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Short Communication

CONSTRUCTION AND ANALYTICAL APPLICATIONS OF LIQUID-MEMBRANE ELECTRODES FOR ATROPINE AND NOVATROPINE

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Summary. Liquid-membrane electrodes sensitive to atropinium and novatropinium cations are described. The atropinium electrode exhibits rapid and near-Nernstian response in the 10^{-2}—3×10^{-5} M range over the pH range 2—8.5; the novatropinium electrode shows near-Nernstian response in the 10^{-2}—3×10^{-6} M range at pH 2—10. Other alkaloids interfere. Direct potentiometry and potentiometric titrations are used to determine atropine and novatropine in pharmaceutical preparations with satisfactory results.

Several sensitive potentiometric sensors for some organic bases of pharmaceutical importance have recently been reported. Electrodes have been constructed that are sensitive to methacholine, neostigmine [1], ephedrine [2], novocaine [3], codeine, morphine, ethylmorphine [4], strychnine [5], and nicotine [6]. Also, a potentiometric method [7] for the determination of various alkaloids with picrate ions using a picrate-selective electrode [8] has been reported.

In this communication, the performance characteristics of new electrodes sensitive to atropinium and novatropinium (methylhomatropinium) cations are described. The liquid ion-exchangers are atropinium tetraphenylboron and novatropinium tetraphenylboron, respectively, dissolved in 2-nitrotoluene. The electrodes proved useful in direct potentiometric determinations of atropine and novatropine in pharmaceutical preparations. The atropinium sensitive electrode was also used to estimate the pK_a value of atropine and in potentiometric titrations of atropine.

Experimental

Instrumentation. The reference electrode was an Orion 90-01-00 Ag/AgCl single-junction electrode, filled with Orion 90-00-01 solution. E.m.f. values were measured with a Corning Model 12 Research pH/mV meter and recorded on a Heath-Schlumberger Model SR-255 B potentiometric recorder. All solutions were measured at ambient temperature with constant magnetic stirring.

Reagents. All solutions were prepared with deionized distilled water from

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reagent-grade materials, unless otherwise stated. The chemicals were atropine sulfate and sodium tetraphenylboron (E. Merck, Darmstadt), novatropine (a gift from Chifar Chemical and Pharmaceutical Co., Athens); sodium tetra(*m*-chlorophenyl)boron was prepared as described by Jarzembowski et al. [9]. Pharmaceutical preparations were obtained from local drugstores. Standard 0.1000 M solutions of atropine, novatropine and sodium tetraphenylboron were prepared by dissolving the appropriate amount of substance in water. Working solutions were prepared by appropriate dilutions. Ionic strength adjustment solutions of 0.10 M and 0.50 M sodium sulfate and 0.10 or 0.20 M phosphate buffer, pH 6.5, were prepared in the usual manner.

Preparation of the liquid ion-exchangers. Atropinium tetraphenylboron was precipitated by mixing 1.0 ml each of aqueous 0.1 M solutions of atropine sulfate and sodium tetraphenylboron. The salt was extracted with 10.0 ml of 2-nitrotoluene, and the organic phase was washed twice with distilled water and dried thoroughly with anhydrous sodium sulfate. For the atropinium tetra(*m*-chlorophenyl)boron, sodium tetra(*m*-chlorophenyl)boron was substituted for sodium tetraphenylboron. Novatropine tetraphenylboron was prepared by mixing 0.5 ml of 0.01 M novatropine solution with 0.5 ml of 0.1 M sodium tetraphenylboron solution. The precipitate was extracted and treated as indicated above for the atropinium salt.

Construction of the electrodes. An Orion liquid-membrane electrode body (model 92) was used as the electrode assembly with a Millipore LCWPO 1300 teflon membrane; the teflon membranes were cut to the appropriate size and a stack of four was used to avoid any leakage of the liquid exchanger. The electrode was assembled in the usual way; the internal reference solution was 0.01 M atropine sulfate—0.1 M NaCl for the atropinium electrode and 0.01 M novatropine—0.1 M NaCl for the novatropinium electrode. The electrodes were conditioned by soaking in 10^{-2} M atropine or novatropine solution, as appropriate, for 24 h before use, and were stored in the same solution when not in use. Their operative lifetime was about one month.

Preparation of the calibration curve. A portion (30.00 ml) of 0.10 M phosphate buffer was pipetted into a 100-ml beaker, the electrodes were immersed, constant stirring (without air bubbles) was started, and 0.001 ml of 0.1 M atropine or novatropine standard solution was added. Readings were made after stabilization to ±0.1 mV. Further increments of 0.1 M standard solution were added to cover the 3.3×10^{-6}—1.2×10^{-2} M range, and the calibration graphs were plotted in the usual way.

