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Drug-selective electrode for ketamine determination in pharmaceutical preparations and electrochemical study of drug with BSA

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Abstract

Ion-selective membrane electrode to the drug ketamine hydrochloride has been constructed using a modified PVC membrane which has ionic end-groups as ion-exchanger sites and which was cast using plasticized with *ortho*-nitrophenyloctyl ether (*o*-NPOE) as plastisizer. This drug electrode show excellent Nernstian responses (59 mV per decade) in the concentration range 1×10^{-5}–1×10^{-2} M with a detection limit of 5×10^{-6} M. Response time was about 10 s for ketamine concentrations between 1×10^{-5} and 1×10^{-2} M. The response is not affected by pH between 4 and 8.5. Selectivity coefficients against various organic and inorganic cations were evaluated. The electrode was applied for determination of ketamine hydrochloride in pharmaceutical preparations using direct potentiometry. The drug electrode has also been used to study the interaction of bovine serum albumin (BSA) with ketamine in buffer solution (phosphate, pH 7). The saturated quantities of ketamine binding were 114, 32 and 25 mol/mol in 0.01, 0.02 and 0.1% of protein, respectively. The Hill equations were applied to obtain co-operative drug bindings to BSA at 27 °C.

Keywords: Drug-selective electrode; Pharmaceutical preparations; Ketamine; BSA; Protein interaction

1. Introduction

Ketamine hydrochloride or 2-(2-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride is widely used as an anesthetic drug that stimulate NMDA (*N*-methyl-D-aspartate) receptors on neuronal cells. Ketamine hydrochloride blocks NMDA receptors, and this action is although to contribute to ketamine's potent anesthetic and analgestic properties. Ketamine occludes the open channel by binding to a site located within the channel pore [1]. Inhibition of potassium channels, especially in central neurons, has been suggested to underlie some of the excitatory effects and emergence phenomena observed with dissociative anesthetic such as ketamine [2]. For the determination of such drugs has been suggested the several methods [3–7]. Conventional method for determination of ketamine hydrochloride was

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non-aqueous titration with perchloric acid [8]. Other methods available in the literature include direct UV–visible spectrophotometry [9,10], gas chromatography–mass spectrometry [11–14], liquid chromatography [15–17] and polarography [18]. Most of these methods involve time-consuming procedures and use of sophisticated instruments.

Ion-selective membrane electrodes are now widely used for the direct potentiometric determination of ion activities or ion concentrations in different samples [19–23]. Particularly, the feasibility, their use in continuous as well as in situ applications impose a strong competition on the currently established methods like flame photometry. Under a variety of membrane type, solvent polymeric membranes have proved to be especially suited for clinical analysis since they can easily be manufactured in different sizes and shapes and are less affected by the response of biological substrates such as protein, enzyme, antibody, … [24–26]. Their advantages are simple design, low cost, adequate selectivity, low detection limit, high accuracy, wide concentration range and applicability to coloured and turbid solutions [27]. In the membranes were made from liquid and plasticized poly(vinylchloride) (PVC) and are based on a water-insoluble ion-pair complex acting as ion-exchanger [28,29]. Some problems have been encountered with the membranes, e.g. the requirement of extensive pre-conditioning treatment, care in storage, sensitivity to some hydrophobic counterions and a relative short lifetime. In connection with study of surfactant-selective electrodes some of the above disadvantages encountered with the above PVC membrane have been overcome by the use modified PVC which has ionic end-groups as ion-exchange sites and a water-immiscible liquid as plasticizer to cast the membrane [30,31]. The former inhibits the dissolution of the ion-exchanger and the latter method yields membranes, which have longer lifetimes.

In this work, with using improved polymeric plasticized PVC method explained above, ketamine-selective electrode was constructed and have been applied for analysis a range of ketamine concentrations in the region 1×10^{-6}–1×10^{-2} M using the Nernstian equation. The selectivity coefficients of this electrode relative to the other drugs such as triflupromazine, antazoline, prometazine, atropine, naphazoline and propranol hydrochloride as well as the some of inorganic cations and the workable pH range of the electrode were also determined. In addition, this electrode was used to the study the interaction of ketamine with BSA in buffer solutions and different concentrations of protein.

