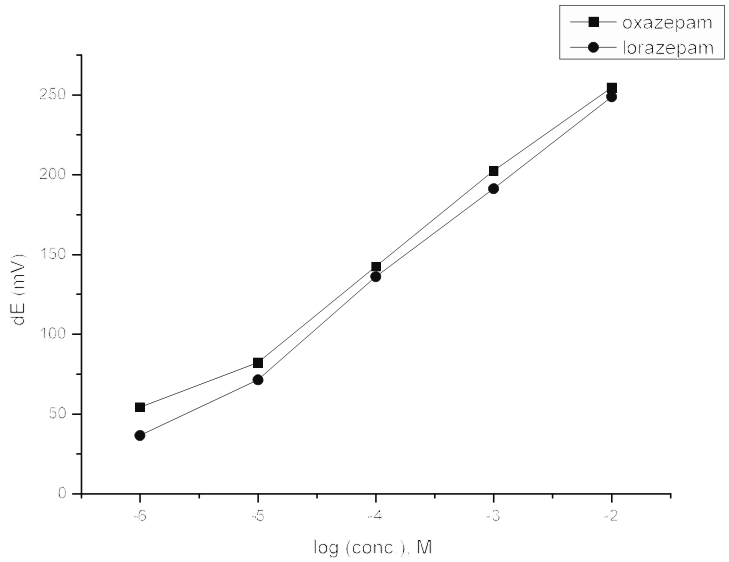


Fig. 3 Calibration curve of *Oxaz*- and *Lora*-membrane ISEs with DBS as plasticizer

The Electrodes and Their Selectivity Characteristics

The selectivity coefficient is the main source of information concerning interferences on the electrode response. In analytical applications, the selectivity for the analyte must be as high as possible, i.e. the selectivity coefficients must be very small, so that the electrode exhibits a Nernstian dependence on the primary ion over a wide concentration range. The selectivity of the ion-exchanger of membrane electrodes depends on the selectivity of the ion-exchange process at the membrane test solution interface and the mobility of the respective ions in the matrix of the membrane. The hydrophobic interactions between the primary ions and the PVC membrane are reflected by the values of the Gibb's energy of transfer for cations between the aqueous and membrane phases. The response of the electrodes towards different substances and ionic species was checked.

Table III Potentiometric selectivity coefficients, $K_{i,j}^{pot}$, for three proposed electrodes based on NPOE (where *i* denotes mediator and *j* enterrfering species

Interfering species (ion/substance)	Olan-sensor $K_{i,j}^{pot}$	Oxa-sensor $K_{i,j}^{pot}$	Lora-sensor $K_{i,j}^{pot}$
Na^+	8.5×10^{-4}	1.6×10^{-3}	7.2×10^{-4}
K^+	7.7×10^{-4}	4.4×10^{-3}	5.6×10^{-3}
Mg^{2+}	5.3×10^{-4}	5.8×10^{-3}	4.1×10^{-3}
Ca^{2+}	8.5×10^{-3}	7.3×10^{-4}	6.3×10^{-4}
Sr^{2+}	1.2×10^{-2}	6.6×10^{-3}	3.3×10^{-3}

Table III — Continued

Interfering species (ion/substance)	Olan-sensor $K_{i,j}^{pot}$	Oxa-sensor $K_{i,j}^{pot}$	Lora-sensor $K_{i,j}^{pot}$
Cu^{2+}	6.3×10^{-2}	5.2×10^{-3}	6.1×10^{-3}
Zn^{2+}	2.3×10^{-2}	8.7×10^{-3}	4.3×10^{-3}
Diphenhydramine	3.4×10^{-3}	2.3×10^{-4}	1.2×10^{-4}
Urea	8.2×10^{-4}	2.2×10^{-4}	2.4×10^{-4}
Starch	2.4×10^{-4}	4.3×10^{-4}	8.1×10^{-4}
Glucose	3.6×10^{-4}	3.6×10^{-4}	7.4×10^{-4}
Maltose	5.4×10^{-4}	6.2×10^{-4}	6.6×10^{-4}
Olanzapine	-	0.7×10^{-2}	3.2×10^{-2}
Oxazepam	3.2×10^{-2}	-	3.1×10^{-2}
Lorazepam	1.3×10^{-2}	4.2×10^{-2}	-