Potentiometric titration of atropine. A 30.00-ml aliquot of the sample was pipetted into the reaction cell, and the stirred solution was titrated with a standard sodium tetraphenylboron solution. For atropine samples in the ranges 3×10^{-2}—5×10^{-3} and 3×10^{-3}—5×10^{-4} M, the sodium tetraphenylboron solutions used were 0.1000 and 0.01000 M, respectively. The inflection point of the titration curve was taken as the end-point.

Determination of atropine or novatropine in pharmaceutical preparations.

Atropine sulfate eye-drops (0.5 or 1%) were diluted 100-fold with water. Atropine sulfate injections (1 mg ml^{-1}) were diluted three-fold. For direct potentiometry of atropine a 15.00-ml aliquot of the diluted sample and 15.00 ml of 0.20 M phosphate buffer, pH 6.5, were pipetted into the reaction cell. The atropine concentration was found from a calibration graph for 0.1 M phosphate buffer, pH 6.5.

The novatropine (4 mg ml^{-1}) and novalumine (1.3 mg ml^{-1}) solutions were diluted 20-fold with water. Novatropine was determined as described above for the atropine. For the determination of novatropine in tablets, fifteen 2.5-mg tablets were powdered and extracted with 300 ml of water. The extract was filtered and diluted to 500.0 ml, and a 15.00-ml aliquot was used for the measurement, as described above for the diluted atropine sample.

Results and discussion

Characteristics of the electrodes. Typical calibration curves for the electrodes under various experimental conditions are shown in Figs. 1 and 2. The response of the atropinium electrode is linear in the 10^{-2}—3×10^{-5} M range with a slope of about 59 mV/concentration decade, at 27°C. Similar calibration curves were obtained when an atropinium electrode with atropinium tetra(*m*-chlorophenyl)boron in 2-nitrotoluene as liquid ion-exchanger was used. Atropinium tetraphenylboron was preferred because sodium tetraphenylboron is readily available. The response of the novatropinium electrode is linear in the 10^{-2}—3×10^{-6} M range with a slope of 61 mV/concentration decade, at 27°C. Considerable deviation from linearity was observed with unbuffered novatropine solution (Fig. 2, curve A).

The electrodes reached stable potential readings (±0.1 mV) within 15 s to 3 min depending on the alkaloid concentration, the buffer, etc. Generally, fast response (less than 1 min) was observed with solutions $>10^{-4}$ M. For

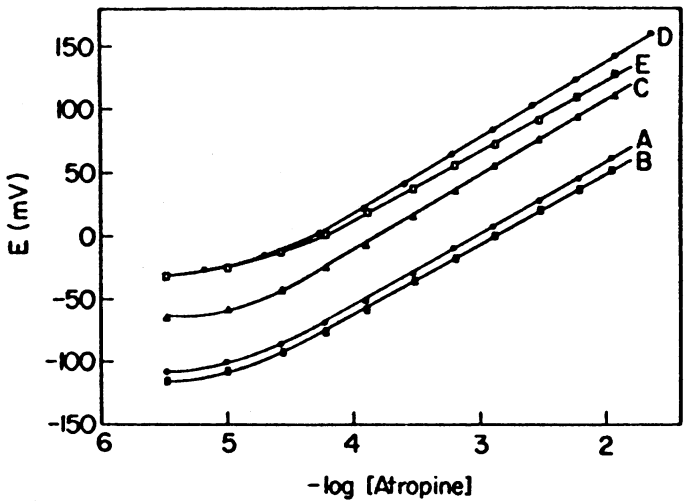

Fig. 1. Calibration curves for the atropinium-selective electrode. (A) In pure atropine sulfate solutions; (B) in 0.10 M Na_2SO_4 solution; (C) in 0.50 M Na_2SO_4 solution; (D) in 0.10 M phosphate buffer, pH 6.5. Curve E shows the response of the atropinium electrode to codeine, in 0.10 M phosphate buffer pH 6.5.

