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POTENTIOMETRIC DETERMINATION OF NICOTINE IN TOBACCO PRODUCTS WITH A NICOTINE-SENSITIVE LIQUID MEMBRANE ELECTRODE

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SUMMARY

A simple potentiometric method with standard additions is described for the rapid determination of nicotine in tobacco products. A nicotine-sensitive electrode with a liquid membrane of nicotine hydrogen tetra(*m*-chlorophenyl)borate dissolved in *o*-nitrotoluene is used. The electrode exhibits near-Nernstian response to monoprotonated nicotine cation activity from 0.08 to 10^{-5} M, in the pH range 4—7. Nicotine down to 2 mg per g of sample can be determined with a standard deviation of about 0.5 mg of nicotine. Comparison with an official method gave satisfactory results.

Ion-selective electrodes are used extensively for the quality control of various products. Commercially available ion-selective electrodes have been successfully applied for determinations of traces of nitrate and ammonia in tobacco, and of hydrogen cyanide, hydrogen sulfide, nitrogen oxides and ammonia in tobacco smoke [1].

Nicotine is the most important alkaloid of tobacco, occurring in relatively large quantities (2—8%) as its malate or citrate salt in tobacco leaves [2]. Nicotine is determined routinely during quality control of tobacco and tobacco products. In all established methods, nicotine is separated from its natural matrix by steam distillation and subsequently determined spectrophotometrically by direct measurements at 259 nm [3].

Recently, several sensitive potentiometric sensors for some organic bases of pharmaceutical importance have been reported. Electrodes have been constructed that are sensitive to the following compounds: methacholine, neostigmine [4], ephedrine [5], novocaine [6], codeine, morphine, ethylmorphine [7], and strychnine [8]. These electrodes are generally sensitive but have a narrow spectrum of possible applications because of their nature and their limited selectivity. This drawback is partially counterbalanced by their relatively convenient and inexpensive construction, making their use acceptable in certain cases.

In this paper, a nicotine-sensitive liquid-membrane electrode is described. The liquid ion-exchanger consists of nicotine hydrogen tetra(*m*-chlorophenyl)borate, $(NicH^+)(TCPB^-)$, dissolved in *o*-nitrotoluene. This electrode

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is sufficiently selective to be used for the determination of nicotine in tobacco products without any prior separation. With a standard addition technique, down to 2 mg of nicotine per g of sample can be determined potentiometrically with a standard deviation of about 0.5 mg of nicotine per g of sample.

EXPERIMENTAL

Apparatus

The nicotine-sensitive electrode was used with an Orion 90-01-00 Ag/AgCl single-junction reference electrode; its chamber was filled with Orion 90-00-01 equitransferent solution. E.m.f. values were measured with an Orion Ion-analyzer (Model 801 digital pH/pIon meter) and pH with a Metrohm pH meter (Model E350B). All solutions were measured at ambient temperature with constant magnetic stirring.

Reagents

All solutions were prepared with deionized distilled water from reagent-grade materials. Sodium tetra(*m*-chlorophenyl)borate, NaTCPB, was prepared as described by Jarzembowski et al. [9].

Standard 0.1000 M nicotine. Dissolve 4.056 g of nicotine (British Drug Houses) in 200 ml of water, adjust the pH to 6.0 ± 0.1 with 1 M HCl and dilute to exactly 250 ml with water (solution A). Store this solution in a refrigerator.

Nicotine electrode liquid ion-exchanger. Dissolve 0.096 g of NaTCPB (0.20 mmol) in 2 ml of water and mix the resulting solution with 2.00 ml of solution A. Extract the resulting heavy precipitate of ($NicH^+$)($TCPB^-$) into 10.00 ml of *o*-nitrotoluene. Filter the organic layer through a filter paper containing 1—2 g of anhydrous sodium sulfate. The filtrate should be clear and light yellow. This solution is approximately 0.020 M in ($NicH^+$) ($TCPB^-$) and is almost saturated.

Phosphate buffer (0.041 M, pH 6.3). Mix 0.20 M sodium hydroxide and 0.055 M phosphoric acid solutions, in a ratio of 1:3.

