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Novel-Selective Bromazepam Membrane Sensors: Application Pharmaceutical Preparations and Biological Fluids

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The construction and performance characteristics of bromazepam selective electrodes were developed. Two types of electrodes: plastic membrane I and coated wire II were constructed based on the incorporation of bromazepam with phosphomolybdic acid. The influence of membrane composition, kind of plasticizer, pH of the test solution, soaking time and foreign ions on the electrodes was investigated. The electrodes showed a Nernstian response with a mean calibration graph slope of 53.22 ± 0.7 and 51.63 ± 0.4 mV decade^{-1} at 25 ºC for electrode I and II, respectively, over bromazepam concentration range from 5.0×10^{-6} to 1.0×10^{-3} and 1.0×10^{-6} to 1.0×10^{-3} mol L^{-1}, with detection limit 2.5×10^{-6} mol L^{-1} and 5.01×10^{-7} mol L^{-1} for electrode I and II, respectively. The constructed electrodes gave average selective precision and accuracy within the pH range 3-8. Interferences from common cations, alkaloids, sugars, amino acids and drug excipients were reported. The results obtained by the proposed electrodes were also applied successfully to the determination of the drug in pharmaceutical preparations.

Key Words: Coated wire electrode, Ion-selective electrode, Bromazepam, Pharmaceutical formulations.

INTRODUCTION

Bromazepam (Fig. 1) is an antianxiety treatment and a sedative. Bromazepam is in the group of drugs known as benzodiazepines, a class of antidepressants, antipanic agents and muscle relaxants. Bromazepam is usually used as a short term treatment for major anxiety, but is not recommended for use to relieve everyday stress or anxiety. This medication may also be used to relieve temporary insomnia, but if used daily, it will become in effective in few weeks[1]. Several methods have been used for the determination of bromazepam. High performance liquid chromatographic methods have been reported for the determination of bromazepam in urine and human plasma[2-8]. Liquid chromatography coupled with tandem mass spectrometry methods have been developed and validated[9-12]. Gas liquid chromatographic methods have been described for the determination of bromazepam in human plasma[13-15]. Sensitive end fast determination of bromazepam in human tissues and blood samples has been reported[16,17]. Other methods have been developed for determination of bromazepam including capillary electrophoresis[18,19], spectrophotometry[20,21], atomic absorption spectrometry[22,23], potentiometry[24,25], voltammetry[26,27] and polarography[28]. This work describes new selective membrane electrodes of two types, plastic membrane I and coated wire electrode II for the determination of bromazepam in pure form and in pharmaceutical preparations.

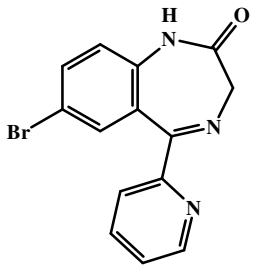

Fig. 1. Chemical structure of bromazepam

EXPERIMENTAL

All chemicals used were of analytical grade, pure grade bromazepam was kindly supplied from Sigma Aldrich Company, USA. The pharmaceutical preparation (calmepame® 3 mg/tablet and lexotanil® 3 mg/tablet) were purchased from local drug stores. Methanol 99 %, acetone 99.9 %, dibutylsebacate (DBS) 99 %, dibutylphthalate (DBP) 99 %, dioctyl-

phthalate (DOP) 99 % and tetrahydrofuran (THF) 97 % were provided by Fluka, Switzerland. Poly(vinyl chloride) (PVC) high molecular weight and phosphomolybdic acid 98 % were purchased from Sigma Aldrich, USA. Stock bromazepam solution (1.0×10^{-1} mol L^{-1}) was prepared daily by dissolving 0.7905 g of the drug in 25 mL acetonitrile. More dilute solutions were prepared by appropriate dilution using double distilled water.

Procedures

Preparation of bromazepam-phosphomolybdate ion-pair: The ion-pair was prepared by mixing 50 mL of 1.0×10^{-2} mol L^{-1} phosphomolybdic acid with 150 mL an equimolar solution of bromazepam, stirred for 10 min. The resulting yellowish precipitate was filtered, washed and dried at room temperature for 24 h. The ion-pair should be stored in a desiccator.