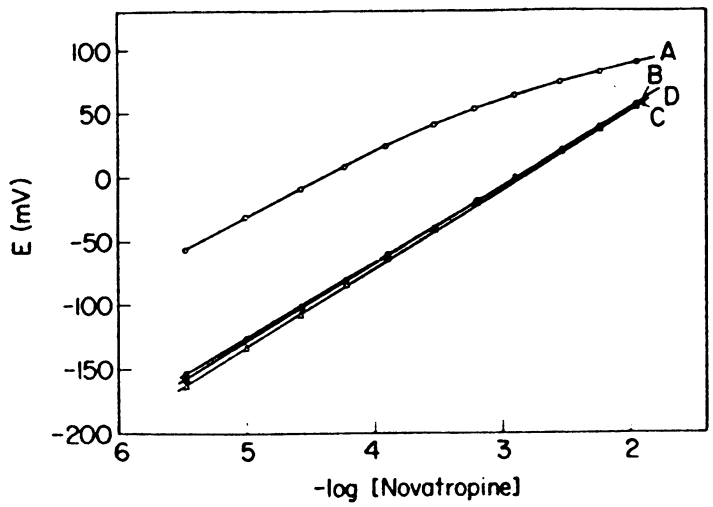

Fig. 2. Calibration curves for the novatropinium-selective electrode. (A) In pure novatropine solutions; (B) in 0.10 M phosphate buffer, pH 6.5; (C) in 0.10 M Na_2SO_4 solution; (D) in 0.50 M Na_2SO_4 solution.

solutions $<10^{-4}$ M the response time increased with increasing ionic strength.

To check the pH-dependence of the potential of the atropinium electrode, potential—pH curves were constructed. The initial solution was made acidic by adding concentrated sulfuric acid in 30.00 ml of atropine solution (10^{-3} or 10^{-4} M), and then the pH was adjusted (pH meter) by addition of small volumes of 10 M NaOH solution. The plots (Fig. 3) show that at pH 2—8.5, the potentials are constant. At higher pH values, the potentials gradually decrease because of the decreased concentration of the atropinium ion which is converted to atropine. Similarly, the potential of the novatropinium electrode was practically independent of pH in the range 2—10. At higher pH values, the electrode potential was unstable. The acidity constant K_a of atropine can be calculated from Fig. 3, as pK_a is equal to the pH value where the initial atropinium concentration, $[AH^+]$, is halved, i.e., when the potential decreases by 17.8 mV (Fig. 3). The pK_a value thus calculated is 9.80 at 27°C, which is in good agreement with the literature value of 9.65 [10].

Potentiometric selectivity coefficients for the atropinium electrode were measured by the mixed solution method and calculated as previously described [11]. The results are presented in Table 1. The atropinium electrode was also found to respond to various other alkaloids, e.g., codeine, papaverine, cocaine, and morphine. A response curve for codeine given by the atropinium electrode is shown in Fig. 1 (curve E).

Analytical applications. The atropinium electrode proved useful in the potentiometric titration of atropine with sodium tetraphenylboron. Typical titration curves are shown in Fig. 4. Amounts of atropine in the range 15—900 μmol were determined with an average error of about 2%. Direct potentiometric determinations on aqueous 10^{-3} M atropine and novatropine solutions from 3-point calibration curves showed imprecision of about 1.5%.

Comparative results for the determination of atropine and novatropine in some pharmaceutical preparations are shown in Table 2. The eye-drops con-