As mentioned in the Experimental part, the selectivity coefficients of the interfering cations were determined by the separate solution method, which is a very simple approach providing a reasonable measure of the degree of interference of foreign species that may be present in the test solution [23,24] — see Table III. The mechanism of selectivity is mainly based on the stereo-specificity and electrostatic environment and it is dependent on how much fitting is present between the locations of the lipophilicity sites in the two competing species in the drug solution side and those present in the receptor of the ion-exchanger.

The inorganic cations do not interfere because of differences in ion size, mobility and permeability. Also, the smaller the energy of hydration of the cation, the greater is the response of the membrane. The electrodes exhibit good tolerance toward sugars, amino acids, and urea as the presence of these species up to 4- or even 5-fold excess did not affect significantly the potential reading. This has indicated that the presence of these species can be tolerated to a high extent. Such a high tolerance can then be attributed to the differences in polarity and lipophilic nature of their molecules relative to those of the drugs under investigation. Though *Olan*, *Oxaz* and *Lora* mutually interfere, these three similar substances so far do not exist in one pharmaceutical preparation.

Effect of pH and of the Type of Buffer

The influence of pH on the response of the proposed drug-selective electrode was checked by measuring the potential displayed by 1×10^{-3} and 1×10^{-4} M drug test solutions over the range of pH 2-11. Small volumes of dilute sodium hydroxide and/or HCl were used for adjustment. It is apparent from the potential-pH profiles that the response of all three sensors was fairly constant in acetate buffer solutions of pH 3.5-6.0. The potential did not vary over this range by more than ± 3mV and the electrode could be applied for the determination of investigated drugs. A considerable decrease in the potential above pH 6 was observed, which was probably due to the decreased concentration of the protonated form of the drug, whereas — at higher pH — a deviation in the potentials reading might occur due to the penetration of the hydroxonium ion into the membrane layer. On the other hand, two types of buffers, i.e., acetate and phosphate media, were investigated on *Olan*-ISE in order to ensure that the electrode would be functioning [25]. The effect of the two buffers was not critical and no marked difference was observed as shown in Fig. 4 and Table IV. However, acetate buffer of pH 4.5 was found to be more suitable because of the fact that the sensor potentials were almost constant and stable within ±3 mV.

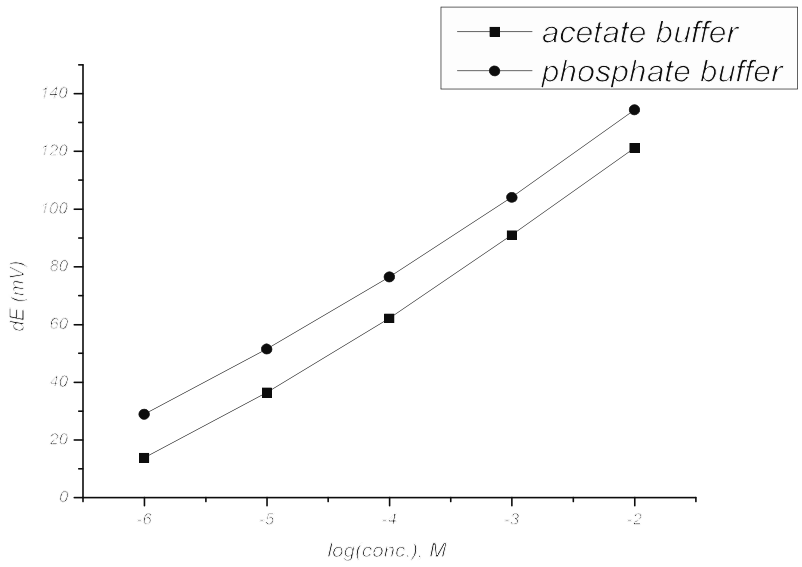


Fig. 4 Calibration curve of *Olan*-electrode obtained in acetate and phosphates buffers