Membrane composition: The membrane composition was studied by varying the percentages (w/w) of the ion pair, poly(vinyl chloride) (PVC) and plasticizer dioctylphthalate (DOP), until the optimum composition that exhibits the best performance characteristics was obtained. The membranes were prepared by dissolving the required amount of the ion-pair, PVC and DOP, in 5 mL of tetrahydrofuran (THF). The solution mixture was poured into a petri dish (3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature. To obtain the uniform membrane thickness, the amount of THF was kept constant and its evaporation was fixed for 24 h.

Electrode construction

Plastic membrane electrode: A punched circular membrane was attached to a polyethylene tube (8 mm in diameter) in an electrode configuration by means of PVC-THF solution. A mixture containing equal volumes of 1.0×10^{-3} mol L^{-1} bromazepam and potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The constructed electrode was pre-conditioned after preparation by soaking for 24 h in 1.0×10^{-3} mol L^{-1} bromazepam and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Ag/AgCl/internal solution /membrane/test solution/ /KCl salt bridge//SCE.

Coated wire electrode: Pure aluminum wire of 4 cm length was tightly insulated by polyethylene tube leaving 1 cm at one end for the coating and 0.5 cm at the other end for connection. The coating solution was described previously under membrane composition. Prior to coating, the polished aluminum surface was washed with a detergent, thoroughly rinsed with water and dried with acetone. Then the wire was rinsed with chloroform and allowed to dry. Afterwards, the aluminum wire was coated by quickly dipping it into the coating solution several times and allowing the film left on the wire to dry for *ca.* 3 min. The process was repeated several times until a plastic membrane of *ca.* 1.0 mm thickness was formed. The prepared electrode was conditioned by soaking for 6 h in 1.0×10^{-3} mol L^{-1} bromazepam solution. All potentiometric measurements were performed using the following cell assembly: Al/membrane/test solution//KCl salt bridge// SCE.

Determination of bromazepam in dosage forms

Bromazepam® tablets: Ten calmepam® 3 mg/tablets or loxetanil 3 mg/tablets were finely powdered and mixed, appropriate weights of each fine powder were shaken with 10 mL of acetonitrile to obtain solution labeled to 1.0×10^{-2} mol L^{-1} drug. The later solution was diluted to obtain solutions of 5.0×10^{-6} to 9.0×10^{-4} mol L^{-1} for plastic membrane and coated wire electrode, respectively. The bromazepam-electrodes were immersed in the solutions and the emf was recorded and drug concentrations were calculated from both calibration graph and standard addition.

Content uniformity assay of calmepam® 3 mg tablet: Ten individual tablets of calmepam® 3 mg/tablet were placed separately in 100 mL of volumetric flask and dissolved in 10 mL of acetonitrile followed by 90 mL of distilled water, The electrode(s) was directly immersed into 10 mL of each sample solution for five times and should be washed with deionized water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

Application to serum and urine: Bromazepam has been determined in human urine and serum using the investigated electrodes using concentration range of 1.0×10^{-5} to 1.0×10^{-3} mol L^{-1}. The determination was carried out without further separation or precondition.

RESULTS AND DISCUSSION

Optimization of membrane composition: In this study three membrane compositions (a), (b) and (c) were investigated and the results were summarized in Table-1. The results showed that the electrode(s) made by membrane of type (c) with 9.5 w % bromazepam-phosphomolybdate ion pair, 42 w % PVC and 48.5 w % plasticizer DOP exhibited the best performance characteristics (53.22 ± 0.7 and 51.63 ± 0.4 mV $decade^{-1}$ at 25 ºC) over bromazepam concentration range from 5.0×10^{-6} to 1.0×10^{-3} and $1.0 \times 1\ 0^{-6}$ to 1.0×10^{-3} mol L^{-1} for plastic membrane and coated wire electrodes, respectively.

