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Potentiometric Sensor for the Determination of Dibucaine in Pharmaceutical Preparations and Electrochemical Study of the Drug with BSA

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Plasticized poly(vinyl chloride), PVCs, with different membrane compositions tested for use in the construction of an ion-selective sensor for the determination dibucaine. A prepared membrane with dioctyl phthalate-PVC and ion-pair of *N*-(1-naphthyl)ethylenediamine dihydrochloride-tetraphenyl borate had a good potential to acts as a potentiometric sensor for the analysis of dibucaine. A linear relationship was obtained between potential and logC varying between 1.0×10^{-6} and 1.0×10^{-2} M dibucaine with a good repeatability and reproducibility. The sensor was applied for the determination of the drug in pharmaceuticals and biological fluids such as plasma and urine samples with satisfactory results. The drug electrode has also been used to study the interaction of bovine serum albumin (BSA) with dibucaine. The saturated quantities of dibucaine binding were 13.04, 5.30 and 9.70 mol/mol in 0.01, 0.02 and 0.1% of protein, respectively.

Key Words : Protein interaction, Dibucaine, Potentiometry, Ion selective electrode

Introduction

Dibucaine hydrochloride, 2-butoxy-*N*-(2-diethylamino-ethyl) quinoline-4-carboxamide hydrochloride is a drug that was first synthesized by Miescher.[1] It is used in various formulations such as ointments, creams, suppositories and injections. In the following application it is the essential part of a haemorrhoidal ointment. Several procedures have been reported in the literature for the analysis of dibucaine. These methods are spectrophotometry,[2,3] fluorimetry,[4] polarozgraphy,[5] high performance liquid chromatography,[6] gas chromatography,[7] thin-layer chromatography,[8] electrophoresis,[9] ion-selective electrodes.[10]

The use of ion-selective electrodes for the determination of pharmaceutical compounds have been reviewed especially with potentiometric titrations based on ion-pair formation.[11] These reviews showed that potentiometric detection based on ion-selective electrodes (ISEs), offers several advantages such as speed and ease of preparation and procedures, simple instrumentation, relatively fast response, wide dynamic range, reasonable selectivity, and low cost.[12] Potentiometric ion-selective electrodes have been reported for the determination of pharmaceutical compounds such as amiloride,[13,14] pentazocine,[15] betahistine,[16] desipramine[17] and venlafaxine.[18] According to our knowledge, only one reported paper presents about potentiometric determination of dibucaine[10] based on dibenzo-24-crown-8 ether-PVC electrode, with a linear calibration range of 0.001-1.0 M.

In this study, a plasticized poly(vinyl chloride) membrane with different compositions using *N*-(1-naphthyl) ethylene-diamine dihydrochloride sodium tetraphenyl borate as an ionophore was used. The potential responses of different membranes sensor towards dibucaine were studied. After optimization of the membrane composition, the potentiometric sensor was successfully used for the determination of dibucaine in body fluids with satisfactory results.

Experimental

Apparatus. Potentials were measured by direct potentiometry at 25 ± 0.1 °C with the help of ceramic junction calomel electrodes and the cell set-up was as follows:

$$Hg/Hg_2Cl_2, KCl(sat'd) \,||\, \text{drug solution} \,|\, \text{Membrane} \,|\, \text{Pt-wire}$$

All potentiometric measurements were made with a pH/mV meter, Corning, Model 140 (Switzerland). All emf measurements were carried out in a 50-mL double walled glass cell with a constant magnetic stirring. Response times were determined after the potential of the solution had become constant, and similar measurements were carried out in another solution of 100-fold lower concentration.

A pH-meter, Corning, Model 140 (Switzerland), with a double junction glass electrode was used to check the pH of the solutions.

Reagents and Solutions. All chemicals used were of analytical reagents grade and were used without further purification. All solutions were prepared by dissolving the chemicals and the salts of metal nitrates in distilled deionized water.

PVCs of high relative molecular weight, sodium tetraphenyl borate (NaTPB), dibutyl phthalate (DBP), dioctyl phthalate (DOP), dioctylsebacate (DOS), tetrahydrofuran (THF), Bovine serum albumin (BSA) and all other chemicals were of highest purity available from Aldrich (Milwaukee, USA), and were used without further purifications, except for THF which was distilled before use.