Effect of the Plasticizer Used

In this work, polyvinyl chloride (PVC) membranes plasticized with DBS or NPOE and containing the lipophilic salt, *Olan-Re, Oxaz-Re* or *Lora-Re*, were prepared. Potentiometric responses of the electrodes based on neutral ionophores and

modified with the investigated drugs were greatly influenced by the polarity of the membrane medium, which was in turn defined by the dielectric constants of the incorporated drug moiety and plasticizer (solvent mediator) used. Regarding the latter, the two dielectric constants were $D_{i(DBS)} = 4.01$ and $D_{i(NPOE)} = 23.6$, indicating a high sensitivity and nearly Nernstian slope for the ISEs with NPOE-plasticized membrane, whereas the ISEs with DBS-plasticized membranes showed anomalous response to the corresponding drug.

Table IV Potential response of *Olan*-ISE with NPOE plasticizer and in buffer of choice

Parameter evaluated	Acetate buffer	Phosphate buffer
Concentration range, mol l^{-1}	10^{-6}-10^{-2}	10^{-6}-10^{-2}
Slope, mV $decade^{-1}$	26.92	26.33
Intercept	175.6	184.4
Limit of detection, mol l^{-1}	1.0×10^{-6}	0.9×10^{-6}
R.S.D., %	2.80	2.76
Correlation coefficient (r^2)	0.9986	0.9983

Composition of the Membranes

In plastic membranes of ISEs studied, the amount of the ion-pair exchanger should be sufficient to obtain reasonable ionic exchange at the gel layer-test solution interface, which is responsible for the membrane potential and its changes. Also, the amount of the plasticizer should be in the extent that produces a membrane of

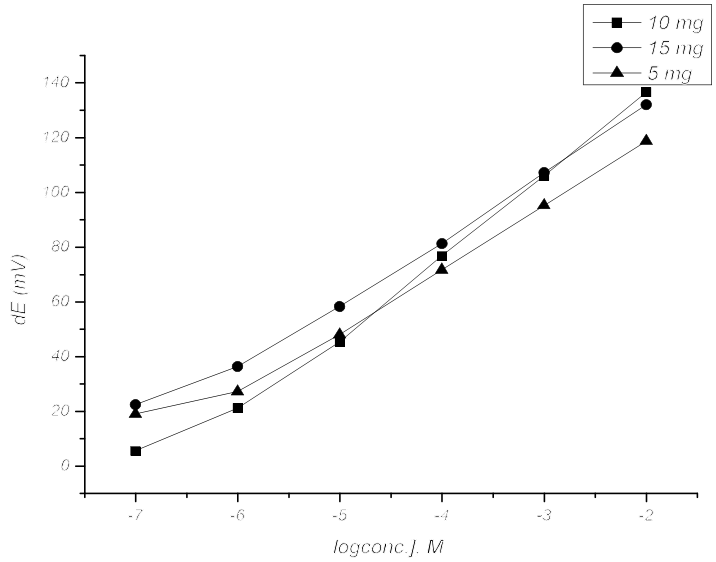


Fig. 5 Effect of composition of ionophore on response of *Olan*-sensors

good physical properties and, at the same time, plays efficiently its role as a solvent mediator for the ion-exchanger. The composition of each of these membranes was varied in such a way as to reach the optimum composition exhibiting the best performance characteristics (calibration-curve slope, rectilinear concentration range, and the response reproducibility).