Procedures

Electrode preparation. An Orion liquid-membrane electrode barrel (Model 92) is used as the electrode assembly with a Millipore LCWPO-1300 teflon membrane. After assembly in the usual manner, the internal reference solution (solution A is suitable) and the liquid ion-exchanger are injected into the appropriate ports. When not in use, the electrode is kept in air. The electrode should be immersed in a dilute nicotine solution (10^{-3}—10^{-2} M, pH 6—7) for 10 min before each series of measurements.

Determination of calibration slope. Pipet 20.0 ml of phosphate buffer solution into the measurement cell (a 50-ml beaker), immerse the electrodes in the solution, start stirring at the maximum speed at which air bubbles

are not formed, and add 30 μl of solution A. Read the e.m.f. when it has stabilized to ±0.1 mV (0.5—2 min). Continue with four more increments of solution A (50, 100, 100, 100 μl) and read the meter after each addition. Calculate the slope, S, of the calibration curve, i.e., E (mV) vs. logarithm of analytical nicotine concentration. Repeat the measurements if the correlation coefficient is less than 0.999. In case of consistent curvature in the lower concentration range, refill the nicotine-sensitive electrode.

Determination of nicotine in tobacco products. Pulverize the tobacco sample (from cigarettes, cigars or pipe tobacco) with a mortar and pestle. Into 20-ml vials with well-fitting stoppers, weigh appropriate amounts of the pulverized samples containing 3—20 mg of nicotine. Pipet 10.00 ml of 0.20 M sodium hydroxide solution into each vial, stopper the vials tightly, shake and immerse them in a water bath, thermostatted at 70 ± 2°C, for 45 min with frequent shaking. Cool the vials and centrifuge at 3000 rpm for 5 min. Transfer to the measurement cell 5.00 ml of the supernatant brown extract and 15.00 ml of 0.055 M phosphoric acid solution, immerse the electrodes in the solution (immersing the electrodes in the alkaline extract before neutralization will cause erratic responses), start stirring, and record the corresponding potential, E_1. Add 100 μl of solution A and record the new potential, E_2.

Calculate the amount of nicotine from the equation: nicotine (mg/sample) $= 3.245\,[1.005 \cdot 10^{(E_2-E_1)/S_4}]^{-1}$. Instead of this rather inconvenient equation, a nomogram (Fig. 1) can be used; if solution A is not 0.1000 M, appropriate corrections must be made.

RESULTS AND DISCUSSION

Membrane material

The nicotine (Nic) molecule forms mono- and di-protonated species:

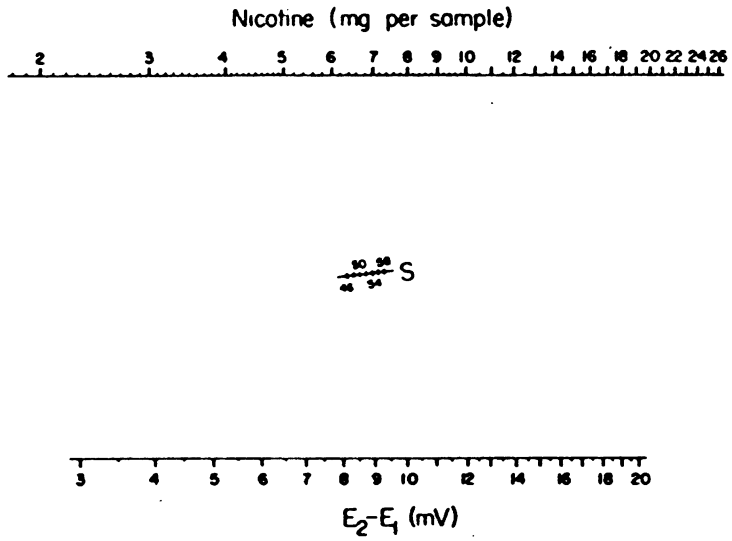

Fig. 1. Nomogram for the direct calculation of nicotine, in mg per sample, for given $E_2 - E_1$ and S values.

$$Nic \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} NicH^+ \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} NicH_2^{2+}$$

For $NicH_2^{2+}$, $pK_1 = 3.04$, $pK_2 = 7.84$, and so $NicH^+$ is the predominant species in the pH range 4—7. If equimolar amounts of nicotine and NaTCPB solutions are mixed in this pH range, $(NicH^+)(TCPB^-)$ precipitates; little of either reactant remains in the supernatant solution which indicates the 1:1 composition. This ion associate is the active ingredient of the liquid ion-exchanger used.

