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A New Tiapride Selective Electrode and Its Clinical Application

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Abstract: The construction and electrochemical response characteristics of a poly(vinyl chloride) (PVC) membrane selective electrode for the determination of tiapride (TPD) are described. The sensing membrane comprised an ion-pair formed between the protonated drug and tetraphenylborate (TPB \(-\)) in a plasticized PVC matrix. The influence of membrane composition on the electrode response was studied. The electrode showed a fast, stable and Nernstian response over a wide tiapride concentration range (1x10\(^{-5}\)-1x10\(^{-2}\) M) with a slope of 57.5±0.2 mV dec\(^{-1}\) of concentration, a detection limit of 4.3x10\(^{-6}\)±2.5x10\(^{-7}\) M, a wide working pH range (2-8) and a fast response time (< 5 s). The electrode showed good selectivity towards tiapride with respect to some inorganic and organic compounds. The electrode has been applied to the determination of tiapride in human urine and iontophoresis solution.

Keywords: Ion-selective electrode, tiapride determination; urine, iontophoresis solution.

1. Introduction

Substituted benzamides have been the first class of atypical anti-psychotics successfully employed both schizophrenia and depression. They are neuroleptics highly selective for dopamine receptors. Tiapride (N-[2-(diethylamino) ethyl]-5-(methylsulfonil)-o-anisamide, Scheme 1) (TPD), is a substituted benzamide with antipsychotic properties. It acts as antagonist of the dopamine D\(_2\) receptors, a property which distinguishes this compound from other anti-psychotic agents. This particular feature
may explain the very low incidence of side effects on the extrapyramidal system. In addition, tiapride has anti-emetic, anti-dyskinetic and anti-hypertensive actions [1].

A review of the literature revealed that several analytical methods have been described for its determination, including chromatographic [2-3], spectrophotometric [4-5], fluorimetric [6], FICHEMILUMINOMETRIC [7], and polarographic [8] methods.

In recent years, potentiometric membrane ion-selective electrodes (IESs) have been used in pharmaceutical and biological analysis [9-16]. This is mainly due to their simple design, low cost, adequate selectivity, good accuracy, wide concentration range and applicability to coloured and turbid solutions. However, a thorough literature survey has revealed no methods involving selective electrodes for the determination of tiapride.

Therefore, the aim of this work was to develop a polymeric ion-selective electrode for tiapride determination and its application for determining this drug in urine and in an iontophoresis solution. Our scope of work is to develop sensors for point of care clinical analysis in the treatment of mentally ill patients.

2. Results and Discussion

2.1. Influence of membrane composition

Several membranes of the different compositions (Table 1) prepared as described in the Experimental were tested. Two plasticizers, NPOE and DOS, with very different dielectric constants were tested as membrane solvent. The calibration graphs obtained for the corresponding membranes, A and B respectively, are shown in Fig. 1.
Table 1. Composition of the membrane.

| Membrane | Percentage (w/w) of components in membranes                      |
|----------|-------------------------------------------------------------------|
|          | PVC          | NPOE          | DOS | TPD-TPB |
| A        | 33.0         | 66.0          | —   | 1.0     |
| B        | 33.0         | —             | 66.0 | 1.0     |
| C        | 32.3         | 64.7          | —   | 3.0     |

As can be seen the membrane plasticized with NPOE presented better response and a lower detection limit. The detection limit of ISEs with dissolved ion exchangers is controlled by the analyte ion concentration present in the solution as a result of the distribution equilibrium for the ion pair between the membrane and the solution [17]. The solubility of the ion-pair in the organic solvent generally increases as the polarity and dielectric constant increases [18], which explain the lower detection limit obtained with NPOE as plasticizer which was selected for further studies.

