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# Development of Membrane Electrode for the Selective determination of Bromazepam in Tablets and Plasma

**Nesma A. Ali,[1,*] Maha M. Abdelrahman,[2] Ibrahim A. Naguib[2,3] and Mohamed R. El Ghobashy[4]**

[1]*Department of Chemical Laboratories, Forensic Medicine Authority, Justice Ministry,114 Bairam El Tounsy St., El SayedaZeinab, Cairo, Egypt*

[2]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Alshaheed Shehata Ahmad Hegazy St., 62514, Beni-Suef, Egypt*

[3]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Tabuk, 71491, Tabuk, Kingdom of Saudi Arabia*

[4]*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., 11562, Cairo, Egypt*

* Corresponding Author, Tel.:+20 01145601737/ 01023063670; Fax:+20 082 2323209

E-Mail: n.aboyazeed@yahoo.com

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**Abstract**- Polyvinyl chloride (PVC) membrane sensor is described and characterized for the determination of a Benzodiazepine drug; bromazepam (BMZ). The sensor based on the use of the ion association complex of BMZ cation with tetraphenyl borate (BMZ-TPB) counter anion as ion exchange sites in the PVC matrix plasticized with dibutylsebathete (DBS) as a solvent mediator. The performance characteristics of thissensor were evaluated according to IUPAC recommendations; achieve a fast, stable and linear response for BMZ over the concentration range $10^{-6}$ to $10^{-2}$ M with slope of 44.13 mV per concentration decade. The direct potentiometric determination of BMZ using the proposed sensor gave average recovery of 100.05±0.66. The sensor is used for determination of BMZ in pharmaceutical formulations and in plasma. Validation of the method shows suitability of the proposed sensor for use in the quality control assessment of BMZ. The developed method was proved to be simple, accurate and precise when statistically compared with a reference HPLC method.

**Keywords**- Bromazepam, Ion selective electrode, PVC membrane, Tetraphenyl borate

## 1. INTRODUCTION

Bromazepam (BMZ) (7-bromo-5-(pyridyl-2-yl)-1,3-dihydro- 2*H*-1,4-benzodiazepin-2-one)[1] Fig. 1, is one of the 1,4-benzodiazepine series with general properties similar to diazepam. It has been used in the short-term treatment of anxiety disorders occurring alone or associated with insomnia [2]. The benzodiazepines inhibitory action on the central nervous system results from its activation of gamma amino butyric acid (GABA) receptors in the brain [3].

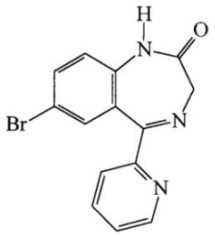
Bromazepam is one of the most commonly prescribed classes of drug for the treatment of anxiety and insomnia so its analysis as one of the most important benzodiazepine derivatives is of high interest [4,5]. Bromazepam is assayed in the British pharmacopeia (BP) by non-aqueous titration [6].Several analytical methods have been revealed in literature for the determination of BMZ either individually or in combination with other benzodiazepines in pharmaceutical or biological fluids including spectrophotometry[7-9], LC-MS[10,11], HPLC [12-23], GC-MS[24], TLC[25] and voltammetric methods [26,27]. Some publications were interested by studying stability of BMZ especially hydrolysis [17,28,29].

Potentiometric determination of BMZ using solid contact ion-selective electrodes was also proposed [30]. Potentiometric determination of BMZ and/or other 1, 4-benzodiazepines using ion association complexes (Drug-tetraphenylborate and/or drug-phosphotungstate) as an electroactive material in poly (vinyl chloride) membrane were also proposed [31, 32].

Being commercially and not expensive, ion selective electrodes have become an item of general equipment of analytical work. This result happens because ion selective electrodes have rapid, simple and low cost and give accurate measurements of ionic species with fast response, wide pH working range, reasonable selectivity, broad concentration range and applicability to turbid and colored solutions [33].

The aim to constructing such an electrode is to produce a sensitive and selective membrane that responds to a specific drug. Such a membrane is usually prepared by incorporating an appropriate ion-exchanger and solvent mediator into a poly(vinyl chloride) (PVC) membrane matrix.