Sodium tetra(*m*-chlorophenyl)borate was chosen because it forms salts, which are generally sparingly soluble in water but soluble in oils, with several compounds containing basic nitrogen atoms and its aqueous solutions are more stable than those of sodium tetraphenylborate [9]. *o*-Nitrotoluene was chosen as the solvent because it gives faster and more complete extraction of $(NicH^+)(TCPB^-)$ than the other solvents tested (1-decanol, nitrobenzene, chlorobenzene, and *o*-dichlorobenzene).

Effect of pH

To check the pH-dependence of the potential of the nicotine electrode, potential—pH curves at various nicotine concentrations were constructed. The pH of the initial solution was altered by addition of very small volumes of hydrochloric acid. The plots (Fig. 2) show that the potential is little affected by pH in the range 4—7, in accordance with the fact that the electrode responds mainly to the monoprotonated nicotine species. At higher pH values there is a gradual decrease in potential because of the gradual increase in the concentration of unprotonated nicotine. Prolonged immersion of the electrode in alkaline solutions causes deterioration of its response, probably because of back-extraction of nicotine from the membrane to the aqueous phase. The movement of the electrode response below pH 4 is inconsistent for the three concentration levels tested. Probably, the electrode becomes progressively sensitive to $NicH_2^{2+}$, so that the slope factor decreases; this is indicated by the gradual decreasing difference in the potentials for the three concentrations.

Characteristics of the electrode

Linear response range. Typical calibration curves for the electrode under different experimental conditions are shown in Fig. 3. The response is linear in the range 0.08—10^{-5} M for pure nicotine solutions (curve A). In the presence of phosphate buffer (curves B and C), the linear range and limit of detection decrease because of electrode response to sodium ions. At pH 2.7 (curve D), there is practically no linear range and the slope is much smaller, indicating response to $NicH_2^{2+}$ species.

Selectivity coefficients. The interference of various ions was studied by the mixed solution method and the selectivity coefficients, $k^{pot}_{NicH^+,j}$, were calculated from

$$k^{pot}_{NicH^+,j} = [(10^{(E_2 - E_1)/S})\alpha_{NicH^+} - \alpha'_{NicH^+}]/\alpha_j^{1/z}$$