Aliquot solution, 0.010 M, dibucaine hydrochloride was prepared by dissolving 0.3799 g of the corresponding compound, respectively, in water and diluted to 100 mL with

water in a calibrated flask. Working standard solutions were prepared by suitable dilution of 1.0×10^{-2} M solutions with water.

Electrode Preparation. For preparation of the membrane, 250 μL of 1.0×10^{-2} M *N*-(1-naphthyl)ethylenediamine solution was added to 25 mL of 1.0×10^{-4} M NaTPB solution. The resulting precipitate (white color) was filtered and washed with deionized water and dried at room temperature while protected from light in a desiccator. Then, about 7.0 mg of the precipitate (*N*-(1-naphthyl)ethylenediamine dihydrochloride-sodium tetraphenyl borate (ion-pair)) was mixed with 31.0 mg PVC and 62.0 mg DOP previously dissolved in 1 mL of THF. Coating process of the platinum wire electrode was performed by dipping Pt-wire twenty times into the mixture. After each coating the membrane was air-dried for 10 min until a thin film was formed.

The sensor was calibrated by immersion in conjunction with the reference electrode in a 5 mL beaker containing 2 mL of water. Then, 0.50 mL aliquot of the drug solution (target ion) was added by continuous stirring, to give a final drug concentration ranging from 1.0×10^{-2} to 1.0×10^{-6} M. The potential was then recorded after its stabilization to ± 0.1 mV. A calibration graph was then constructed by plotting the recorded potentials as a function of log[dibucaine].

Determination of the Drug in Plasma and Urine. Drug-free human serum and urine used in this study was obtained from healthy volunteers. The urine and blood samples were separately centrifuged at 8000 rpm to remove the blood cells and other dead cells. The serum was kept in a freezer at –20 °C until analysis. The samples of human urine and serum were diluted 2 times with water and were analyzed directly as described in the recommended procedure. The samples were spiked just before analysis.

Results and Discussion

Influence of Membrane Composition. In a preliminary experiment, membranes with and without carrier were constructed. The membrane response was not reliable. However, in the presence of the ion-pair, the optimized membrane demonstrated a Nernstian response. Besides the critical role of the nature of the ion-carrier in preparing membrane-selective sensors, some other important features of the PVC membrane include the amount of the ionophore, nature of the solvent mediator (plasticizer), and plasticizer to PVC ratio are important. Thus, several membrane compositions were investigated by varying the ratios of PVC, plasticizer, and the ionophore. The potentiometric response of the membrane was greatly improved in the presence of the ionophore.

In general, the sensitivity of an ion-selective electrode is strongly influenced by the nature and amount of the plasticizer and lipophilic additive. The effect of the plasticizer on the characteristics of this electrode was investigated by using three kinds of plasticizers: DOP, DBP, and DOS. The electrodes plasticized with DOP exhibited a better sensitivity response than with DBP. The potential of the ion-selective electrodes to dibucaine are shown in Table 1. It is also known that the nature and amount of lipophilic additive strongly influence the response of this electrode. The results show that using the liophilic anion in cation-selective membrane electrodes diminished the ohmic resistance[19] and also increased the sensitivity of the electrode. The final results showed that 31.0 wt %. PVC, 62.0 wt %. DOP, and 6.90 wt % .Ion pair (No. 1, Table 1) resulted in the best sensitivity with a Nernstian slope of 58.9 ± 0.1 mV per decade over a wide linear dynamic range.

Influence of pH. The effect of pH on the electrode potentials was investigated by recording the e.m.f values of the drug-selective electrode in 1.0×10^{-3} M dibucaine solution. The pH of this solution was altered by adding very small amounts of concentrated hydrochloric acid and sodium hydroxide solutions. Figure 1 indicates that the e.m.f values are independent with respect to pH variations in the range of

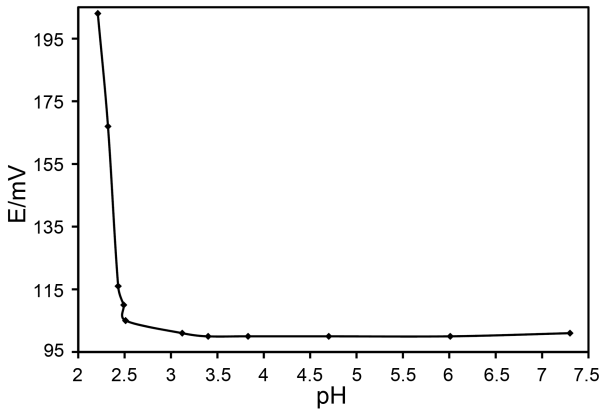

Figure 1. Influence of pH on the response of the membrane (1.0×10^{-3} mol L^{-1} dibucaine).