In the present work, development of ion selective membrane sensorfor bromazepam was establishedbased on the use of ion association complexes of BMZ with tetraphenyl borate. The high lipophilicity and remarkable stability of this complex suggested itsselective use as electroactive materials in PVC matrix membrane sensor for the determination of BMZ in the presence of excipients and plasma without the need of preliminary extraction and separation steps. Moreover, it offers sensitive, selective and convenient technique for the determination of BMZ in its pure and as a quality control analysis of BMZ tablets.




**Bromazepam (BMZ)**

$C_{14}H_{10}BrN_3O$

Mol. Wt. 316.2

**Fig. 1.** Chemical structure of bromazepam

## 2. EXPERIMENTAL

### 2.1. Apparatus

Potentiometeric measurements were made at 25±1 ˚C with aHanna (Model 211) pH/mV meter. A single junction calomel reference electrode (Model HI 5412) was used in conjunction with the drug sensor. A WPA pH combined glass electrode ModelCD 740 was used for pH measurements.

### 2.2. Reagents and solvents

All chemicals were of analytical grade and bidistilled water was used. Tetrahydrofuran (THF) 99% (Lab Scan), high molecularweight (10,000) polyvinyl chloride (PVC) powder (Aldrich) and tetraphenyl borate (TPB) were obtained from Aldrich. Dibutylsebathete was obtained from Sigma. Phosphatebuffer pH 3,it was prepared by dissolving 34 g of potassium dihydrogen orthophosphate in 250 ml of water and adjust pH with orthophosphoric acid[6].

### 2.3. Materials

*2.3.1. Pure samples*

Bromazepam was kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO), Cairo, Egypt. The purity was found to be 99.72% according to the company analysis certificate.

*2.3.2. Market samples*

Lexotanil® tablets manufactured by: La Roche S.p.A. Milan, Italy. Batch No. (M1139B01) labeled to containing 3 mg bromazepam/tablet were purchased from a local market.

Calmepam® tablets manufactured by Glaxo SmithKline S.A.E., Cairo, Egypt,batch No. (A506716) labeled to containing 3 mg bromazepam/tablet were purchased from a local market

### 2.4. Prepared solutions

A stock solution of $10^{-2}$ M Bromazepam was freshly prepared by dissolving 0.158 g of Bromazepam in 1.5 ml of 0.01 N HCl then complete the volume of 50 ml with either water or phosphate buffer pH 3.

Bromazepam working solutions ($10^{-7}$–$10^{-3}$ M) were prepared by suitable dilution from its stock solution using either water or phosphate buffer pH 3.

### 2.5. Procedures

#### *2.5.1. Preparation of bromazepam-tetraphenyl borate membrane (BMZ-TPB)*

Ten milliliter of $10^{-2}$ M bromazepam aqueous solution was mixed with 10 ml of a saturated aqueous solution of tetraphenyl borate (TPB). The resulting precipitate was filtered, washed with cold water, allowed to dry at room temperatureand grounded to fine powder.
In a glass petri dish (5 cm diameter), 10 mg of the previously prepared ion association complex was mixed thoroughly with 0.35 ml of dibutylsebathetethen 0.19 g of poly vinyl chloridewas added (PVC). This mixture was dissolved in 5ml tetrahydrofuran (THF), covered with a filter paper and left to stand overnight to allow slow evaporation of the solvent at room temperature, thus a master membrane with 0.1mm thickness was formed [34].

#### *2.5.2. Electrode assembly*

A disk of an appropriate diameter (about 8 mm) was cut from the BMZ-TPB previously prepared master membrane and cemented to the flat end of PVC tubing with THF. A mixed solution consisting of equal volumes of $10^{-2}$ M bromazepam and$10^{-2}$ M sodium chloride was used as an internal reference solution. Ag/AgCl coated wire (3mm diameter) was employed as an internal reference electrode. The sensor was conditioned by soaking for 24 h in a solution of $10^{-2}$ M of drug and stored in the same solution when not in use.

#### *2.5.3. Effect of pH on the electrode response*

The effect of pH on the potential values of the electrode system was studied over pH range of 1–12 at 1-pH interval by immersing electrodes in $10^{-3}$ and $10^{-4}$ M BMZ solutions. The pH was gradually increased or decreased by adding aliquots of diluted sodium hydroxide or hydrochloric acid solutions, respectively.The potential obtained at each pH was recorded.