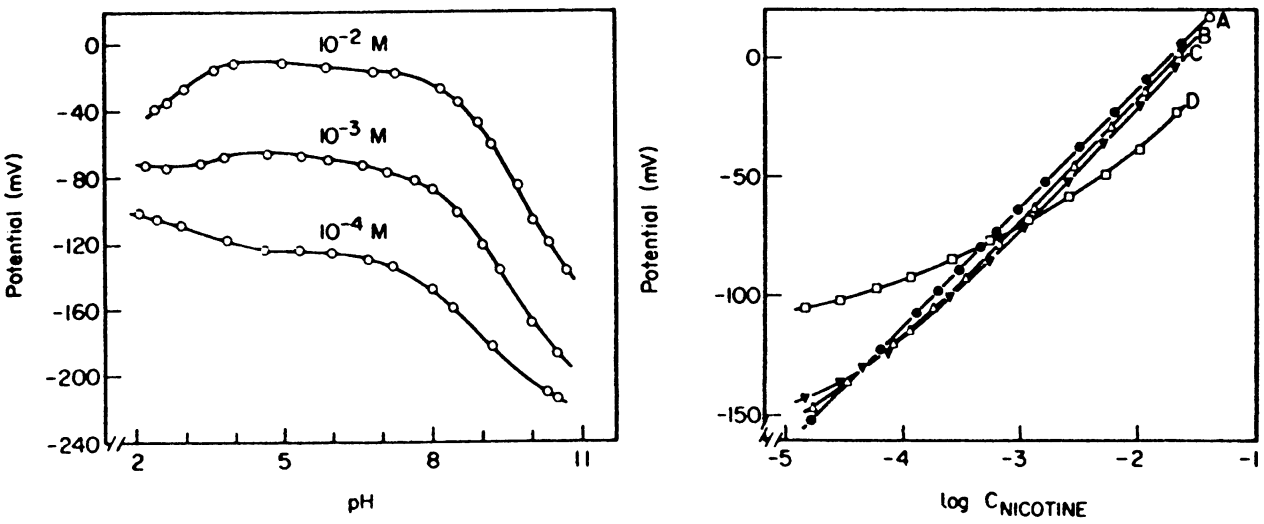

Fig. 2. Effect of pH on the potential of the nicotine-sensitive electrode.

Fig. 3. Calibration curves for the nicotine-sensitive electrode: (A) in pure nicotine solutions adjusted with HCl to pH 6—6.5; (B) in NaH_2PO_4—Na_2HPO_4 buffer, 0.059 M, pH 6.3; (C) as in B, but with a total phosphate concentration of 0.0083 M; (D) in sodium citrate—citric acid buffer, 0.059 M, pH 2.7.

where E_1 is the potential in a nicotine solution of activity α_{NicH^+}, E_2 is the potential in a solution containing nicotine and interfering cation j of activities α'_{NicH^+} and α_j, respectively, S is the electrode slope, and z is the valency of the cation j. The selectivity coefficients obtained are presented in Table 1.

Response times. The electrode provides stable potential readings (±0.1 mV) within 30 s to 2 min, depending on the actual nicotine concentration, presence of interferences, pH, etc. Generally, fast response (less than 1 min) is observed when the electrode is used in the proposed buffer solution.

Stability of response. The electrodes exhibited a day-to-day reproducibility within ±2 mV for nicotine concentrations in the range 2×10^{-4}—2×10^{-3} M,

TABLE 1

Potentiometric selectivity coefficients for the nicotine-sensitive electrode

Interference j	[j] (M)	[$NicH^+$] (M)	$k^{pot}_{NicH^+,j}$
Li^+	1.7×10^{-2}	4.2×10^{-4}	1.6×10^{-2}
Na^+	1.7×10^{-2}	4.2×10^{-4}	1.6×10^{-2}
K^+	1.7×10^{-2}	4.2×10^{-4}	1.4×10^{-2}
NH_4^+	1.7×10^{-2}	4.2×10^{-4}	1.1×10^{-2}
Ca^{2+}	1.7×10^{-2}	4.2×10^{-4}	4.0×10^{-3}
Mg^{2+}	1.7×10^{-2}	4.2×10^{-4}	3.5×10^{-3}
$(CH_3)_4N^+$	1.7×10^{-3}	4.2×10^{-3}	2.5
Pyridine H^+	1.7×10^{-3}	4.2×10^{-3}	1.0
Pyrrolidine H^+	1.7×10^{-3}	4.2×10^{-3}	0.18
Piperidine H^+	1.7×10^{-3}	4.2×10^{-3}	0.36

actually used in the analytical application, provided that the electrode was not used under unfavorable extreme conditions, i.e., in highly acidic or alkaline solutions or in the presence of strong interferences.

Electrode aging has no effect on the response time but has a marked effect on the slope S. The electrodes tested had an initial slope of about 58 mV/concentration decade but in one month of normal use, the slope decreased to about 48 mV/concentration decade. This not unusual disadvantage must be allowed for by determining the slope before each series of measurements because the slope must be accurately known for the standard addition method.

The electrode must be refilled when the liquid ion-exchanger has been exhausted or the supporting membrane has been damaged, and stable potentials are no longer obtainable.

Potentiometric titration of nicotine

The electrode can also be used as indicator electrode for the precipitation titration of nicotine with sodium tetraphenylborate. Such titrations can be used for the determination of the titer of relatively concentrated (ca. 0.01 M) nicotine solutions. In Fig. 4 two typical titration curves of nicotine with sodium tetraphenylborate are shown.