TABLE-1
OPTIMIZATION OF MEMBRANE COMPOSITION (wt/wt %)

Type of sensor	m	PVC (wt %)	DOP (wt %)	Ion-pair (wt %)	Slope	RSD (%)	r	Linear conc. range
Plastic membrane electrode	(a)	42.0	55.0	3.0	50.48	1.4	0.9992	$1.0 \times 10^{-3} - 5.0 \times 10^{-5}$
	(b)	45.0	45.0	10.0	49.98	1.1	0.9987	$1.0 \times 10^{-3} - 9.0 \times 10^{-5}$
	(c)	48.5	42.0	9.5	53.22	0.7	0.9999	$1.0 \times 10^{-3} - 5.0 \times 10^{-6}$
Coated wire electrode	(a)	42.0	55.0	3.0	48.26	0.9	0.9994	$1.0 \times 10^{-3} - 1.0 \times 10^{-5}$
	(b)	45.0	45.0	10.0	50.49	0.6	0.9993	$1.0 \times 10^{-3} - 1.0 \times 10^{-5}$
	(c)	48.5	42.0	9.5	51.63	0.4	0.9998	$1.0 \times 10^{-3} - 1.0 \times 10^{-6}$

Nature and response characteristics of the electrodes: Bromazepam reacts with phosphomolybdic acid to form a stable bromazepam-phosphomolybdate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as active material with DOP as a solvent mediator in a poly(vinyl chloride) membrane response for bromazepam. The critical response characteristics of plastic membrane and coated wire electrodes were determined and results are summarized in Table-2. The electrode(s) exhibited a Nernstain response over the concentration range from 5.0×10^{-6} to 1.0×10^{-3} and 1.0×10^{-6} to 1.0×10^{-3} mol L^{-1} bromazepam for electrode I and II, respectively with a slope 53.22 ± 0.7 and 51.63 ± 0.4 mV decade^{-1} at 25 ºC change in concentration for electrode I and II, respectively (Fig. 2). The choice of THF as membrane solvent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and ability to dissolve ion-pair complex.

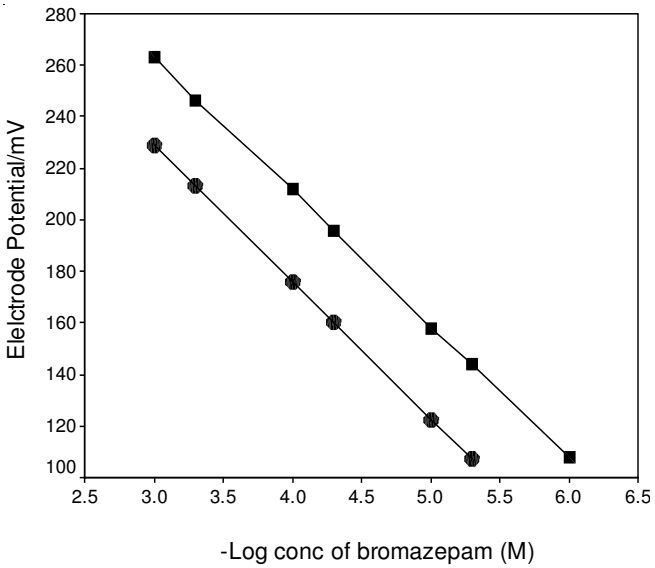

Fig. 2. Typical calibration graph of bromazepam sensors: (●) plastic membrane electrode, (■) coated wire electrode

Life time: The response time of the electrode(s) was tested for 1.0×10^{-6} to 1.0×10^{-1} mol L^{-1} bromazepam solutions. The electrode(s) exhibited a fast and dynamic response of 25 and 20 s, for a period of 20 and 28 days for electrode I and II, respectively.

Effect of plasticizer: In this study, three plasticizers, dibutylsebacate (DBS), dioctylphthalate (DOP) and dibutylphthalate (DBP) were used to examine the optimization of the membrane with plasticizer entailed the use of plasticizer content ratio, 55.0, 42.0 and 45.0 w %, the use of PVC contents of 42.0, 45.0 and 48.5 w % and the electroactive compound (bromazepam-phosphomolybdate) contents of 3, 10 and 9.5 w %. The results obtained showed that the response performances of the prepared membranes were rather different depending on the use of plasticizer, the proportion of the plasticizer toward PVC and of the electroactive compound. The typical potential responses of the electrodes constructed with three plasticizers were given in Fig. 3. As shown in Fig. 3, the DOP-PVC electrodes were superior to DBS-PVC and DBP-PVC electrodes in both the response slope and linear concentration range. So DOP was selected as the plasticizer of the membranes.

Effect of soaking: The optimum soaking time in 1.0×10^{-3} mol L^{-1} drug solution was found to be 6 h and 4 h at which the slope of the calibration curve was 53.22 ± 0.7 and 51.63 ± 0.4 mV decade^{-1} at 25 ºC for plastic and coated wire, respectively. The calibration plot slopes decreased upon prolonged soaking (Figs. 4 and 5).