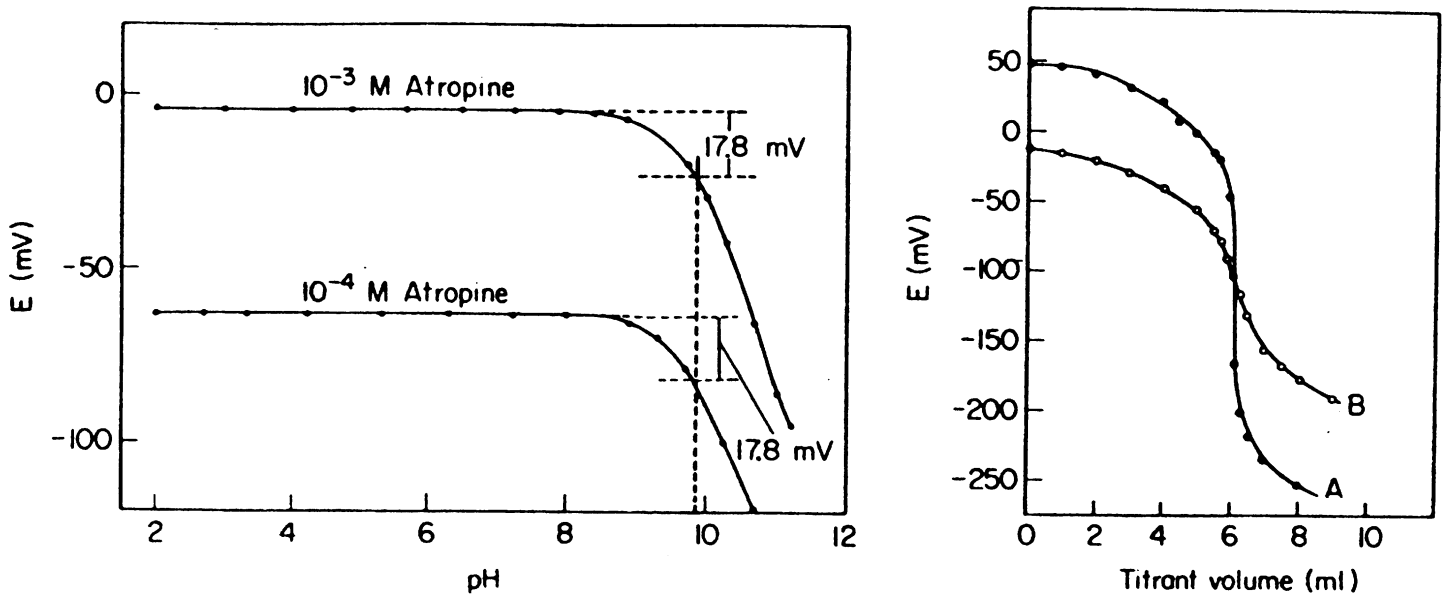

Fig. 3. Effect of pH on the potential of the atropinium electrode.

Fig. 4. Titration curves for the potentiometric titration of 30 ml of atropine sulfate with sodium tetraphenylboron, at pH 6. (A) 2×10^{-2} M atropine with 10^{-1} M sodium tetraphenylboron; (B) 2×10^{-3} M atropine with 10^{-2} M sodium tetraphenylboron.

TABLE 1

Potentiometric selectivity coefficients for the atropine electrode[a]

Possible interference	a_j, ($\times 10^{-2}$ M)	a'_{AH^+} ($\times 10^{-4}$ M)	$k^{pot}_{AH^+,j}$	Possible interference	a_j, ($\times 10^{-2}$ M)	a'_{AH^+} ($\times 10^{-4}$ M)	$k^{pot}_{AH^+,j}$
Na^+	6.8	7.6	$<10^{-4}$	Pb^{2+}	1.8	6.7	7×10^{-4}
K^+	6.8	7.6	1×10^{-4}	Mg^{2+}	1.8	6.7	8×10^{-4}
NH_4^+	6.8	7.6	2×10^{-4}	Cd^{2+}	1.8	6.7	2×10^{-3}
Ni^{2+}	1.8	6.7	5×10^{-4}	Sr^{2+}	1.8	6.7	3×10^{-3}
Ca^{2+}	1.8	6.7	6×10^{-4}				

[a] a_j, a_{AH^+} and a'_{AH^+} are the activities of the possible interference, atropinium in pure solutions, and atropinium in mixed atropine—interfering ion solutions, respectively. For full details, see [11]. Solutions of all possible interfering ions were prepared from their corresponding nitrate salts. a_{AH^+} was 10^{-3} M in all cases.

tained unspecified additives, in order to be isotonic with tears. There is satisfactory agreement between the results obtained by the proposed method and the official methods. In contrast to most of the common methods used for the determination of atropine and novatropine in pharmaceutical preparations, which are time-consuming and require large samples, the proposed method is simple, fast and sensitive.

TABLE 2

Determination of atropine and novatropine in pharmaceutical preparations

Pharmaceutical preparation	Atropine sulfate or novatropine (mg ml^{-1})		
	Present method	Reference method	Nominal
Atropine sulfate injection (aqueous)	0.92	0.93[a]	1
0.5% Atropine sulfate (eye-drops)	6.06	5.64[a]	5
1% Atropine sulfate (eye-drops)	9.8	9.3[a]	10
Novatropine solution (aqueous)	4.08	3.97[b]	4
Novalumine solution[d]	1.27	1.29[c]	1.3
Novatropine tablets[e]	2.27	2.35[c]	2.5

[a]U.S.P. (titrimetric) [13]. [b]Gravimetric (AgBr) [12, 14]. [c]The reference gravimetric method was not applicable to these samples. The bromide content was found by potentiometric titration with $AgNO_3$. [d]Oily solution also containing phenobarbital (16 mg ml^{-1}). [e]mg/tablet; unspecified excipients.

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