2. Experimental

2.1. Reagents and materials

Ketamine hydrochloride used in the present work was obtained from Sigma. Relative high molecular weight PVC (1.8% carboxyl content), *o*-nitrophenyloctyl ether (*o*-NPOE) and sodium tetraphenylborate were purchased from Fluka. The other drugs used as interferences were purchased from Fluka and Sigma. All the solutions were prepared in doubly distilled deionised water and stored in the dark to prevent photochemical oxidation of drug [32]. Bovine serum albumin (BSA) was purchased from Sigma. All the test solution for drug–protein interaction study was made in phosphate buffer at pH 7, with ionic strength of 0.01 M.

2.2. Apparatus

The potentiometric measurements were accomplished by use of a digital pH/mV meter (Corning ion analyzer 250). All determinations were performed at the temperature of 27 ± 0.1 °C and with using an Ag/AgCl (Orion) double junction (d.j.) reference electrode. Activities were calculated according to the Debye–Huckel procedure [33].

2.3. Preparation of membrane

For the preparation of the polymeric plasticized PVC membrane, the first stage involve of the modification of PVC for use with respective drug. This procedure involves exchanging the hydrogen ions of the $-CO_2-H^+$ end charge groups with the

drug cation [29]. For carry out this step, i.e. ion-exchange step, 0.4 g of powdered PVC was dissolved in 25 cm^3 THF and then was added to it 20 cm^3 of (0.02 M) aqueous ketamine hydrochloride solution, gradually. This results in the precipitation of the conditioned PVC in a fiber from which was filtered and washed thoroughly with water and was dried in a vacuum desicator for 24 h. The second stage contained dissolution of certain amounts of this PVC–drug complex as well as *o*-NPOE as polymeric plasticizer and a little amount of sodium tetraphenylborate (NaTPB) in 20 cm^3 THF. The resulting mixture was transferred into a flat-bottomey beaker and the solvent was evaporated slowly at room temperature. Finally, a membrane disc of 10 mm diameter was cut by using a master membrane and was fixed to the Teflon tubing.

2.4. Cell assembly

The electrochemical system was as follows:

1. Ag/AgCl/internal solution/membrane/test solution/Ag/AgCl (d.j.) reference electrode.
2. The internal filling solution contained 1×10^{-3} M ketamine hydrochloride and 0.01 M NaCl. In all the experiments the temperature was controlled to within ± 0.1 °C by circulating thermostat water through the double glass cell and the test solution (20 cm^3 of 0.01 M NaCl) was continuously stirred during measurement.

2.5. Measurements of the electrode potentials

The concentration of the test sample solution was changed successively by adding a known amount of ketamine solution to initial sample (0.02 M) with using of a Hamilton micropipette. The responses of the electrode was tested in the concentration range 1×10^{-6}–1×10^{-2} M at the temperature of 27 °C. The electrode potential was recorded as a function of ketamine concentration (log[drug]). The calibration plot obtained was used for subsequent measurements of unknown ketamine concentrations (Fig. 1).

3. Result and discussion

3.1. Electrode responses

The overall electrode operating characteristics were investigated on the basis of the calibration curves obtained by plotting the measured e.m.f values against the $-\log$[drug]. The tests were carried out over the concentration range 10^{-6}–10^{-2} M in solution with 0.01 M NaCl for adjusting of ionic strength. The influence of the membrane composition on the potential response of ketamine ion-selective electrode was investigated. Then the optimum relative amounts of PVC, *o*-NPOE and NaTPB as a suitable additive in construction of drug-selective electrode were investigated and the results are summarized in Table 1. The data presented correspond to the average of three values obtained from the electrode. The response times for the all concentrations were about 10 s. Deviations from linear behavior of Nernstian equation are observed at low and high drug concentrations. Deviation in low concentrations are due to the sensor becoming insensitive in this region of the membrane potential [34,35] and deviations in high concentrations are presumably due to aggregation of the

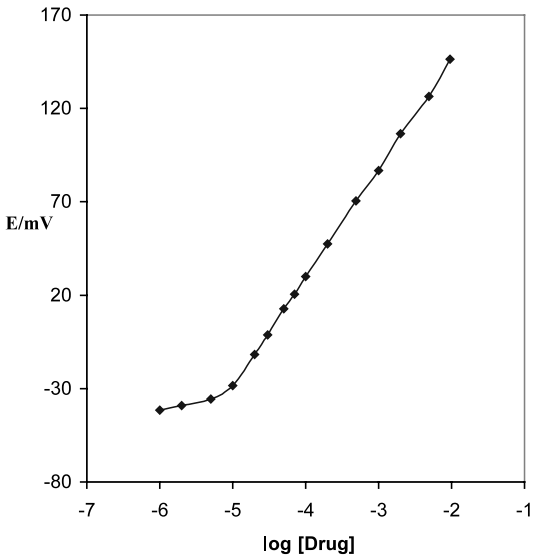

Fig. 1. Calibration curve for ketamine hydrochloride electrode obtained in 0.01 M NaCl solution.