Regeneration of the electrode: The regeneration of the bromazepam-phosphomolybdate membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in solution that was 1.0×10^{-2} mol L^{-1} phosphomolybdic acid, followed by 3 h in 1.0×10^{-2} mol L^{-1} bromazepam solution (Figs. 6 and 7). It was found that the lifespan of the regenerated electrode(s) is limited to 2 h.

Effect of pH: The effect of pH of the bromazepam solution using 1×10^{-3} mol L^{-1} bromazepam on the electrode(s) potential was investigated. The solution was acidified by the addition of small volumes of 0.1 mol L^{-1} hydrochloric acid then the pH value was increased gradually using 0.1 mol

TABLE-2
CRITICAL RESPONSE CHARACTERISTICS OF BROMAZEPAM-PHOSPHOMOLYBDATE SENSORS

Parameter[a]	Bromazepam-phosphomolybdate plastic membrane electrode	Bromazepam-phosphomolybdate coated wire electrode
Slope (mV per decade)	53.22 ± 0.7	51.63 ± 0.4
Intercept	388.68	417.49
Correlation coefficient r	0.9999	0.9998
Linear range (M)	$1.0 \times 10^{-3} - 5.0 \times 10^{-6}$	$1.0 \times 10^{-3} - 1.0 \times 10^{-6}$
Detection limit (M)	2.5×10^{-6}	5.01×10^{-7}
Response time for 10^{-3} M (s)	25	20
Working pH range	3-8	3-8
Lifetime/day	20	28
Accuracy (%)	99.36	99.25
Standard deviation (%)	0.4	0.5
Repeatability (CV_w %)	0.5	0.6
Between day variability (CV_b %)	0.8	0.7
Robustness[b]	99.94 ± 0.2	99.96 ± 0.3
Ruggedness[c]	99.89 ± 0.1	99.54 ± 0.1

[a]Mean of three measurements; [b]A small variation in method parameters were studied as pH of buffer; [c]Comparing the results by those obtained by different sensors assemblies using-Orion 420 A.

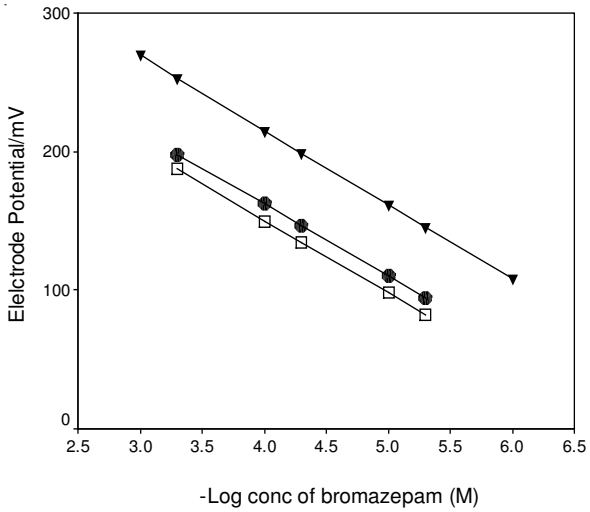

Fig. 3. Optimization of plasticizers. DBS (□) (PVC membrane composition: DBS 55.0 w %, PVC 42.0 w %, ion-pair, 3.0 w %) DBP (●) (PVC membrane composition: DBP 45.0 w %, PVC 45.0 w %, ion pair, 10.0 w %) DOP (▼) (PVC membrane Composition: DOP 42.0 w %, PVC 48.5 w %, ion-pair, 9.5 w %)

