

## Short Communication

# Potentiometric determination of diazepam with a diazepam ion-selective electrode

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### Introduction

Diazepam (7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-2-one) is a benzodiazepine tranquilliser with anticonvulsant, sedative, muscle relaxant, and amnesic properties. A number of analytical methods for the assay of diazepam are available including HPLC [1], spectrophotometry [2], differential pulse polarography [3], non-aqueous titration [4], gas chromatography [5] and infrared spectrophotometry [6]. However, each of these methods has its shortcomings such as lack of selectivity, need for an expensive instrument or being time-consuming. The ion-selective electrode method has the advantage of selectivity, simplicity and rapidity. A variety of drug ion-selective electrodes has been proposed [7]. However, no electrode sensitive to diazepam has been reported. In the present paper, diazepam ion-selective electrodes made with various ion-pair complexes of diazepam are described and their performances compared. The electrodes are suitable for direct potentiometry and potentiometric titration of diazepam.

### Materials and Methods

#### *Apparatus and reagents*

Potentiometric measurements were made with a PHS-3A model precision pH-meter (Shanghai Analytical Instrument Factory No.

2), a diazepam ion-selective electrode and a double-junction saturated calomel electrode with 1 M sodium nitrate in the outer compartment. Diazepam and other drugs tested were of pharmacopoeial quality [4]. Silicotungstic acid, phosphotungstic acid, phosphomolybdic acid, picrolonic acid and picric acid were of laboratory reagent grade. Other chemicals used were of analytical reagent grade. Double-distilled water was used.

*Standard diazepam solutions.* To 71.2 mg of diazepam, 20 ml of a solution containing 0.1 M sodium nitrate and 0.005 M hydrochloric acid was added. 2 M Hydrochloric acid was added dropwise under continuous stirring until complete dissolution of diazepam was achieved. The pH was adjusted to about 2.3 with dilute sodium hydroxide solution, then diluted in a volumetric flask to 50 ml with the same solution containing 0.1 M sodium nitrate and 0.005 M hydrochloric acid. This is a 5 mM standard solution of diazepam. Other standard diazepam solutions are prepared by serial dilution with the same diluent (pH 2.3,  $I = 0.11$  M).

*Sodium tetraphenylborate (0.05 M).* This solution was prepared and standardized by potentiometric titration with 0.05 M silver nitrate, using a silver/silver sulphide electrode in conjunction with a double-junction saturated calomel electrode.

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### Preparation of ion-pair complexes

**Diazepam tetraphenylborate.** This was prepared by mixing 0.02 M diazepam in 0.01 M hydrochloric acid with an equal volume of 0.02 M sodium tetraphenylborate, and leaving the mixture overnight. The mixture was filtered through a porosity-4 sintered-glass crucible; the residue was washed with distilled water until no chloride ion was detected in the washing, then dried under vacuum for 48 h. Diazepam reineckate, diazepam silicotungstate, diazepam phosphotungstate and diazepam phosphomolybdate were prepared similarly except that the precipitate with the hetero-polyacid anion was filtered off after precipitation was complete.

**Diazepam dipicrylamine.** An appropriate amount of dipicrylamine suspension was dissolved in dilute sodium hydroxide solution. After adjusting to about pH 8, the solution was added to 20 ml of 0.02 M diazepam until complete precipitation was achieved. The mixture was left for 30 min and treated as indicated above.

**Diazepam tetra-iodobismuthate.** 0.85 g of bismuth subnitrate and 10 ml of acetic acid were dissolved in 20 ml of 32% (m/v) potassium iodide solution; 20 ml of water and 12 g of potassium iodide were added. The solution was added dropwise to 15 ml of 0.02 M diazepam. The precipitate was treated as indicated above for diazepam dipicrylamine.

**Diazepam tetra-iodomercurate.** 2.5 g of mercuric chloride and 9 g of potassium iodide were dissolved in 60 ml of water. The procedure was then carried out as indicated for diazepam tetra-iodobismuthate.

### Electrode construction

The poly(vinylchloride) membrane electrode was prepared as described previously [8]. The internal reference solution was 0.01 M sodium chloride saturated with silver chloride; 5 mM ion-pair complex in dibutyl phthalate (as solvent and plasticizer) was used. The electrode was preconditioned in 0.5 mM diazepam for 5 h and was then ready for use. When not in use, the electrode can be stored in air.

### Measuring cell

Diazepam ion-selective electrode-sample solution (pH 2.3,  $I = 0.11$  M)–1 M sodium nitrate-saturated calomel electrode.