Two different TPD-TPB concentrations, 1.0 and 3.0 %, in the membrane were tested, (membranes A and C respectively). The corresponding calibration graphs are shown in Fig.1. As can be seen, the slopes in the linear range are very similar but the detection limit of membrane A (4.3x10^-6 M) is lower than that of membrane B (1.7x10^-5 M), which is due to the lower TPD concentration in the aqueous solution as a result of the distribution equilibrium of the TPD-TPB. Taking all these results into account, membrane A was selected for further studies.
2.2 Influence of pH

The effect of pH on the electrode potential at various tiapride concentration in the range $2.5 \times 10^{-5}$-$1 \times 10^{-2}$ M, was studied. The pH was varied by adding HCl or NaOH and the results are shown in Fig. 2. As can be seen, the electrode potential was independent of pH in the range 2-8 for all the tiapride concentrations assayed, and in this range the electrode can be safely used for tiapride determination. At higher pH values, the potential decreased due to the gradual increase in the concentration of the unprotonated form of the TPD. The pH of the tiapride working solutions prepared as described in 2.2, is between 6-7 and therefore lies within the range of constant response.

![Figure 2: Influence of pH at different tiapride concentrations](image)

2.3 Response characteristics

Ion-selective electrode characterization carried out with a mathematical and computational program has been shown to be very useful for the determination of detection limits and selectivity constants, among others [19]. Non-linear curve fitting using available common software was used for the determination of the ISE characteristics [20]. The slope and the detection limit for tiapride of the selected electrode were determined by fitting calibration data to equation (1):

$$E = E_i^0 + S \log(LOD + C_{TPD})$$

(1)

The calibration parameters were evaluated from repeatedly making calibration graphs between tiapride $1 \times 10^{-6}$ and $1 \times 10^{-2}$ M. The calibration parameters obtained are shown in Table 2. As can be seen, a near-Nernstian response within a three decade concentration range, with low detection limit and good calibration reproducibility, was obtained.
The repeatability and reproducibility of the calibration parameters were studied by making successive calibrations with the same membrane on the same day (n=5) and on different days (n=5). The results obtained for the LOD were 4.3x10\(^{-6}\) ± 2.5x10\(^{-7}\) M and 6.1x10\(^{-6}\) ± 4.2x10\(^{-7}\) M, respectively, and for the slopes, 57.5 ±0.2 and 57.4 ±0.3 mV per decade of concentration, respectively.

The response time was obtained from the dynamic potential response corresponding to tiapride concentration steps to obtain a 10-times more concentrated solution. The values obtained for different tiapride concentrations are included in Table 2, and show a very rapid response even for low concentrations.

| *Slope (mV per dec) ± S.D. | 57.5 ± 0.2 |
|--------------------------|------------|
| Linear range (M)         | 1x10\(^{-3}\) to 1x10\(^{-2}\) |
| *Detection limit (M) ± S.D | 4.3 × 10\(^{-6}\)± 2.5x10\(^{-7}\) |
| Response time (s) 10\(^{-6}\)-10\(^{-2}\) (M) | \(t_{95\%} \leq 5\) |
| Working pH range (10\(^{-6}\)-10\(^{-2}\) M) | 2 – 8 |
| Lifetime (months)        | >3         |

*Mean of five calibrations

The tiapride selective electrode worked for at least 3 months, during which time no appreciable change in the calibration characteristics or response time was observed.

2.4 Selectivity

Selectivity coefficients for different ions with respect to tiapride were determined by the activity ratio method [21], in which the selectivity coefficient is measured as the ratio of ion activities or concentrations that generate the same membrane potential when measured in a separate solution type experiment. A tiapride calibration graph carried out in water was used to calculate the concentration of tiapride (\(C_{TPD}\)) that corresponds to the potential observed for a certain concentration (\(C_j\)) of interfering ion. The selectivity coefficients were calculated as the ratio of these concentrations, \(K_{TPD,j} = C_{TPD} / C_j\), where \(j\) is the interfering ion.

The resulting selectivity coefficients are shown in Table 3. Ca\(^{2+}\), Mg\(^{2+}\), lactose, glucose and sucrose did not produce any change in the potential up to 1x10\(^{-1}\)M with respect to the blank. When the concentration of these species was substantially increased, the potential decreased (i.e. it provided negative interference).
Table 3. Selectivity coefficients.

| Interfering species | Log $K_{TPD,j}$ | [j] / M assayed |
|---------------------|-----------------|-----------------|
| Na$^+$              | -5.16           | 2               |
| K$^+$               | -4.75           | 1               |
| Ca$^{2+}$, Mg$^{2+}$|                 | No interference up to 1x $10^{-1}$ M |
| Lactose, glucose, sucrose |             | No interference up to 1x $10^{-1}$ M |

2.5 Applications

The new tiapride-selective electrode was satisfactorily applied to the determination of tiapride in human urine and in HEPES saline buffer, a medium usually used for iontophoresis samples.