Furthermore, it comprised the different compositions of ionophore cooperating in the membrane and the slope (mV decade^{-1}) of the obtained calibration graphs for *Olan*-sensor plasticized with NPOE. The membrane was prepared three times and the relative standard deviation (R.S.D.) calculated for the slope values was always fairly low, indicating good reproducibility of the preparation process and comparable slope values of the calibration graphs obtained with ISEs differing in composition. As shown in Fig. 5, it is evident that the change of composition of ionophore has significantly affected the response of the electrode. The results proper are gathered in Table V, showing that the optimal composition of ionophore is 10 mg, offering an ISE with the best performance.

Table V Effect of ionophore on response of *Olan*-ISE with NPOE as plasticizer

Parameters evaluated	Composition, mg		
	5	10	15
Slope, mV decade^{-1}	20.75	26.91	22.38
Intercept	156.7	186.3	173.7
R.S.D., %	5.1	3.83	4.6
Limit of detection, mol l^{-1}	6.2×10^{-7}	3.1×10^{-7}	4.6×10^{-7}
Correlation coefficient (r^2)	0.9839	0.9988	0.9923

Regeneration of the Ion-Selective Electrodes Prepared

From the above discussion, it is clear that soaking of the electrodes in their respective drug solutions for a long time has a negative effect on the response of the membrane towards the ions investigated. The same effect appears after working with the electrode for a long time. The regeneration of such "exhausted" electrodes was tried by simply re-loading of the ion-exchangers of the *Olan*-RE, *Oxa*-RE, and *Lora*-RE type, in the external gel layer of the membrane [26]. This was accomplished by soaking the drained electrodes for 24 hours in a solution of 0.01 M A-RE, followed by saturation in a solution of the respective drug. As found, the soaking in 0.01 M solution of each drug for ca. three hours was sufficient for regeneration and, seen from the slopes of the renewed electrodes, increasing from 21.32, 45.70, and 43.33 up to 24.51, 52.16 and 50.01 mV decade^{-1}

for *Olan*-, *Oxaz*-, and *Lora*-ISEs, respectively. Generally, it has been found that the life time of regenerated electrodes is limited up to few hours (60-180 min.). This was due to the ease of leaching of lipophilic salts from the gel layer at the electrode surface compared to a situation when they had been firmly attached to the PVC network through the solvent mediator with strong binding forces.

Choice of the Method for Quantitative Analysis and Potentiometric Determination of the Drugs of Interest Using BZD/RE-modified PVC-Membrane ISEs.

In fact, three principal methods could be considered as potentially applicable to the quantitative analysis with the three *Drug*/RE-ISEs developed during these studies. Such a choice comprised: (i) direct calibration measurements, construction of the corresponding plot and the calculation of the concentration from the Nernst equation; (ii) potentiometric titration involving the use of counter ions as the titrant which seemed more accurate, depending essentially on the use of the ISE as end-point detector, but more time consuming (in an estimate, at least 20-30 min. for the average analysis); (iii) the standard addition(s) method, which is often most reliable, due to the most effective suppression of matrix effects, and therefore quite frequently applied in practical determinations with ISEs.

Direct Potentiometry: The individual drugs contained in commercial pharmaceutical preparations had represented the first part of practical samples that were analyzed and their concentrations determined with the respective ISE when using the standard addition method.
The results obtained are listed in Table VI. By applying the least-squares method for five replicate determination (i.e., n = 5) at a 95 % confidence level, the corresponding regression equations can be written as follows

$$Y = 0.984(\pm 0.0037)X + 0.0133(\pm 0.0718) \quad \text{for } \textit{Olanzapine} \tag{6}$$

$$Y = 1.003(\pm 0.0060)X + 0.2699(\pm 0.1076) \quad \text{for } \textit{Oxazepam} \tag{7}$$

$$Y = 0.995(\pm 0.3101)X + 0.2138(\pm 0.0137) \quad \text{for } \textit{Lorazepam} \tag{8}$$

when all three regression plots have exhibited nearly ideal correlation (with r^2 = 0.9999) and where X is the average reference assay and Y is the average found by the proposed electrode / method. Also, the RSDs obtained were highly satisfactory, ranging from ±0.8 to ±2.1 %, and the same can be stated about the recovery rates varying from 97.93 to 99.27 %, with the variation coefficients within an interval of 0.81-1.66. Finally, there is also a fine agreement with the results of the official reference method [27].