Table 1. The potentiometric response of the membrane in the presence of the several solvent mediators

No	Composition (%)			Slope (mV decade^{-1})	Dynamic range (M)	R^2
	PVC	Ion pair	Plasticizer			
1	31.0	7.0	62.0, DOP	58.9	$1.0 \times 10^{-2} - 1.0 \times 10^{-6}$	0.997
2	23.0	7.0	70.0, DOP	44.2	$1.0 \times 10^{-2} - 1.0 \times 10^{-5}$	0.978
3	30.1	8.2	61.7, DOS	46.3	$1.0 \times 10^{-2} - 1.0 \times 10^{-6}$	0.977
4	26.7	4.6	68.7, DOP	70.6	$1.0 \times 10^{-2} - 1.0 \times 10^{-5}$	0.964
5	31.0	7.2	61.8, DBP	67.5	$1.0 \times 10^{-2} - 1.0 \times 10^{-6}$	0.935
6	33.3	-	66.7, DOP	19.4	$1.0 \times 10^{-3} - 1.0 \times 10^{-5}$	0.830

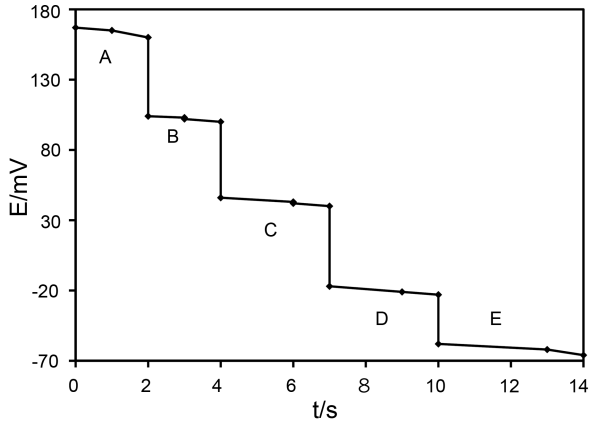

Figure 2. Response time of the electrode. (A) 1.0×10^{-2}, (B) 1.0×10^{-3}, (C) 1.0×10^{-4}, (D) 1.0×10^{-5}, and (E) 1.0×10^{-6} dibucaine.

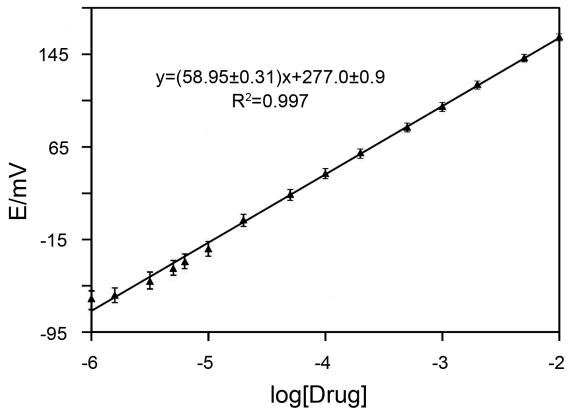

Figure 3. Calibration graphs of the membrane for dibucaine under the optimum conditions (each point was measured three times).

2.5-7.3. In this range, the electrode can be applied for the determination of dibucaine. The considerable decrease of the potential observed at pH levels higher than 7.3 is due to the decreased concentration of the protonated form of dibucaine.

Response Characteristics of the Electrode for Dibucaine. The response time is an important factor for any ion-selective electrode. The response time was recorded at different concentrations of dibucaine in the sample solution. The potentiometric response of the sensor was recorded by changing the solution from a lower (1.0×10^{-6} M dibucaine) to a higher (1.0×10^{-2} M dibucaine) concentration (Fig. 2). The response time for the electrode to reach the final equilibrium value was different for different concentrations. The response time of the sensors was found to be less than 3 s at various concentrations of the test solution and remained stable for several days without much drift in the potentials.