Table 1
The composition and response characteristics of the drug-selective membrane electrodes

Membrane	Membrane composition (% w/w)			Slope (mV per decade)[a]	LLLR (M)[b]	LLD (M)[c]	RSD (%)[a]
	PVC	*o*-NPOE	NaTPB				
1	40	60	–	47.3	1×10^{-4}	5×10^{-5}	1.45
2	40	59	1	51.5	5×10^{-4}	1×10^{-5}	1.20
3	38	60	2	59.1	1×10^{-5}	5×10^{-6}	1.27
4	35	63	2	56.1	2×10^{-5}	8×10^{-6}	1.33
5	37	60	3	55	1×10^{-5}	5×10^{-6}	1.30

[a] Mean and relative standard deviation values of slopes for three replicate measurements.
[b] Lower limit of linear range.
[c] Lower limit of detection.

drugs [25]. The recorded electrode potentials were maintained over 20 min. This electrode showed good Nernstian behavior and were linear over a wide concentration range (1×10^{-5}–1×10^{-2} M) with a slope of 59 mV per decade.

3.2. Effect of pH on the electrode responses

The effect of pH on the electrode potentials was investigated by recording the e.m.f values of the drug-selective electrode in 5×10^{-4} M ketamine hydrochloride solutions and 0.01 M NaCl. The pH of this solution was altered by adding very small values of concentrated hydrochloride acid and sodium hydroxide solutions. Fig. 2 indicates that the e.m.f values were remained constant with respect to altering the pH over range 4.0–8.5. In this variety of the pH, the electrode can be applied for determination of ketamine hydrochloride. The considerable decrease of the potential observed in the pH of higher than 8.5; it is due to the decreased concentration of the protonated form of ketamine.

3.3. Selectivity of the electrode

The selectivity coefficients, $K^{\mathrm{Pot}}_{\mathrm{Drug},\,J^{z+}}$, of ketamine-selective electrode towards other drugs and inorganic cations as interfering ions (J^{z+}) were evaluated by the mixed solution method (MSM) that the fixed solution is containing 1×10^{-3} M from ketamine hydrochloride as primary ion [36]. The, $K^{\mathrm{Pot}}_{\mathrm{Drug},\,J^{z+}}$, values are listed in Table 2.

3.4. Lifetime and reproducibility

The lifetimes of electrodes were investigated by performing the calibration periodically with standard solutions and calculating the response slopes. It was indicated that the electrode can be used continuously for about 2 months without considerable decrease in its slope values. This kind of the membrane electrodes do not require any preconditioning in the solutions of corresponded drugs or maintenance before use. The membranes of electrodes were washed with water after each application and stored in a desicator under atmospheric condition and kept far from the light.

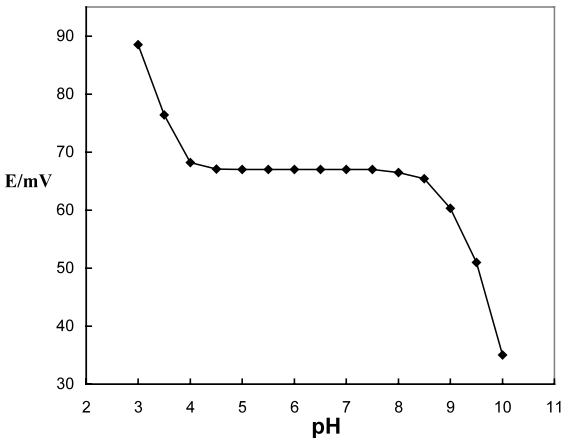

Fig. 2. Effect of pH on the potential of ketamine electrode in 5×10^{-4} M ketamine hydrochloride and 0.01 M NaCl solution.