## Results and Discussion

### Comparison of electroactive materials

Diazepam ion-selective electrodes with different electroactive materials were investigated in order to compare their electrode performances. The results obtained with ion-pair complexes of diazepam with tetraphenylborate, dipicrylamine, hetero-polyacid anions and halogeno-metal complex acid anions are summarized in Table 1. Linearity ranges were computed by means of an IBM PC-XT computer and a program for regression analysis. Detection limits were defined according to the IUPAC definition [9]. Although there are differences between linearity ranges and response slopes for different ion-pair complexes tested as the electroactive material, the differences are insignificant. The experimental results indicate that, for all electrodes tested, a nearly Nernstian cationic response to diazepam is obtained over approximately three orders of magnitude in concentration, and the linearity range extends down to about  $10^{-5}$  M diazepam. This fact implies that the electrode

**Table 1**  
Response characteristics of diazepam ion-selective electrodes with various electroactive materials

Electroactive material	Slope (mV/log C)	Linearity range (M)	Detection limit (M)
Diazepam tetraphenylborate	59.4 ± 0.8	$5 \times 10^{-3}$ – $5 \times 10^{-6}$	$1.3 \times 10^{-6}$
Diazepam dipicrylamine	60.2 ± 0.4	$5 \times 10^{-3}$ – $5 \times 10^{-6}$	$1.3 \times 10^{-6}$
Diazepam tetra-iodobismuthate	57.2 ± 0.4	$5 \times 10^{-3}$ – $2 \times 10^{-5}$	$4 \times 10^{-6}$
Diazepam tetra-iodomercurate	58.2 ± 0.3	$5 \times 10^{-3}$ – $2 \times 10^{-5}$	$4 \times 10^{-6}$
Diazepam silicotungstate	59.6 ± 0.2	$5 \times 10^{-3}$ – $4 \times 10^{-6}$	$1 \times 10^{-6}$
Diazepam phosphotungstate	58.6 ± 0.1	$5 \times 10^{-3}$ – $4 \times 10^{-6}$	$1 \times 10^{-6}$
Diazepam phosphomolybdate	58.4 ± 0.3	$5 \times 10^{-3}$ – $5 \times 10^{-6}$	$1 \times 10^{-6}$
Diazepam reineckate	58.6 ± 0.2	$5 \times 10^{-3}$ – $1 \times 10^{-5}$	$2 \times 10^{-6}$

response characteristics are not significantly influenced by the type of the ion-pair complex tested. Besides tetraphenylborate and dipicrylamine which are widely employed as the electroactive material for drug ion-selective electrodes, other ion-pair complexes proposed here can also be recommended as promising electroactive materials for the development of drug ion-selective electrodes.

It has been noted that some ion-pair complexes such as diazepam phosphotungstate and diazepam phosphomolybdate are sparingly soluble in nitrobenzene and other membrane solvents and, therefore, their respective liquid-membrane electrodes cannot be prepared with sufficiently good response characteristics. However, since these substances can be dispersed homogeneously in the plasticized poly(vinylchloride) membrane, their respective poly(vinylchloride) membrane electrodes can still be prepared; those electrodes exhibit relatively good electrode performances.

#### Effect of pH

The effect of solution pH on electrode potentials was investigated in diazepam hydrochloride solutions in which the pH was varied by addition of sodium hydroxide and/or hydrochloric acid solution from a 10- $\mu$ l microsyringe. At pH 1.9–2.8 no significant effect on membrane potentials was observed (Fig. 1). The calibration graph retains linearity but with different slopes at pH 2.8–3.0 and is non-linear below pH 1.9. Above pH 3, formation of the very slightly soluble diazepam base caused the electrode potentials to decrease; at pH values

below 1.5–1.9, the electrode began to respond to hydrogen ions.

#### Response time

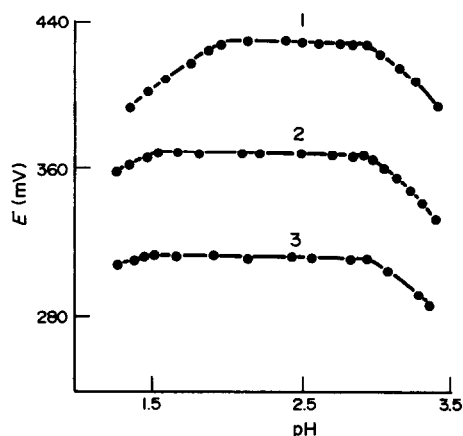
The electrode response times were measured for diazepam hydrochloride solutions of different concentrations. All the poly(vinylchloride) membrane electrodes made with the ion-pair complexes show fast responses. The response time ranges from less than 20 s in  $10^{-2}$ – $10^{-5}$  M solutions to 30–50 s for solutions  $<10^{-5}$  M. Electrodes made with an ion-association complex of diazepam with a hetero-polyacid anion give a faster response than do other diazepam ion-selective electrodes.

#### Reproducibility, stability and life-span

The reproducibility of the potential measurements in  $10^{-4}$  M standard diazepam hydrochloride was checked periodically. A shift of less than 1 mV/day in the absolute millivolt values was observed. The electrode potential was repeatedly measured in  $10^{-5}$ – $10^{-3}$  M diazepam hydrochloride; a standard deviation of  $<0.6$  mV was obtained for six measurement cycles. The electrode can be used for not more than 3 months.