The reproducibility of the calibration parameters were studied by making calibrations with the same membrane on different days (n = 3) and with different membranes (n = 3). The standard deviation obtained for the slope was ± 0.41 mV per decade with the same membrane and ± 0.60 mV per decade with different membranes.

The electrode used over a period of 2 months without any significant effects on the membrane potential. The lifetime of the electrode was determined by reading the potential of the calibration solutions and plotting the calibration curves for periode of 2-month. The results showed that the slope of the sensor was changed from 58.9 ± 0.1 to 57.7 ± 0.1 after 60 days (just about 1% changed) without affecting on the linear dynamic range. The slope of the electrode (58.9 mV per decade change) was observed to show a gradual decrease after 60 days when it reached approximately 0.1 mV per decade. We may, therefore, conclude that the lifetime of the proposed electrode is at least 2 months. After this period, a slight change was observed in the slope. The lifetime of ion selective electrodes mainly depend on the type of ionophore and plasticizers used, and also on the number of times it is used.[20] After 2 months, the electrode response deteriorated, which may be attributed to the aging of the PVC matrices, the ionophore, and the plasticizer.[20]

The limit of detection for this membrane was calculated from the intersection of the two extrapolated segments of the calibration curve, as recommended by IUPAC, and determined as 7.1×10^{-7} M of dibucaine.

Calibration Graph. Figure 3 shows the calibration graph obtained for dibucaine studied. As can be seen, the electrode exhibited a Nernstian response for dibucaine within the concentration range of 1.0×10^{-2} to 1.0×10^{-6} M with a regression equation of E(mV) = (58.95 ± 0.31)logC + (277.0 ± 0.9), r^2 = 0.997), where C is dibucaine concentration in M.

Selectivity Study. The selectivity behavior is obviously one of the most important characteristics of an ion selective electrode, determining whether a reliable measurement in the target sample is possible. Potentiometric selectivity coefficients $K_{I,J}^{POT}$ describing the preference of the membrane for an interfering ion J relative to dibucaine I were determined by the separate solution method (SSM).[13,14] The potentiometric selectivity coefficients of the proposed electrode are summarized in Table 2.

Determination of the Drugs in Real Samples. The proposed membrane sensor was found to work well under laboratory conditions. It can be seen that the amount of

Table 2. Values of selectivity coefficients of dibucaine selective electrode

Interfering substance	$\log K_{I,J}^{POT}$	Interfering substance	$\log K_{I,J}^{POT}$
Pb^{2+}	-2.41	Lactose	-4.25
Fe^{3+}	-3.63	Fructose	-4.54
K^{+}	-2.97	Sucrose	-4.73
Ba^{2+}	-3.95	Glucose	-4.89
Na^{+}	-3.96	Ascorbic acid	-3.49
Mg^{2+}	-3.95	Amiloride	-3.47
Cu^{2+}	-5.72	Amitriptyline	-1.95
Mn^{2+}	-5.49	Diphenylhydramine	-1.74
Ni^{2+}	-4.25	Fluoxetine	-1.64
Cd^{2+}	-3.27	Clomipramine	-2.11
Sr^{2+}	-4.85	Imipramine	-2.87
Citric acid	-3.51	Ampicillin	-5.42

Table 3. Recoveries of dibucaine in urine and human plasma. samples

Sample	Added	Found	Recovery (%)
Plasma	-	< limit of detection	-
Plasma	1.00×10^{-6} (mol L^{-1})	$1.03(\pm 0.05) \times 10^{-6}$ (mol L^{-1})	103.0
Plasma	1.00×10^{-3} (mol L^{-1})	$0.98(\pm 0.04) \times 10^{-3}$ (mol L^{-1})	98.0
Urine	-	< limit of detection	-
Urine	3.00×10^{-5} (mol L^{-1})	$2.78(\pm 0.10) \times 10^{-5}$ (mol L^{-1})	91.0
Urine	7.00×10^{-3} (mol L^{-1})	$7.14(\pm 0.12) \times 10^{-3}$ (mol L^{-1})	102.0