*2.5.4. Sensor calibration*

The prepared electrode was immersed in conjunction with the single junction calomel reference electrode in aqueous solutions of bromazepam in the range of $10^{-7}$to $10^{-2}$ M starting from low to high concentrations.It was allowed to equilibrate whilst stirring and recording the emf readings within ±1 mV. The membranesensor was washed between measurements with water. ThemV–concentration profiles were plotted. The regression equationsfor the linear part of the curves were computed and used for subsequent determination of unknown concentrations of bromazepam.

*2.5.5. Selectivity measurements*

Potontiometry selectivity coefficients $K_{BMZ,B}^{pot}$ were evaluated according to IUPAC guidelines using the separate solutions method [35] in which the potential of cell compromising the membrane electrode and a reference electrode is measured with two separate solutions, A and B where A (BMZ ions) and B (interfering ion) at the same activity aA=aB. The emf for A and B are measured values, respectively. Different interfering anions at a concentration of $1\times10^{-3}$ M at a suitable pH were utilized and the results were obtained using the equation

$$\log k_{A,B}^{pot} = \frac{EB - EA}{S} + \frac{1 - ZA}{ZB} logaA$$

Where $K_{A,B}^{pot}$ is the potentiometric selectivity coefficient, *S* the slope of the calibration plot, aA the activity of BMZ and ZA and ZB are the charges on BMZ and the interfering ions, respectively.

*2.5.6. Application to pharmaceutical formulations*

Ten tablets of each Calmepam and Lexotanil tablets were weighed and powdered separately. An amount of the powdered tablets equivalent to 15.8 mg of Bromazepam was accurately transferred separately to a 50 ml volumetric flask dissolved in 1.5 ml of 0.01 N HCL then the volume was completed to the mark with phosphate buffer pH 3to prepare a $10^{-3}$ and $10^{-4}$ M solutions of bromazepam. The emf produced by immersing the prepared electrode in conjunction withsingle junction calomel reference electrode in the prepared solutionswas determined then the concentration of bromazepam was calculated from the regression equation of the corresponding electrode.

*2.5.6. Application to plasma sample*

4.5 ml of human plasma were placed into two Stoppard shaking tubes then 0.5 ml of $10^{-2}$ and $10^{-3}$ M bromazepam were added separately and shacked. The membrane sensor was

immersed in conjunction with the single junction calomel reference electrode in these solutions. Wash the membrane sensor with water between measurements. The emf produced for each solution was measured by the proposed electrode then the concentration of bromazepam was determined from the corresponding regression equations.

## 3. RESULTS AND DISCUSSION

The advantages of ion-selective electrodes (ISEs) of simple design and operation, fast response, reasonable selectivity, low detection limit, high accuracy, wide concentration range, applicability to colored and turbid solutions, and possible interfacing with automated and computerized systems make the development and application of it is continue to be of interest for pharmaceutical analysis. Recently ion selective electrodes were used to solve some analytical problems such as direct determination of drugs in presence of their degradation products or related substances [36-38].

In the present work, membrane belonging to the type of supported ion exchangers was fabricated with PVC as a polymer matrix. BMZ reacted with tetraphenyl borate as a cation to form stable 1:1 water insoluble ion association complex BMZ-TPB, with low solubility and suitable grain size precipitate. This ratio was confirmed by the Nernstian response of the suggested sensor which was about 60 mV; the typical value for monovalent drugs[35]. Deviations from the ideal Nernstian slope (60 mv) stems from the fact that the electrode responds to the activity of the drug rather than its concentration.

**Table 1.** Response characteristics of bromazepam–tetraphenyl borate (BMZ-TPB) electrode

| Parameter | BMZ-TPB sensor |
|---|---|
| Slope(mV/decade) | 44.13 |
| Intercept(mV) | 168.78 |
| Correlation coefficient (r) | 0.9999 |
| Detection limit [M] | $3.1\times10^{-7}$ |
| Response time (s) | 20–30 |
| Life span (weeks) | 4–6 |
| Working pH range | 2–4 |
| Concentration range [M] | $10^{-6}$ to $10^{-2}$ |
| Average recovery (%) | 100.05 |
| R.S.D.%[a] | 0.66 |

[a] Average of five determinations

PVC acts as standard support matrix and as traps for the sensed ions. It has the advantages of chemical inertness, high tensile strength and low cost, but its use makes a need for a plasticizer [39]. In the present work, dibutylsebathete was as a plasticizer from diesters of carboxylic acids. It plasticize the membrane with PVC, dissolve the ion association complex, and adjust both permittivity of the final organic membrane and mobility of the ion exchange sites. Such adjustments influence the partition coefficient of the studied drug with subsequent effect on electrode selectivity. The membrane constituents were dissolved in THF that was slowly evaporated at room temperature leading to the membrane formation.