The results show that the proposed electrodes can be used to determine *Olanzapine, Oxazepam and Lorazepam* in pure samples or pharmaceutical preparations with both high accuracy and high recovery; all without time-consuming pre-treatment procedures required, e.g., to minimize the undesirable matrix effects. Also, the target drugs were successfully determined in spiked urine

Table VI Quantification of content of *Olanzapine*, *Oxazepam* and *Larazepam* in pharmaceutical preparations using respective ISE with NPOE as plasticizer

Method proposed				Reference method*		
Taken μg ml^{-1}	Recovery %	*R.S.D.* C.V.	*t*-value	Recovery %	±R.S.D %	*F*
Olanzapine, *Zyprexa* tablet, 10 mg						
12.5	98.4	0.20	0.6	99.11	0.56	1.14
		1.62				
18.75	98.66	0.15	0.85	98.32	0.41	0.13
		0.81				
25.00	98.42	0.41	2.34	100.1	0.37	1.22
		1.66				
Oxazepam, *Serenid-D* (Weyth) tablet, 15 mg						
11.46	98.22	0.43	1.10	100.02	0.33	1.69
		0.83				
17.20	98.58	0.47	1.18	97.12	0.40	1.38
		1.27				
22.93	99.27	0.36	0.86	98.65	0.25	2.07
		1.51				
Lorazepam, *Lorazem* tablet, 2 mg						
12.85	97.93	0.26	1.80	99.54	0.22	1.39
		1.20				
19.30	98.44	0.66	1.18	100.21	0.54	1.49
		1.42				
29.70	98.83	0.44	0.76	98.35	0.34	1.67
		1.49				

*Reference method for Olanzapine

samples with the mean recoveries within 97.67-99.31 %, with the RSDs in an interval of 0.16-0.53 %, respectively. The recovery and the RSDs characterising the method tested are summarized in Table VII, surveying also other useful details. These results have shown that all the electrodes can detect the drugs in the spiked urine samples with high accuracy and precision, as well as with s high recovery; again, without any additional pre-treatment procedures.

Table VII Potentiometric determination of *Olanzapine*, *Oxazepam* and *Lorazepam* in urine samples using respective ISE with NPOE as plasticizer

Method proposed				Reference method*		
Taken μg ml^{-1}	Recovery %	±*R.S.D.* %	*t*-value	Recovery %	±R.S.D %	*F*
Olanzapine						
12.50	97.67	0.50	0.27	98.11	0.56	0.79
18.75	97.78	0.41	0.53	98.32	1.03	0.15
25.00	98.23	0.47	2.36	100.21	0.25	3.52
Oxazepam						
11.46	97.68	0.26	1.72	99.42	0.33	0.63
17.20	98.12	0.32	0.22	98.00	0.23	1.93
22.93	99.31	0.49	0.68	98.65	0.36	1.85
Lorazepam						
12.85	98.24	0.16	1.37	99.54	0.22	0.53
19.30	99.12	0.40	1.15	100.21	0.34	1.38
29.70	98.00	0.53	0.41	98.35	0.34	2.42

*Reference method for *Olanzapine*

Potentiometric Titrations: Though the determination by potentiometric titrations may be time consuming, it offers the advantage of high accuracy and precision, when the end-point can easily be determined *via* the sharp potential break. In addition, partially exhausted electrode can also be employed because the actual/exact potential value during titration and at its end-point is of secondary interest [28]. Representative titration curves for the determination of all three drugs using the respective electrodes are shown in Figs 6, 7, and 8. Notable is the fact that as the concentration of the drug had increased, the inflection of the break-point became sharper than those for lower concentrations of the drugs. Then, these

electrodes could be successfully used to indicate properly the potentiometric titrations of the drugs investigated.