Table 2
Selectivity coefficients of the drug-electrode calculated by the MSM, in 1×10^{-3} M of ketamine hydrochloride at temperature of 27 °C

Interferent	$K^{Pot}_{Drug,\,jz+}$	Interferent	$K^{Pot}_{Drug,\,jz+}$
Li^+	5.31×10^{-3}	Cu^{2+}	2.54×10^{-4}
Na^+	3.28×10^{-3}	Antazoline	0.31
K^+	2.78×10^{-3}	Trifluprom azine	0.24
NH_4^+	6.50×10^{-3}	Propranolol	0.87
Mg^{2+}	8.93×10^{-4}	Atropine	0.55
Ca^{2+}	7.47×10^{-4}	Prometazine	0.35
Fe^{2+}	5.26×10^{-4}	Naphazoline	0.76

3.5. Analytical applications

This membrane electrode can be successfully used for analysis of ketamine hydrochloride in pharmaceutical preparations. The determinations were made on a type sample, i.e. in ampoule. As the conventional method for determination of ketamine (titration in non-aqueous solvents) was difficult and time-consuming as well as using of expensive solvents, but this method (potentiometric determination) is easy, fast and inexpensive (Table 3). One of the important applications of this drug-selective electrode would have the study and investigation of interactions between ketamine and proteins.

Table 3
Determination of ketamine in some pharmaceutical preparations using ketamine electrode

Samples[a]	Amount founded (mg/ml)[b]	Recovery (%)	RSD (%)[c]
1	50.9	101.7	1.34
2	50.6	101.3	1.27
3	50.8	101.6	1.01
4	51.4	102.6	1.35
5	51.4	102.5	1.13
Average	51.0	101.9	1.22

[a] Chemical works of gedeon Richter Ltd., Budapest-Hungary.
[b] Amount taken was 50.0 mg/ml.
[c] SDs of three determinations.

3.6. Drug binding to protein

Serum albumin is the principle protein component of plasma and is remarkable for its power to bind a great variety of molecules, including bilirubin, fatty acids, tryptophan, metal ions and numerous drugs [37,38]. Investigation of the mechanism by which small molecules (drug in particular) bind to serum albumin is indispensable for understanding the transport function of albumin. It is also very important to obtain quantitative characteristics of this interaction for practical purposes. The interaction between serum albumin and a number of drugs is reversible, as has been shown by equilibrium dialysis technique [39]. In the present work, we report the results obtained from an electrochemical study of the interaction of ketamine with BSA, using a ketamine ion selective electrode. It is noteworthy that, in recent years, we have used a hexadecylpyridinium, selective electrode for study of complexation of some crown ethers and protein interaction with hexadecyl pyridinium [40,41]. The binding of ketamine with BSA was measured in the drug concentration range 1×10^{-5}–1×10^{-2} M in presence of 0.01, 0.2 and 0.1% of BSA at 27 °C. The measurement was carried out using the following procedure. First the e.m.f of the drug electrode relative to the reference electrode was measured as a function of drug concentration up to the high concentration limit of electrode. The experiment was then repeated by measuring the relative e.m.f of the drug electrode in the presence of a constant amount of BSA. Each point was obtained after attainment of equilibrium value which required 10 min. From these data, it is possible to evaluate the free drug concentration, at each total concentration of the drug. Result of the potentiometric titration of BSA by drug at various concentrations of protein is shown in Fig. 3. The calibration curve clearly shows the excellent performance of the drug electrode. In the presence of protein, the deviation from the calibration curve in the log[drug] axis, where [drug] is the added drug concentration, allows us to calculate the amount of bond drug. Fig. 4 shows the plots of ΔE vs. log $\bar{v}$, where ΔE is the potential difference of electrode in the presence and absence of protein at each total concen-

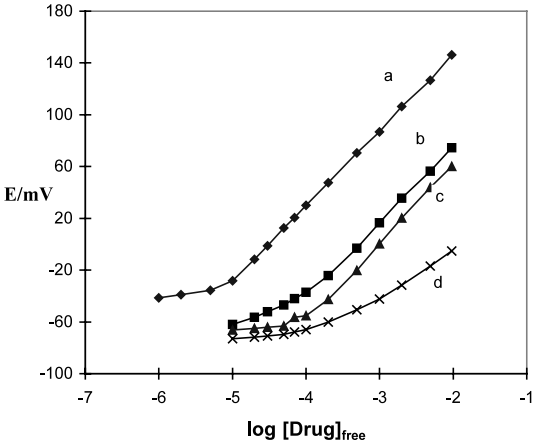

Fig. 3. E.m.f response of ketamine electrode in various concentrations of BSA: (a) 0; (b) 0.01; (c) 0.02; (d) 0.1%.