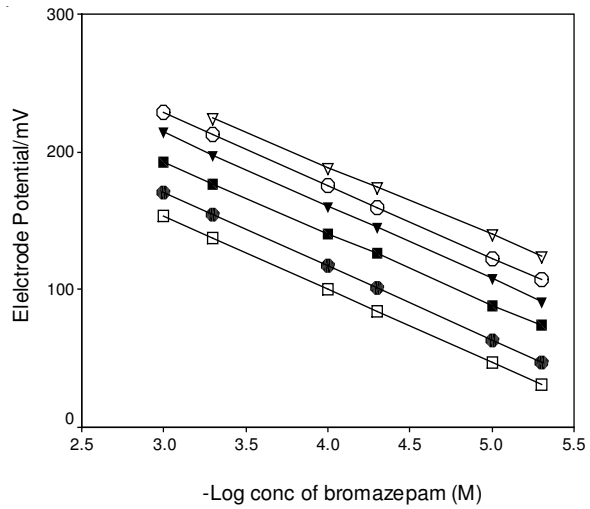

Fig. 4. Calibration graphs obtained at 25 ± 1 °C after soaking the bromazepam-phosphomolybdate plastic membrane electrode for (□) 24 h, (●) 7 days, (■) 10 days, (▼) 15 days, (○) 20 days, (▽) 25 days

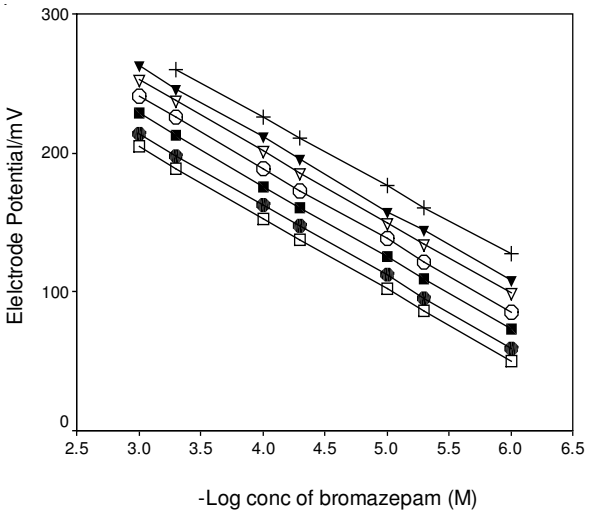

Fig. 5. Calibration graphs obtained at 25±1°C after soaking the bromazepam phosphomolybdate coated wire electrode for (□) 24 h, (●) 7 days, (■) 10 days, (○) 15 days, (▽) 25 days, (▼) 28 days, (✚) 30 days

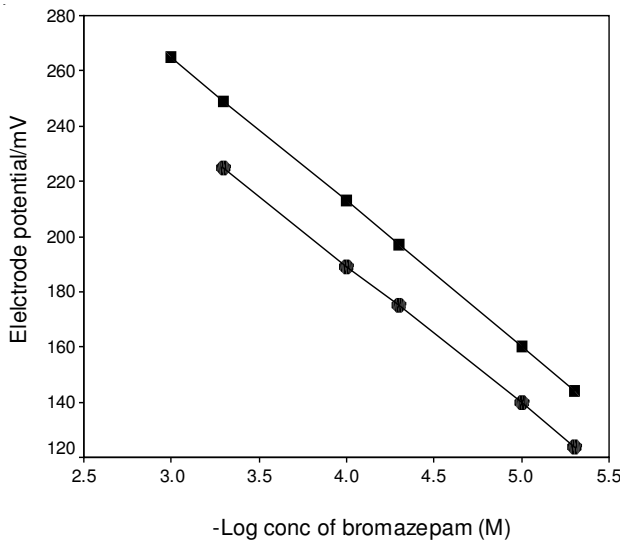

Fig. 6. Regeneration of bromazepam-phosphomolybdate plastic membrane sensor (●) exhaust electrode (■) regenerated electrode

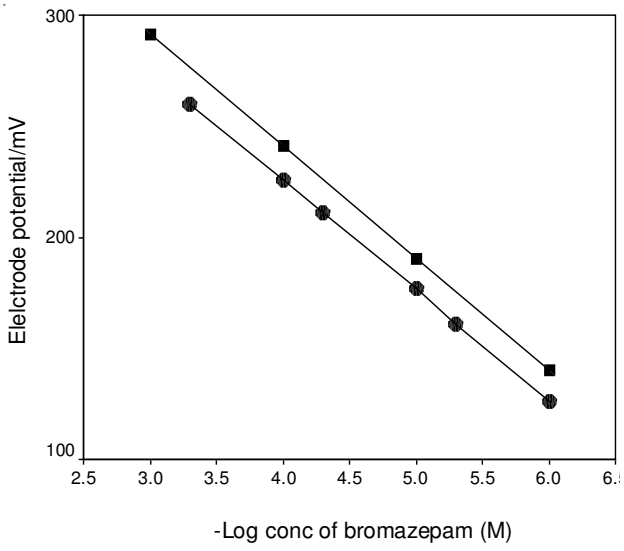

Fig. 7. Regeneration of bromazepam-phosphomolybdate coated wire membrane sensor (●) exhaust electrode (■) regenerated electrode