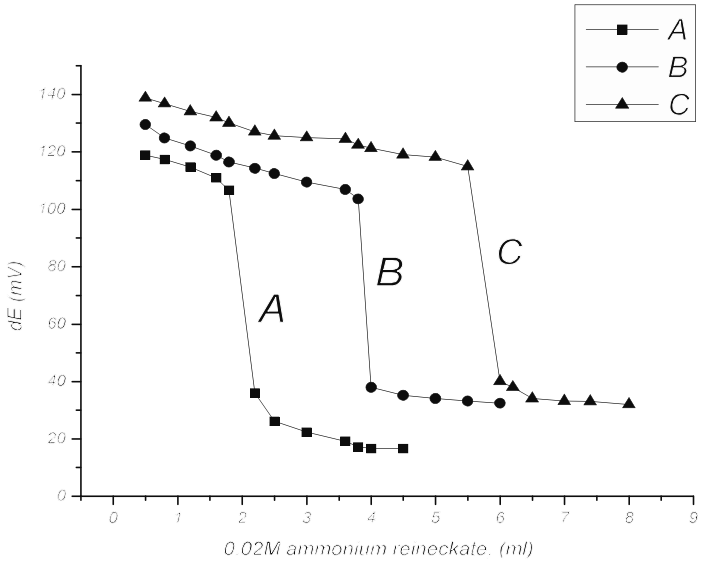


Fig. 6 Typical potentiometric titration curves of (A) 2.0 ml; (B) 4.0 ml and (C) 6 ml 0.01 M *Olanzapine* with standard solution of 0.01 M ammonium reineckate (A-RE) as titrant and using *Olan*-ISE as indicator electrode

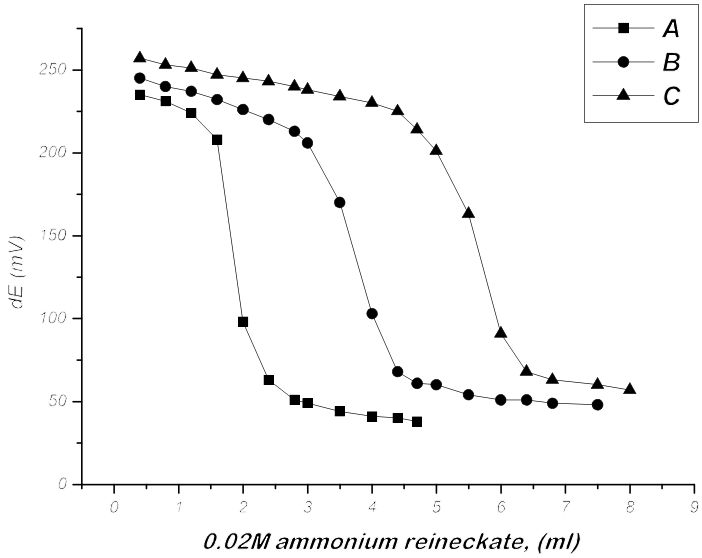


Fig. 7 Typical potentiometric titration curves of (A) 2.0 ml; (B) 4.0 ml and (C) 6 ml 0.01 M *Oxazepam* with standard solution of 0.01 M A-RE using *Oxaz*-ISE

Statistical Evaluation of Both Methods for Quantitative Analysis

The results of conventional *F*-and *t*-tests are shown in Table VIII. The obtained values were substantially lower than theoretical (tabulated) values; i.e., the

methods applied do not exhibit significant differences compared to those obtained by the official method [27].