tration of drug, for which the measurements were taken. The $\bar{v}$ is the drug-bonded per mole of protein. The resulting plots show a distinct break at the v_s values (overall stoichiometric binding constant) characteristics of saturated quantities of the drug binding, which are dependent on the concentration of protein (114, 39 and 25 for 0.01, 0.02 and 0.1%, respectively). The protein concentration dependent bindings have been previously reported [42,43], which can be described as

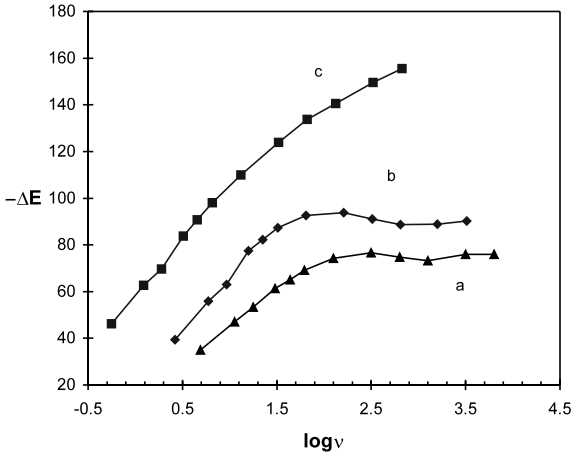

Fig. 4. Potential difference of ketamine electrode (ΔE) in the presence and absence of protein at each total concentration of drug as a function of the log v: (a) $v_s = 114$ for 0.01%; (b) $v_s = 39$ for 0.02%; (c) $v_s = 25$ for 0.1%.

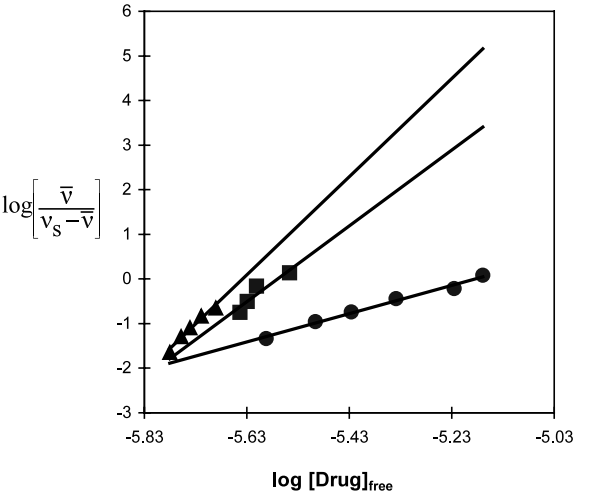

Fig. 5. Hill plot for binding of drug to BSA in 0.01, 0.02 and 0.1% of protein: (a) $n_H = 3.18$, $\log K = 5.19$ for 0.01%; (b) $n_H = 8.5$, $\log K = 5.57$ for 0.02%; (c) $n_H = 11.0$, $\log K = 5.64$ for 0.1%.

protein–protein interaction. Hill equation [44] has been used to calculate the intrinsic Hill binding constant (K) and Hill coefficient (n_H) according to 1:

$$\log\left[\frac{\bar{v}}{v_s - \bar{v}}\right] = n_H \log K + n_H \log [\text{drug}]_{\text{free}} \quad (1)$$

We observe three n_H which changes from 3.18 to 11 and suggests possible positive co-operative binding sites. We used v_s of each saturated site corresponding to protein concentration in Eq. (1) for calculation of n_H and $\log K$ (Fig. 5).

4. Conclusion

The proposed ketamine selective electrode was successfully applied for fast and accurate determination of ketamine in pharmaceutical. The results of the present study indicate that the electrochemical method can be used to the study of the interaction of drug with proteins. This method has been unique in its high sensitivity to the change of concentration of drug on protein binding. The complete binding data may be largely due to the long period necessary to attain dialysis equilibrium (at least about 98 h). The binding

data obtained in the present study were measured by the potentiometric technique, which required only 100–150 min, and thus seems to be the most reliable method among that so far reported.

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