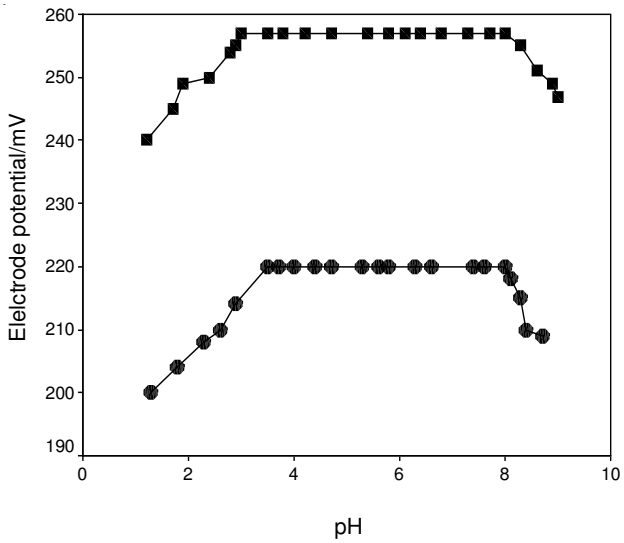

Fig. 8. Effect of pH on potential/mV of bromazepam sensors using 1×10^{-3} M (●) electrode potential/mV for plastic membrane electrode, (■) electrode potential/mV for coated wire electrode

L^{-1} sodium hydroxide for each pH value, the potential was recorded and thus the potential-pH curves for bromazepam concentration were constructed (Fig. 8). The pH range was 3-8 for both electrodes.

Selectivity of the electrode: Interference from many sugars, inorganic cations, certain alkaloids and amino acids was studied using separate solution method[29], Table-3 showed that the proposed bromazepam-phosphomolybdate membrane electrode(s) is highly selective toward bromazepam. The electrode(s) showed no response to a number of potentially interfering ionic excepients as (K^+, Na^+, Ca^{2+} and Mg^{2+}) and non-ionic usually used in the manufacturing of the pharmaceutical preparations, such as starch and lactose. The inorganic cations did not interfere may be due to the differences in their mobilities and permiabilities from bromazepam cation while, amino acids did not interfere due to their different polarity and lipophilic character. In addition some related drugs as paroxetine and gabapentin were non-interferent.

TABLE-3
SELECTIVITY COEFFICIENTS OF THE BROMAZEPAM-PHOSPHOMOLYBDATE SENSORS CALCULATED BY THE SEPARATE SOLUTION METHOD (1×10^{-3} M OF BOTH BROMAZEPAM AND INTERFERENT AT 25 ºC)

Interferent	$K^{pot}_{Brom.}$	
	Bromazepam-phosphomolybdate plastic-membrane electrode	Bromazepam-phosphomolybdate coated-wire electrode
Sodium chloride	4.2×10^{-4}	3.3×10^{-4}
Potassium chloride	3.5×10^{-4}	2.4×10^{-4}
Magnesium sulphate	2.3×10^{-3}	1.2×10^{-3}
Calcium chloride	2.9×10^{-4}	4.4×10^{-3}
Urea	8.7×10^{-3}	9.2×10^{-3}
Glucose	4.9×10^{-3}	4.0×10^{-3}
Lactose	2.2×10^{-3}	6.9×10^{-3}
Starch	8.8×10^{-4}	7.1×10^{-4}
Quinidine	9.2×10^{-4}	3.4×10^{-4}
Caffeine	5.6×10^{-3}	1.7×10^{-3}
L-Cystin	3.3×10^{-4}	3.2×10^{-4}
L-leucin	1.9×10^{-3}	4.3×10^{-3}
Pseudoephedrine hydrochloride	8.0×10^{-4}	2.9×10^{-4}
Gabapentin	4.1×10^{-3}	1.4×10^{-5}
Paroxetine hydrochloride	5.2×10^{-3}	3.6×10^{-3}
Sulfathiazole	1.2×10^{-4}	3.2×10^{-4}

Quantification of bromazepam: The direct potentiometric determination of bromazepam in pure form using the proposed electrodes gave average recovery of 99.12-99.36 ± 0.388-0.553 % and 99.18-99.25 ± 0.479-524 % for plastic and coated wire electrode, respectively. Furthermore, the results obtained were compared with the official method[30], (potentiometric titration using 0.1 M perchloric acid, for determination of bromazepam) and the results were listed Table-4.