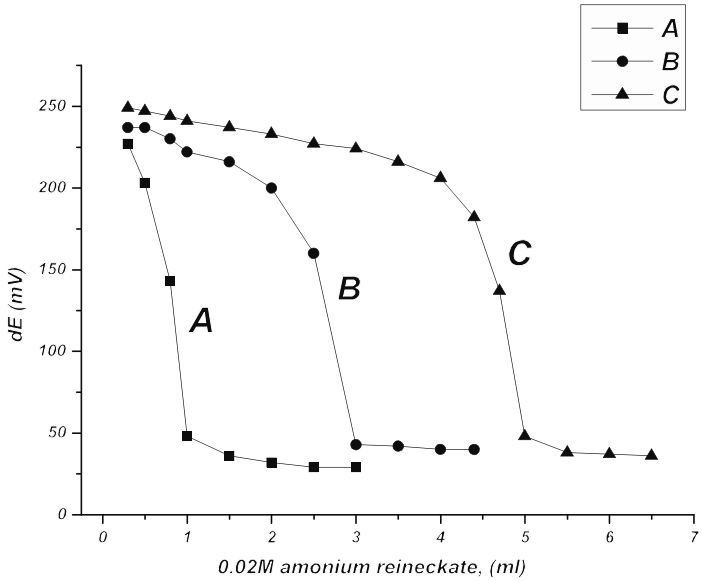


Fig. 8 Typical potentiometric titration curves of (A) 1.0 ml; (B) 3.0 ml and (C) 5 ml 0.01 M *Lorazepam* with standard solution of 0.01 M A-RE using *Lora*-ISE

Table VIII Statistical treatment of data obtained from determination of three drugs at electrodes proposed in comparison with reference method employing HPLC.

Parameters evaluated	Reference method (based on HPLC)	*Olanzapine* electrode	*Oxazepam* electrode	*Lorazepam* electrode
Working range mol l^{-1}	0.5-18 μg ml^{-1} *	10^{-7}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}
	12-40 μg ml^{-1} **			
	20-130 μg ml^{-1} ***			
LOD *** mol l^{-1}	0.12 μg ml^{-1} *	3.2×10^{-7}	4.6×10^{-6}	3.7×10^{-6}
	3.5 μg ml^{-1} **			
	5.0 μg ml^{-1} ***			
Accuracy %	98.89 ± 1.3*	98.40-98.66	98.22-99.27	97.93-98.83
	98.56 ± 1.1**			
	98.41 ± 1.3***			

* Reference method (used) for *Olanzepine* (see Ref. [27])
** Reference method for *Oxazepam* and *Lorazepam* [27]
*** Limit of detection; average of three determinations

To check the accuracy, the new potentiometric method was compared with standardized procudure with the aim to find if there is any significant difference which could reveal the extent into which potential deviation(s) may affect the applicability of the new method(s) compared to the already existing method(s). As found out, the method examined did not exhibit any significant differences, as confirmed by the data in the last Table.

Conclusion

The new ISEs based on the functioning of the BZD/RE ion-associate in the PVC-membrane have been shown to be applicable in potentiometric analysis of three pharmaceuticals of the benzodiazepine family, allowing one to determine as low as 1×10^{-7} M *Olanzapine* and 1×10^{-6} M *Oxazepam* or *Lorazepam*. The electrodes proposed offer the advantages of low cost, ease of fabrication, high stability, fast response over a wide concentration of the analyte(s) being almost independent of pH, as well as adequate selective in the presence of related species.

The whole procedure has also been found sufficiently reliable for quantitative analysis, when combined either with the calibration curve method or with the standard addition(s) procedure. Last but not least, the drugs of interest can be determined either in pure (powdered) form or in the commercial dosage (tablet) forms without any special pre-treatment and the employment of all three ISEs developed seems to be feasible also in analyses of turbid or intensively coloured sample solutions.

It can be concluded that the procedure described in the previous sections is a valuable contribution to the palette of similar methods already developed for analysis of pharmaceuticals with familiar structures, showing also that potentiometric indication may offer a possible alternative to more common voltammetric measurements (see, e.g., Refs [29,30]).

Acknowledgements

The authors would like to thank Prof. Dr. Ivan Švancara for his interest in the article, kind help in its final editing, and — last but not least — for enabling our paper to appear in the Sci. Pap. Univ. Pardubice.

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