To determine TPD in human urine a previous study of the possible interference from the sample matrix was carried by making calibration graphs applying the procedure described in 3.5 to spiked blank urine sample with different amounts of TPD. The results obtained showed lower slopes and lower recoveries then those obtained in the absence of urine matrix. Therefore, the prior TPD extraction step described in 3.6, was carried out. Known amounts of TPD were added to urine samples and the analyses were performed following the procedure described in 3.6. The results obtained are summarized in Table 4. Good recoveries were obtained.

Table 4. Determination of tiapride hydrochlorhide in different samples.

| Sample          | Tiapride (mg L$^{-1}$)* | Recovery |
|-----------------|-------------------------|----------|
|                 | Added       | Found     | %        |
| Human urine     | 3.42        | 3.50±0.02 | 102.3    |
|                 | 34.2        | 33.8±1.9  | 99.0     |
| HEPES saline    | 0.34        | 0.36±0.03 | 105.9    |
|                 | 2.06        | 2.09±0.10 | 101.4    |
|                 | 5.42        | 5.24±0.25 | 96.6     |
|                 | 22.2        | 21.6±0.7  | 97.7     |
|                 | 54.7        | 54.9±2.7  | 100.4    |

*Mean ± SD (n=3)

Tiapride is rapidly and efficiently absorbed after oral administration and is eliminated principally by hepatic metabolism and subsequent urinary excretion. The normal dose for adults is 300-1200 mg/day. Tiapride undergoes only limited metabolism, nearly 70 % of an orally administered dose is excreted unchanged in urine. The concentration of TPD in urine passed during 24h lies within the range of TPD determination of the potentiometric method proposed.

Iontophoresis is the application of a small electric current to enhance the transport of both charged and neutral compounds across the skin [22]. HEPES buffer (derived from N-(2-hydroxyethyl)-
piperazine-N’-2-ethanesulfonic acid) is a physiological buffer used in clinical analysis as an alternative
to conventional phosphate buffer and HEPES saline buffer is a collector vehicle frequently used in
iontophoresis reverse. To determination of tiapride in this medium, known amounts of TPD were
added to HEPES saline buffer samples at pH 7.5 and the analyses were performed by direct
potentiometry following the procedure described in 3.7. The results obtained are summarized in Table
4. Good recoveries were obtained.

3. Experimental Section

3.1. Apparatus

Potentials were measured with an Orion 960 Autochemistry System, the recorder output of which
was connected to a personal computer via a DGH Corporation 1121 module analogue-to-digital
converter (Manchester, UK). An Orion 90-02 double junction silver-silver chloride reference electrode
containing 0.1 M KCl solution in the outer compartment was used.

3.2. Reagents and solutions

Polyvinyl chloride (PVC) of high molecular mass, 2-nitrophenyl octyl ether (NPOE), bis (2-
ethylhexyl) sebacate (DOS) and tetrahydrofuran (THF) were Selectophore products from Fluka.
Tiapride hydrochloride powder, sodium tetraphenylborate (NaTPB), sodium salt of (N-[2-
hydroxyethyl] piperazine-N’-[2-ethane suphonic acid]) (HEPES) and sodium sulphate were from
Sigma. Al other reagents used were of analytical reagent grade, and nanopure water (18-MΩ) prepared
with a Milli-Q (Millipore) system was used throughout.

3.2.1. Standard tiapride hydrochloride solution 5x10^{-1} M

This was prepared by dissolving 4.5613 g of pure drug (Sigma) in 25.0 ml of water. Working
solutions (1x10^{-2} to 1x10^{-6} M) were prepared by appropriate serial dilutions with 1x10^{-1} M sodium
sulphate.

3.2.2. HEPES- buffered saline solution of  pH 7.4

This was prepared by dissolving 1.63 g of N-2 hydroxyethylpiperazine-N’-2 ethanosulfonate sodium
(HEPES sodium salt) and 1.94 g of NaCl in 250 ml of water. The pH 7.4 was adjusted with diluted
HCl. The resulting solution contained 2.5x10^{-2} M HEPES and 1.3x10^{-1} M NaCl.