dibucaine can be accurately determined using the proposed sensor. To assess the applicability of the proposed sensor to real samples, an attempt was made to determine dibucaine in plasma and urine. Each sample was analyzed in triplicate by standard addition method using the sensor. The results are presented in Table 3, which shows that the amounts of dibucaine recovered with the help of the sensor are satisfactory, thereby reflecting the utility of the proposed method. The optimized dibucaine selective electrode was successfully applied as an indicator electrode in the potentiometric titration of dibucaine hydrochloride solution with NaTPB solution. Typical results for the titration of a 50.0 mL 1.0×10^{-3} M dibucaine hydrochloride solution with 1.0×10^{-2} M NaTPB is shown in Figure 4. This figure confirms that the ion selective electrode can be used in potentiometric titration determination of dibucaine, with sharp titration curve around the equivalence point.

Drug Binding to Protein. Serum albumin is the principle protein component of plasma and remarkable for its power to bind a great variety of molecules including tryptophan, bilirubin fatty acids, metal ions, and numerous drugs.[21,22] For understanding the transport function of albumin, understanding of the mechanism by which small molecules (drug in particular) bind to serum albumin is indispensable. In addition, it is also very important for practical purposes to obtain quantitative characteristics of this interaction. The interaction between serum albumin and dibucaine has been shown by equilibrium dialysis technique.[23] hexadecylpyridinium-selective electrode was used for the study of complexation of some crown ethers and protein interaction with hexadecylpyridinium.[24] In the study, the results obtained from potentiometric study of the interaction of dibucaine with BSA, using an ion selective electrode, is reported.

Measurement of dibucaine and BSA binding was accomplished using the following procedure with a drug concentration range of 1.0×10^{-6} - 1.0×10^{-2} M in the presence of 0.01, 0.020 and 0.10% of BSA at 25 °C. Initially, 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 the electrode. Then, the relative e.m.f of the drug electrode was measured in the presence of a constant amount of BSA. 10 min was allowed to attain the equilibrium value before each point was obtained. Free drug concentration can be evaluated at each total concentration of the drug using these data Figure 5 presents the result of the potentiometric titration of BSA by drug at various concentrations of protein.

The excellent performance of the drug electrode is witnessed by the calibration curve. The amount of bond drug in the presence of protein can be calculated from the deviation from the calibration curve in the log[drug] axis, i.e. in the case where [drug] is the added drug concentration. The plots of ΔE *vs*. log ν is shown in Figure 6, where ΔE is the potential difference of the electrode in the presence and absence of protein at each total concentration of the drug for which the measurements were taken and ν is the drug-bonded per mole of protein. A distinct break is observed in the resulting plots at the ν_s values (overall stoichiometric

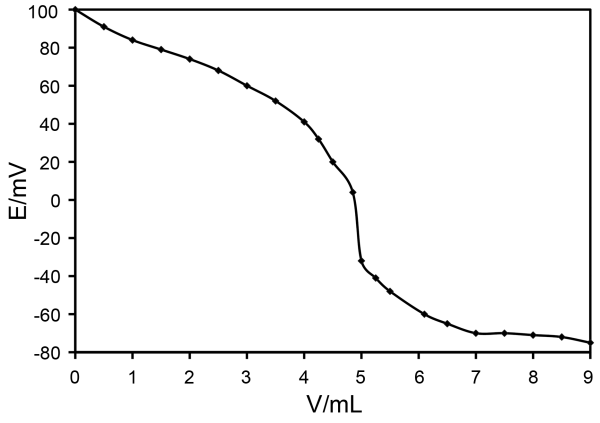

Figure 4. Potentiometric titration of dibucaine hydrochloride solution with NaTPB solution. Typical results for the titration of a 50.0 mL 1.0×10^{-3} M dibucaine hydrochloride solution with 1.0×10^{-2} M NaTPB.