Electrochemical performance characteristics of the proposed sensor were evaluated according to the IUPAC recommendation data [35], Table 1. The response time of the electrodes were tested for concentrations of BMZ from $10^{-6}$ to $10^{-2}$ M. The electrode displayed constant potential readings within 2 mV from day-to-day and the calibration slopes did not change by more than 2 mV per decade over a period of 1 month for the sensor. The measurements was characterized by a fast stable response within 20–30 s for concentrations less than $10^{-4}$ M and 10–20 s for concentrations more than $10^{-4}$ M.

The effect of pH on the electrode potential was investigated and it was found that the electrodes gave a stable potential over a pH range from 2 to 4, Fig. 2. Above and below this pH range, the potential displayed by the electrode was noisy. The potentiometeric response of the studied electrode at the optimum pH was linear with constant slope over a drug concentration range of $10^{-6}$ to $10^{-2}$ M; Fig. 3. The accuracy of the proposed membrane sensor for the quantification of blind samples of BMZ was assessed with average recovery of 100.05±0.662.

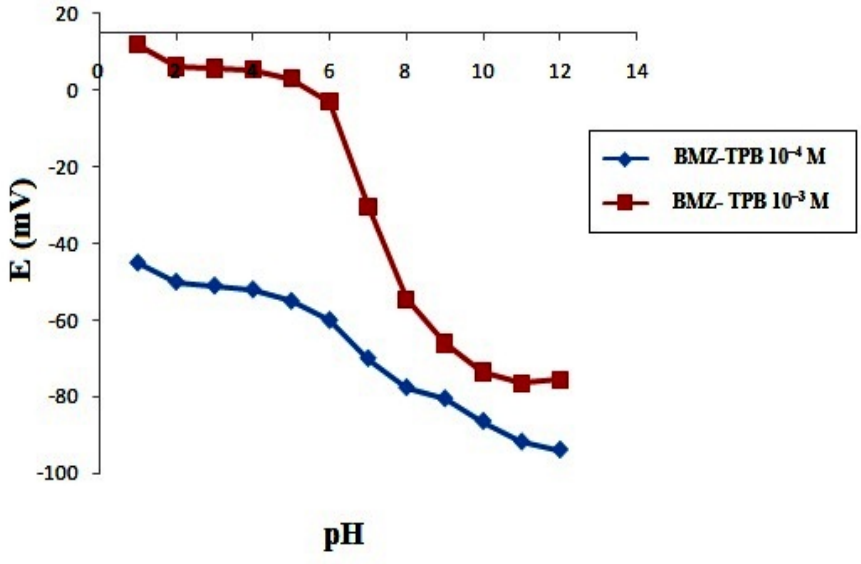



**Fig. 2.** Effect of pH on the response of bromazepamtetraphenyl borate electrode

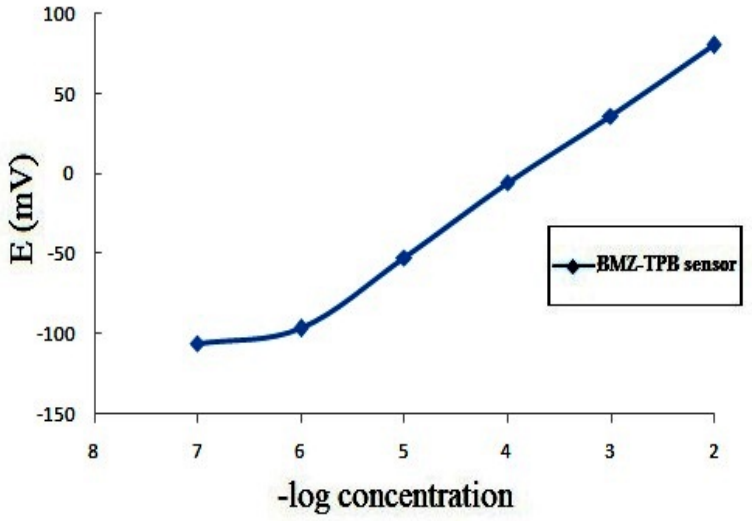



**Fig. 3.** Profile of the potential in mV to the −log concentration of bromazepam tetraphenyl borate electrode

The performance of the sensor in the presence of Pharmaceutical additives, excipients, organic and inorganic related substances was assessed by measuring and comparing the potentiometric selectivity coefficient values ($K_{BMZ,B}^{pot}$). The solutions method [35] with a separate and fixed interferent concentration ($10^{-3}$) was used for the selectivity evaluation. The potentiometric selectivity coefficients of the proposed sensor, Table 2, showed high selectivity for the sensor for bromazepam in presence of tablet excipients, diluents, organic and inorganic related substances and ingredients commonly used in drug formulations; the results revealed that there is no significant interference was observed from interfering species.

**Table 2.** Potentiometric selectivity coefficients$K_{BMZ,B}^{pot}$for the proposed electrode

| Interfering substance | Selectivity coefficient |
|---|---|
| $Na^+$ | $38.3\times10^{-3}$ |
| $K^+$ | $34.3\times10^{-3}$ |
| $NH_4^+$ | $37.1\times10^{-3}$ |
| $Ca^{2+}$ | $46.7\times10^{-3}$ |
| glycine | $34.8\times10^{-3}$ |