3.3 Ion pair preparation

The tiapride tetraphenylborate (TPD-TPB) ion pair was prepared by reacting 10 ml of 1x10^{-2} M
tiapride hydrochloride solution with an equal volume of 1 x 10^{-2} M sodium tetraphenylborate solution.
The mixture was filtered through a porous sintered glass crucible. The residue was washed first with
distilled water until no chloride ion was detected in the washing solution, then with ether and finally
dried at room temperature.
3.4 Construction and conditioning of the electrode

The membranes were prepared by dissolving 3.1 or 9.3 mg of TPD-TPB ion pair, 100 mg PVC and 200 mg of the plasticizer (NPOE or DOS) in 3 ml of tetrahydrofuran. This solution was poured into a Fluka glass ring (inner diameter 28 mm, height 30 mm) on a Fluka glass plate. The solution was allowed to evaporate overnight. A 7 mm diameter piece was cut out with a Fluka punch for ion-selective membranes and incorporated into a Fluka electrode body ISE containing $1 \times 10^{-2}$ M sodium chloride and $1 \times 10^{-3}$ M tiapride and saturated with excess AgCl as internal filling solution. The compositions of the different membranes assayed are shown in Table 1.

The electrodes were conditioned by soaking with constant stirring in a solution containing $1 \times 10^{-3}$ M tiapride in $1 \times 10^{-1}$ M Na$_2$SO$_4$ until the electrode gave a constant potential. When not in use, the electrode was kept immersed in the same dissolution.

3.5 General procedure (calibration of the electrode)

Standard tiapride solutions of $1 \times 10^{-6}$-$1 \times 10^{-2}$ M in $1 \times 10^{1}$ M Na$_2$SO$_4$ were prepared. The tiapride-selective and reference electrodes were immersed and the potential of each sample solution was directly measured. The measured potentials were plotted versus logarithmic values of concentrations and the calibration parameters were calculated by fitting calibration data to the equation shown in 3.3. For the dynamic response studies, the electrode was calibrated by injecting, while stirring, adequate small volumes of tiapride standard solution in 50 ml of $1 \times 10^{-1}$ M Na$_2$SO$_4$ to obtain final concentrations in the range $1 \times 10^{-6}$-$1 \times 10^{-2}$ M.

3.6 Procedure for the determination of tiapride in human urine

In the absence of human urine samples containing tiapride, known amounts of tiapride hydrochloride were added to 50 ml of blank urine samples and the pH was adjusted to about 11 with 1.0 M NaOH before adding 10 ml of dichloromethane. The solution was shaken and the aqueous phase was discharged. The organic phase was shaken with 40 ml of $1 \times 10^{-2}$ M HCl solution and the aqueous phase was separated for further use. The pH of this phase was adjusted to about 7 with 1.0 M NaOH and diluted to 50 ml with water in a calibration flask. The tiapride-selective and reference electrodes were immersed and the tiapride concentration was determined by the standard addition technique.

3.7 Procedure for the determination of tiapride in iontophoresis solution

Frequently, iontophoresis experiments are carried out using a buffer HEPES saline solution. In the absence of human iontophoresis samples containing tiapride, known amounts of tiapride hydrochloride were added to 50 ml of buffer HEPES saline solution of pH 7.5 to obtain final concentration in the range $1 \times 10^{-6}$-$1.5 \times 10^{-4}$ M tiapride. The tiapride-selective and reference electrodes were immersed and the tiapride concentration was determined by direct potentiometry using a calibration graph prepared in the same medium as the sample.
4. Conclusions

The new ion selective electrode developed, based on cation transfer across a plasticized poly (vinyl chloride) (PVC) membrane that contained the ion-pair formed between the protonated tiapride and tetrphenyl borate provides a rapid, sensitive, precise and inexpensive method for direct potentiometric determination of tiapride. The proposed method allows the determination of tiapride, in human urine samples, within the physiological concentration range obtained after the usual therapeutic dose of tiapride has been administered, and in a collector vehicle frequently used in the iontophoresis reverse.

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