Validation of the proposed ion selective electrode method[31]

Linearity: Under the optimal experimental ion selective electrode conditions, a linear relationship exists between the electrode potential/mV and the logarithm of corresponding concentration of the investigated drug. The regression data, correlation coefficient (r) and other statistical parameters were listed in Table-2.

Detection limit: The detection limit was calculated to be about 2.5×10^{-6} according to IUPAC[12] recommendation. Table-2 indicates that the proposed method is sensitive for detection of very small concentrations of bromazepam.

Robustness and ruggedness: Adjusting pH to 5 using 0.1N NaOH gave accuracy % of about 99.36 ± 0.553 and using phosphate buffer of pH 6 it gave 99.94 ± 0.217 indicating that the proposed method was robust. The closed results obtained by using Orion 420 A pH-meter to those obtained using Jenway 3040 pH-meter proved the ruggedness of the method (Table-2).

Accuracy: Table-5 showed mean percentage recoveries of pure drug of 99.27-99.38 ± 0.362-0.513 from spiked placebo samples of magnesium stearate.

Precision: RDS % for determination of the investigated drug in its pharmaceutical preparations "three batches" to nine replicates were 0.482, 0.633 and 0.356 % for determination of bromazepam in calmepam® 3 mg/tablets using plastic membrane and 0.747, 0.364 and 0.486 % in calmepam® 3 mg/ tablets using coated wire electrode. The above RSD % values are less than 2 % indicating good precision.

Analytical applications of the proposed method

Bromazepam tablets: The proposed method could be adopted for the determination of the investigated drug in its pharmaceutical preparations without interference from the coformulated adjuvants. Table-6 showed the results obtained from the determination of bromazepam in tablets in comparison with official method[30].

Content uniformity assay of calmepam® tablets: The proposed method described good accuracy and precise for the

TABLE-4
DETERMINATION OF BROMAZEPAM IN PURE FORM USING BROMAZEPAM-PHOSPHOMOLYBDATE SENSORS IN COMPARISON WITH OFFICIAL METHOD

Statistical parameter	Bromazepam-phosphomolybdate plastic membrane electrode			Bromazepam-phosphomolybdate coated wire electrode		
	Official method[30]	Direct potentiometry		Official method[30]	Direct potentiometry	
		Calibration method	Standard addition method		Calibration method	Standard addition method
Mean %	99.23	99.12	99.36	99.46	99.18	99.25
N	6	6	6	7	6	6
Variance	0.154	0.306	0.151	0.107	0.275	0.229
SD	0.392	0.553	0.388	0.327	0.524	0.479
SE	0.160	0.226	0.158	0.124	0.214	0.196
RSD	0.395	0.558	0.390	0.329	0.528	0.483
"t"		0.397 (2.228)*	0.578 (2.228)*		1.132 (2.201)*	0.905 (2.201)*
F		1.99 (5.05)*	3.58 (5.05)*		2.57 (4.39)*	2.14 (4.39)*

*Theoretical Values of "t" and F at P = 0.05

TABLE-6
COMPARATIVE ANALYTICAL RESULTS OF THE PROPOSED AND REFERENCE METHOD FOR THE TESTED DRUG IN SOME PHARMACEUTICAL PREPARATIONS

