

New modified polymeric electrodes selective to local anaesthetic compounds

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Abstract: New polymeric electrodes responding to the cationic forms of tetracaine (TC), lidocaine (LD), and procaine (PC) were constructed by incorporating their ion-pair complexes (the salts of TC, LD and PC with phosphotungstic acid) into ethylene–vinyl acetate (E/VAC) copolymer. Other ion pairing agents investigated were silicotungstate and tetraphenylborate. The phosphotungstic acid resulted in the best linear and Nernstian response. A 1:1 (v/v) mixture of dioctyl phthalate (DOP) and nitrobenzene (NB) was used as plasticizer. The electrodes exhibited linear response over the concentration ranges 10^{-2} – 5.6×10^{-6} , 10^{-2} – 2.5×10^{-5} and 10^{-2} – 1.8×10^{-5} M of TC, LD and PC, respectively. pH did not affect the electrode performances within the ranges 2.7–6.3, 2.6–6.7 and 2.8–7.5 for the three electrodes, respectively. Interferences are negligible for many organic base and alkali metal cations. Cations of similar structure interfere with LD and PC, but not appreciably with TC. Direct potentiometry was used to determine these compounds in pharmaceutical preparations with accurate results.

Keywords: *Ethylene–vinyl acetate; ion-selective electrodes; phosphotungstic acid; local anaesthetics; direct potentiometry.*

Introduction

Considerable attention has been paid to research in the field of ion-selective polymer-membrane electrodes [1, 2]. These electrodes are finding numerous applications in pharmaceutical analysis [2]. Generally, these sensors contain an ion-pair complex formed with a solvent mediator incorporated in a support matrix, usually poly(vinyl chloride) (PVC) [3–5]. Many other polymers such as poly(methacrylate) [6], poly(vinyl 2-methyl-propylether) [7], methyl methacrylate [8], and polyamide [9] or polyurethane [10] have been reported as suitable matrix materials for ISE construction, but these are not as widely used as is PVC.

The aim of this study was to investigate the use of a new copolymer, ethylene–vinyl acetate (E/VAC), in the construction of electrodes responsive to some local anaesthetic compounds: tetracaine (TC), lidocaine (LD) and procaine (PC). The preparation of these electrodes is easier than the method recommended for conventional plastic membrane ISEs [11]. The use of phosphotungstic acid as a counter-ion in the membranes is compared

with silicotungstic acid and sodium tetraphenylborate.

The dependence of electrode performance on the plasticizers used is discussed. The electrodes are used in direct potentiometric determination of TC, LD and PC in pharmaceutical preparations.

Experimental

Reagents

All reagents were of analytical-reagent grade, and were used as received. Tetracaine hydrochloride, procaine hydrochloride and lidocaine were supplied by Sigma. E/VAC, 40% by wt of the vinyl acetate monomer (Levapren 400), was obtained from Bayer. The other reagents and suppliers are as follows: phosphotungstic acid (PT), silicotungstic acid (ST), sodium tetraphenylborate (TPB) and nitrobenzene (NB) from Merck; tetrahydrofuran (THF) and dioctyl phthalate (DOP) from Janssen. 0.1 M solutions of TC and PC were prepared by dissolving the appropriate amount of pure substance in deionized water, while LD was dissolved in 0.16 M HCl. Stan-

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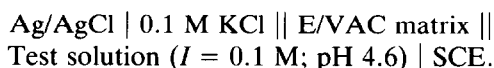
dard solutions were freshly prepared by accurate dilution of 0.1 M solutions.

Construction of the electrodes

The TC, LD and PC-phosphotungstate ion pairs were prepared by the same method as described previously [12]. A 25-ml portion of 1×10^{-2} M local anaesthetic was mixed at room temperature with an equal volume of 1×10^{-2} M phosphotungstic acid. The precipitates obtained were filtered in a sintered-glass crucible (G4), washed with deionized water and dried at 50°C. The polymeric membranes were constructed by simply dipping the end of the tip of a micropipette into the E/VAC solution, which was prepared as follows. About 320 mg of E/VAC was dissolved in 4 ml of the THF, and then 1 ml of DOP and 1 ml of NB were added as plasticizing reagent. A 20-mg portion of the appropriate ion-pair complex was added to this mixture, and was dispersed homogeneously into the matrix by exposing the polymer mixture to ultrasound. After drying for about 2 h, the other end of the tip of the micropipette was filled with an internal reference solution (1 M KCl), and a Ag/AgCl electrode was used as the internal reference electrode.

Potential measurements

The composition of the electrochemical cell was as follows:



Potential measurements were made with the same apparatus as described previously [9].

A 10-ml portion of 0.1 M acetate buffer solution (pH 4.6) was transferred into a cell. The working electrode together with the standard calomel electrode (SCE) was immersed in the solution with constant stirring (without air bubbles), and a 100- μl aliquot of 10^{-5} – 10^{-1} M aqueous local anaesthetic compound was added to give concentrations of 10^{-7} – 10^{-3} M in the measurement cell; 1.1 ml of 0.1 M standard was added to give 10^{-2} M. The potential readings were recorded after stabilization to 1 mV min^{-1} , and the calibration graphs were plotted in the usual way. The slopes of the electrode responses were found by regression analysis of the linear part of the graph. Typical calibration graphs for the electrodes described are shown in Fig. 1.