| sucrose | $39.0\times10^{-3}$ |
| Citric acid | $0.9\times10^{-3}$ |

**Table 3.** Determination of bromazepam in Lexotanil® and Calmepam® tablets by the proposed electrode

| Pharmaceutical formulation | Concentration [M] | Recovery%[a] |
|---|---|---|
| Lexotanil® tablets (batch no.M1139B01) | $1\times10^{-3}$ | 99.43±1.16 |
| | $1\times10^{-4}$ | 100.44±1.02 |
| Calmepam® tablets (batch no.A506716) | $1\times10^{-3}$ | 99.50±0.87 |
| | $1\times10^{-4}$ | 101.64±0.71 |

[a] Average of three determinations

**Table 4.** Determination of bromazepam in spiked human plasma by the proposed electrode

| Concentration [M] | Recovery %[a] |
|---|---|
| $1\times10^{-3}$ | 98.96±1.91 |
| $1\times10^{-4}$ | 97.29±1.85 |

[a] Average of three determinations

**Table 5.** Statistical analysis of the results obtained by the proposed method and the reference HPLC method [14] for the analysis of bromazepam

| Parameters | BMZ-TPB sensor | Reference HPLC method [14] |
|---|---|---|
| Mean | 100.05 | 100.17 |
| R.S.D. | 0.66 | 0.93 |
| n | 5 | 6 |
| Variance | 0.436 | 0.865 |
| *t*-Test (2.262)[a] | 0.239 | – |
| *F*-Test (6.256)[a] | 1.984 | – |

* The values between parentheses are corresponding the theoretical values at the 95% confidence level

So, analysis of pharmaceutical formulations was carried out without prior treatment or filtration. BMZ-TPB sensor was successfully used for the determination of bromazepam in lexotanil® and calmepam® tablets, Table 3.Moreover the electrode gave stable results of its application to the biological fluids; it is revealed by high precision and accuracy of recoveries of the spiked plasma samples, Table 4. Statistical evaluation of the results of analysis of pure BMZ by the proposed electrodes and the reference HPLC method [14] showed that there is no significant difference between the proposed and reference method in terms of accuracy and precision, Table 5.

## 4. CONCLUSION

The presented BMZ-TPB electrode could provide simple and selective quantitative method for determination of bromazepam at a wide concentration range ($10^{-6}$ to $10^{-2}$ M) in pure, pharmaceutical formulations and in plasma. The use of the proposed sensor offers the advantages of fast response, low detection limit, elimination of drug pretreatment and separation steps, and direct determination of drugs in turbid or colored solutions. They can therefore be used for routine analysis of BMZ in quality control laboratories.

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