Sample and source	Statistical parameter	Bromazepam-PM plastic membrane electrode			Bromazepam-PM coated wire electrode		
		Official method[30]	Direct potentiometry		Official method[30]	Direct potentiometry	
			Calibration method	Standard addition method		Calibration method	Standard addition method
Calmepam®3 mg/mL tablets	Mean %	99.18	99.07	99.48	99.10	99.39	99.13
	N	6	7	7	6	7	7
	Variance	0.188	0.458	0.354	0.143	0.145	0.303
	SD	0.434	0.677	0.595	0.378	0.381	0.550
	SE	0.177	0.256	0.225	0.154	0.144	0.208
	RSD	0.438	0.683	0.598	0.381	0.383	0.555
	"t"		0.353 (2.201)*	1.048 (2.201)*		1.375 (2.201)*	0.116 (2.201)*
	F		2.44 (4.39)*	1.88 (4.39)*		1.01 (4.39)*	2.12 (4.39)*
Lexotanil®3 mg/mL tablets	Mean %	99.62	99.43	99.44	99.72	99.13	99.45
	N	6	6	6	6	6	6
	Variance	0.213	0.057	0.309	0.201	0.311	0.056
	SD	0.461	0.239	0.556	0.448	0.558	0.237
	SE	0.188	0.098	0.227	0.183	0.237	0.097
	RSD	0.463	0.240	0.559	0.449	0.563	0.238
	"t"		0.896 (2.228)*	0.611 (2.228)*		1.970 (2.228)*	1.304 (2.228)*
	F		3.74 (5.05)*	1.45 (5.05)*		1.55 (5.05)*	3.59 (5.05)*

*Theoretical values of "t" and F at P = 0.05

TABLE-5
DETERMINATION OF BROMAZEPAM IN BROMAZEPAM-SPIKED PLACEBO SAMPLES USING BROMAZEPAM-PHOSPHOMOLYBDATE SENSORS

Types of electrodes	Sample of tablets placebo		
	Added (M)	Found -log conc. (M)	Recovery (%)
Plastic membrane electrode	5×10^{-6}	5.29	99.79
	9×10^{-6}	5.00	99.09
	1×10^{-5}	4.97	99.40
	3×10^{-5}	4.45	98.89
	5×10^{-5}	4.28	99.51
	1×10^{-4}	4.01	99.12
	3×10^{-4}	3.99	99.75
	5×10^{-4}	3.26	98.76
	1×10^{-3}	3.02	99.16
Statistical parameters	N	9	
	Mean	99.27	
	SD	0.362	
	RSD	0.365	
Coated wire electrode	5×10^{-6}	5.24	98.85
	9×10^{-6}	5.03	99.69
	1×10^{-5}	4.97	99.40
	3×10^{-5}	4.28	99.51
	5×10^{-5}	4.04	99.86
	1×10^{-4}	3.98	99.50
	3×10^{-4}	3.25	98.76
	5×10^{-4}	3.05	100.14
	1×10^{-3}	2.96	98.67
Statistical parameters	N	9	
	Mean	99.38	
	SD	0.513	
	RSD	0.516	

quality control tests, the content uniformity assay showed that the (RSD < 2 %), with mean recoveries of 99.43 ± 0.547 and 99.27 ± 0.515 for plastic membrane and coated wire electrodes, respectively.

Application to serum and urine: When the proposed ISE was applied to determine of bromazepam in human serum and urine, good results were obtained and summarized in Table-7.

TABLE-7
DETERMINATION OF BROMAZEPAM-SPIKING TECHNIQUE USING BROMAZEPAM-PHOSPHOMOLYBDATE IN HUMAN SERUM AND URINE

Types of sensors	Statistical parameter	Direct potentiometry	
		Calibration method	Standard addition method
Plastic membrane electrode	Serum sample		
	Mean ± SD	99.09 ± 0.219	98.89 ± 0.175
	N	5	6
	Variance	0.048	0.031
	SE	0.098	0.071
	RSD	0.221	0.177
	Urine sample		
	Mean ± SD	99.60 ± 0.462	99.10 ± 0.299
	N	5	6
	Variance	0.213	0.089
	SE	0.207	0.122
	RSD	0.464	0.302
Coated wire electrode	Serum sample		
	Mean ± SD	99.16 ± 0.672	99.38 ± 0.237
	N	5	6
	Variance	0.452	0.056
	SE	0.301	0.097
	RSD	0.678	0.238
	Urine sample		
	Mean ± SD	99.11 ± 0.374	99.01 ± 0.199
	N	5	6
	Variance	0.139	0.040
	SE	0.172	0.081
	RSD	0.377	0.201

Conclusion

The described potentiometric methods have simple workup procedure and require no sophisticated instrumentation. The results obtained also show that the constructed electrodes provide response suitable for analytical use in the determination of bromazepam in drug bulk powder, dosage forms and biological fluids. A part from showing linear response within wide pH and concentration ranges with high accuracy and sensitivity, they also has high selectivity and reproducibility.

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