Treatment of samples and analytical results

Extraction of nicotine from tobacco products was incomplete with water or buffer at any temperature. Extraction with 0.2 M sodium hydroxide was incomplete at room temperature. The selected temperature and time of the alkaline extraction ensure complete separation of nicotine from its natural matrix provided that the sample is finely powdered. Erratic results were obtained when the electrodes were directly immersed in the tobacco suspension. Centrifugation was found to be the best way to remove the fine tobacco particles.

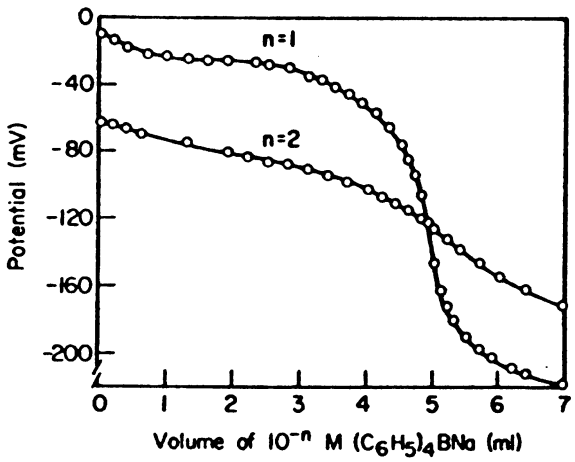

Fig. 4. Titration curves of 50.0 ml of $1.00 \times 10^{-(n+1)}$ M nicotine with 10^{-n} M sodium tetraphenylborate at pH 6.0.

TABLE 2

Potentiometric determination of nicotine in pure aqueous solutions with the nicotine-sensitive electrode
(Results are given as mg/20 ml)

Taken	0.65	0.97	1.61	2.26	2.58	3.21
Found[a]	0.69	0.96	1.63	2.30	2.63	3.32
Found[b]	0.69	0.97	1.56	2.20	2.55	3.07

[a]Based on the calibration curve obtained for the determination of S. [b]Based on the determined value of S and the difference of potential response after the addition of 100 μl of solution A (1.62 mg of nicotine).

For the determination of nicotine in aqueous solutions either the calibration curve method or the standard addition method can be used. Results for both methods are shown in Table 2.

The method was applied to the determination of nicotine in various brands of cigarettes, cigars and pipe tobacco. Nicotine was determined by both the proposed method and the official AOAC method [3]. Typical results are shown in Table 3. Results are generally in good agreement but the potentiometric method is less precise than the official method.

The accuracy of the proposed method was further checked by means of recovery experiments carried out on one representative tobacco sample. The results are shown in Table 4.

Conclusions

The potentiometric method for the determination of nicotine eliminates prior separation steps involving steam distillation techniques. Consequently a large number of samples may be processed simultaneously. The selectivity

TABLE 3

Comparison of potentiometric and AOAC methods for the determination of nicotine in tobacco products

Sample	Nicotine found (mg g^{-1})		Difference
	Present method[a]	AOAC method[b]	
1[c]	3.3 ± 0.2	2.7	0.6
2	10.0 ± 0.3	9.8	0.2
3	13.5 ± 0.3	12.9	0.6
4	14.8 ± 0.2	14.3	0.5
5	15.9 ± 0.6	16.6	−0.7
6	16.1 ± 0.5	15.8	0.3
7	16.7 ± 0.3	15.9	0.8
8	20.6 ± 0.9	19.9	0.7
9	20.8 ± 0.8	21.2	−0.4
			Av. 0.53

[a]Average and standard deviation of five measurements. [b]The standard deviation for the AOAC method was about 0.1 mg g^{-1}. [c]Cigarette tobacco partially extracted with ether.

TABLE 4

Recovery of nicotine added to tobacco samples

Amount of nicotine (mg per sample)			Recovery (%)
Initially present	Added	Found	
1.82	0.97	2.83	104.1
2.74	0.97	3.79	108.2
1.62	1.95	3.48	95.4
2.93	1.95	4.73	92.3
1.40	2.92	4.29	99.0
2.75	2.92	5.52	94.9
			Av. 98.9

and the sensitivity of the described nicotine-sensitive electrode are sufficient for the proposed application. This electrode may find a number of possible applications in the tobacco industry, such as in the quality control of tobacco products and in the process control by continuous monitoring of the nicotine content of various effluents and wastes.

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