#### Selectivity

When diazepam is determined by non-aqueous titration [4], many other basic substances interfere with the determination, and these must be removed before the analytical measurement. The interfering effects of some of these substances and other common compounds on the response of the diazepam dipicrylamine electrode were studied using the mixed-solution method. The selectivity coefficients obtained with more than 26 substances are represented in Table 2. The electrode exhibits fairly good selectivity toward diazepam. Only propantheline, berberine, chlordiazepoxide, chlorpheniramine, glycopyrrolate, tetramisole and tetrabutylammonium are likely to cause interference. However, these substances are rarely formulated in combination with diazepam. No significant interference was caused by other substances tested including sorbitol, propylene glycol and glucose which may be formulated in combination with diazepam. For symmetric quaternary ammonium cations, an approximately linear relationship between the logarithm of the selectivity coefficient and the number of carbon atoms was observed. This



**Figure 1**  
Effect of solution pH on the potential of the diazepam ion-selective electrode: (1)  $5 \times 10^{-3}$  M; (2)  $5 \times 10^{-4}$  M; (3)  $5 \times 10^{-5}$  M.

**Table 2**  
Selectivity coefficients obtained with other substances

Substance	$K_{ij}$	Substance	$K_{ij}$
Potassium chloride	$1.3 \times 10^{-4}$	Chloroquine phosphate	$1.3 \times 10^{-3}$
Sodium chloride	$3.4 \times 10^{-5}$	Piperaquine phosphate	$2.0 \times 10^{-3}$
Ammonium chloride	$6.6 \times 10^{-5}$	Proprantheline bromide	39.0
Calcium chloride	$5.5 \times 10^{-7}$	Glycopyrrolate	0.29
Zinc chloride	$2.0 \times 10^{-6}$	Atropine sulphate	$2.8 \times 10^{-2}$
Aluminium sulphate	$2.5 \times 10^{-4}$	Chlorpheniramine maleate	0.32
Barium chloride	$4.2 \times 10^{-6}$	Thiamine hydrochloride	$2.7 \times 10^{-2}$
Glucose	$1.9 \times 10^{-6}$	Pyridoxine hydrochloride	$1.4 \times 10^{-2}$
Urea	$2.4 \times 10^{-6}$	Tetramisole hydrochloride	0.25
Tetramethylammonium iodide	$7.8 \times 10^{-4}$	Berberine hydrochloride	169
Tetraethylammonium iodide	$1.9 \times 10^{-2}$	Strychnine sulphate	$1.3 \times 10^{-2}$
Tetrabutylammonium iodide	83.7	Starch (1%)	No interference
Propylene glycol	$1.0 \times 10^{-6}$	Sorbital	$1 \times 10^{-6}$
Primaquine phosphate	$1.8 \times 10^{-2}$	Chlordiazepoxide	0.86

**Table 3**  
Assay of diazepam

Direct potentiometry			Potentiometric titration		
Taken (mg ml <sup>-1</sup> )	Found (mg ml <sup>-1</sup> )	Recovery (%)	Taken (mg ml <sup>-1</sup> )	Found (mg ml <sup>-1</sup> )	Recovery (%)
0.0757	0.0766	101.2	0.459	0.454	98.9
0.106	0.104	98.1	0.360	0.360	100.0
0.291	0.284	97.6	0.426	0.424	99.5
0.359	0.358	99.7	0.393	0.386	98.2
0.522	0.506	96.9	0.554	0.558	100.7
0.676	0.694	102.7	0.298	0.295	99.0

phenomenon was also demonstrated in previous studies on dibazol [10] and carbapentane [11] ion-selective electrodes.

#### Diazepam assay

Results obtained from the direct potentiometric determination of diazepam using the calibration curve method are quoted in Table 3. A solution containing 0.01 M glucose and 0.1 M sodium chloride (pH 2.3) was used as the background. The mean recovery was 99.4%; the standard deviation was 2.3%. Diazepam can also be determined by potentiometric titration with sodium tetraphenylborate using the diazepam ion-selective electrode as indicator electrode. For these measurements, the mean recovery was 99.4%; the standard deviation was 0.9%.

#### Assay of diazepam tablets

Diazepam tablets were analysed using the method reported previously [10]. A sample of 30 tablets was weighed and finely powdered. A portion of the powder, equivalent to about 8 mg of diazepam, was transferred to a 100-ml

volumetric flask; 10 ml of water and a minimum amount of 2 M hydrochloric acid were added to dissolve the diazepam. After adjusting to about pH 2.3, the mixture was diluted to volume with a solution containing 0.1 M sodium nitrate and 0.005 M hydrochloric acid. Potentiometric measurements were made in replicate on the sample solution and on a standard solution containing approximately the same concentrations of diazepam and of the background electrolytes until reproducible ( $\pm 0.1$  mV) values were obtained. The mean results of the potentiometric analyses of diazepam tablets with nominal amounts of 2.5 mg and 5 mg in each tablet using the diazepam tetraphenylborate poly(vinylchloride) membrane electrode were 2.46 and 4.87 mg in each tablet, respectively; the standard deviations for five determinations were  $\pm 0.07$  and  $\pm 0.10$  mg, respectively. The corresponding results by the pharmacopoeial method [4] were  $2.42 \pm 0.07$  and  $4.85 \pm 0.09$  mg/tablet.

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