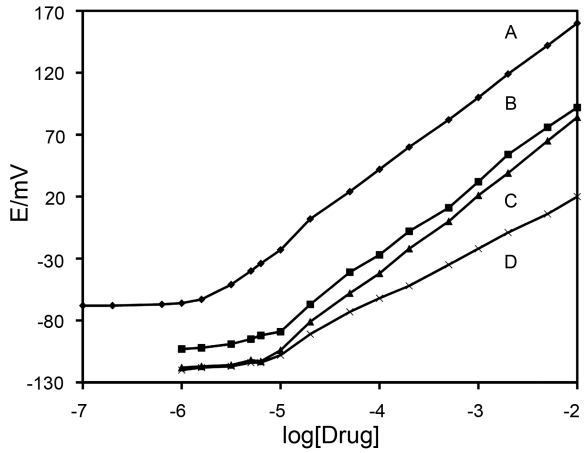

Figure 5. E.m.f. response of the ion selective electrode for dibucaine in various concentration of BSA: (A) 0; (B) 0.01; (C) 0.020; and (D) 0.10%.

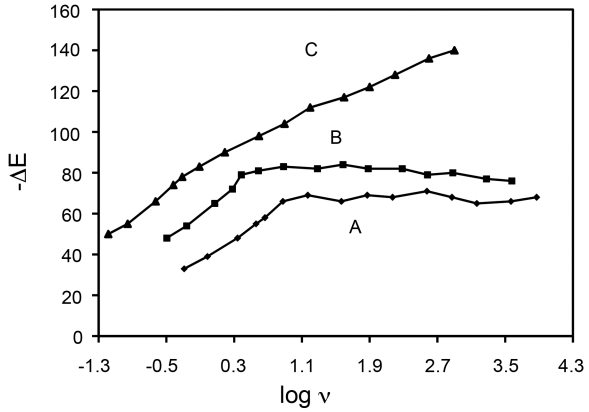

Figure 6. Potential difference ion selective electrode for dibucaine (ΔE) in the presence and absence of protein at each total concentration of drug as a function of the logν: (A) ν_s = 14.79 for 0.01%; (B) ν_s = 3.86 for 0.02%; (C) ν_s = 0.77 for 0.1%.

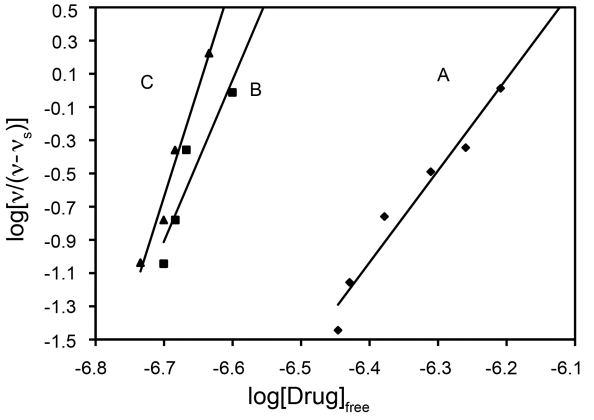

Figure 7. Hill plot for binding of drug to BSA in 0.01, 0.02 and 0.1% of protein: (A) n_H = 5.30, log K = 6.21 for 0.02%; (B) n_H = 9.70, log K = 6.60 for 0.1%; (C) n_H = 13.04, log K = 6.65 for 0.01%.

binding constant) characteristics of saturated quantities of the drug binding. These quantities are dependent on protein concentrations (14.79, 3.86 and 0.77 for 0.01, 0.02 and 0.1%, respectively). The intrinsic Hill binding constant (K) and Hill coefficient (n_H) were calculated according to Hill equation[25] as follows:

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

Here, three n_H values are observed changing from 5.30 to 13.04 and suggesting possible positive cooperative binding sites. We used ν_s of each saturated site corresponding to protein concentration in Eq. (1) above to calculate n_H and log$K$ (Fig. 7).

Conclusion

A membrane with a 1:2 (w/w) dioctyl phthalate to PVC ratio, doped with 6.9% (w/w) *N*-(1-naphthyl)ethylenediamine dihydrochloride-sodium tetraphenyl borate, performed well in the development of an ion-selective electrode for the determination of dibucaine. The sensor is fast, selective, and more sensitive (1000-fold) than the present potentiometric method,[10] and can be used in real sample analysis. The results of the present study indicate that the electrochemical method can be used to study the interactions of dibucaine and proteins. This method has been unique in its high sensitivity to changes in the drug concentration on protein binding. The proposed electrode permits the determination of dibucaine in pharmaceuticals.

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