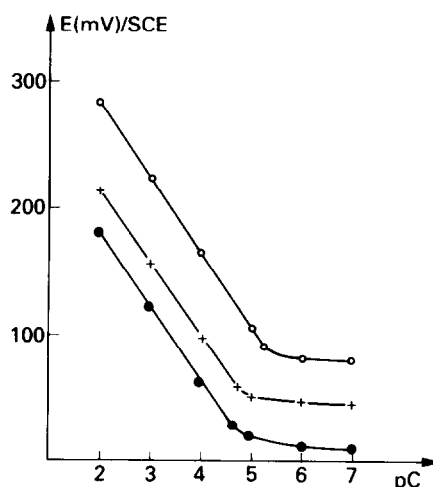


Figure 1 Potential response curves for the local anaesthetic-selective electrodes: lidocaine (●), procaine (+) and tetracaine (○); pH 4.6.

Results and Discussion

Ethylene-vinyl acetate copolymer

An E/VAC copolymer membrane electrode has been recently studied as a membrane matrix for calcium ion-selective electrode preparation [13]. Compared with PVC membranes, it exhibited similar characteristics in terms of sensitivity, slope and pH effect. Selectivity coefficient values were generally similar, although the E/VAC polymer resulted in improved selectivity over Zn^{2+} and Cu^{2+} . These studies suggested that E/VAC membranes should be suitable for other electrodes.

Selection of active materials and plasticizer

Many anions react with local anaesthetics to form ion-pair complexes. TPB [14], tetrakis (3-chlorophenylborate) [15], dinonylnaphthalene sulphonate [16] and reineckate [17] have been used as active materials in PVC-based as well as liquid membrane electrodes. In this study, TPB, silicotungstate and phosphotungstate were tested as counter ions for TC-, LD- and PC-selective electrodes plasticized by the DOP-NB mixture. The response characteristics of these electrodes are summarized in Table 1.

Phosphotungstate-based electrodes showed better linear response, with Nernstian slopes, than silicotungstate- and TPB-based electrodes. This may be due to the fact that TC, LD and PC-phosphotungstate ion-pairs are more hydrophobic than the corresponding TPB and silicotungstate ion-pairs.

Table 1
Response characteristics for various E/VAC, NB + DOP-based local anaesthetic-selective electrodes

Ion pairing agents:	Tetracaine electrode			Lidocaine electrode			Procaine electrode		
	PT	ST	TPB	PT	ST	TPB	PT	ST	TPB
Slope (mV dec ⁻¹)	59	48	54	59	45	52	58	42	45
Linear range (M)	10^{-2} - 5.6×10^{-6}	10^{-2} - 7×10^{-6}	10^{-2} - 7×10^{-6}	10^{-2} - 2.5×10^{-5}	10^{-2} - 10^{-4}	10^{-2} - 5×10^{-5}	10^{-2} - 1.8×10^{-5}	10^{-2} - 5.6×10^{-5}	10^{-2} - 7×10^{-5}
Detection limit (M)	3.5×10^{-6}	4×10^{-6}	4.5×10^{-6}	1.4×10^{-5}	4×10^{-5}	3×10^{-5}	6.3×10^{-6}	1.4×10^{-5}	3×10^{-5}
Corr. coef. (r)	0.9999	0.9994	0.9997	0.9999	0.9999	0.9975	0.9997	0.9999	0.9996

Table 2
Response characteristics for various E/VAC, PT-based local anaesthetic-selective electrodes

Plasticizers:	Tetracaine electrode			Lidocaine electrode			Procaine electrode		
	DOP + NB	NB	DOP	DOP + NB	NB	DOP	DOP + NB	NB	DOP
Slope (mV dec ⁻¹)	59	57	57	59	58	56	58	52	43
Linear range (M)	10^{-2} - 5.6×10^{-6}	10^{-2} - 4.5×10^{-6}	10^{-2} - 4.5×10^{-6}	10^{-2} - 2.5×10^{-5}	10^{-2} - 7×10^{-5}	10^{-2} - 5.6×10^{-5}	10^{-2} - 1.8×10^{-5}	10^{-2} - 5.6×10^{-5}	10^{-2} - 10^{-4}
Detection limit (M)	3.5×10^{-6}	2.5×10^{-6}	2.8×10^{-6}	1.4×10^{-5}	2×10^{-5}	2.5×10^{-5}	6.3×10^{-6}	2.5×10^{-5}	4.5×10^{-5}
Corr. coef. (r)	0.9999	0.9999	0.9997	0.9999	0.9999	0.9998	0.9997	0.9997	0.995

Two solvents were tested as plasticizers for electrodes containing phosphotungstate. The response characteristics of these electrodes are summarized in Table 2. The best results are found by using a mixture of DOP–NB as plasticizer, rather than the individual plasticizers. While the linear range and the detection limit of TC-selective electrodes are somewhat improved by using NB or DOP alone, the electrodes containing a single plasticizer suffer from poor reproducibility and unstable potential readings, especially for DOP plasticized electrodes. Also, the lifetimes of these DOP or NB plasticized electrodes do not exceed 1 day.

No evidence was found that the plasticizers used were involved in determining the selectivities of the electrodes towards the investigated cations. Prior studies [18, 19] indicate that the use of an electron-deficient plasticizer like NB causes the membrane to respond to anion alone. Our TC, LD and PC electrodes containing NB as a single plasticizer show excellent response for these cations. Kang and Wang [20], using NB or dimethyl phthalate as single plasticizer for a nicotine-selective electrode, reported better results with NB. On the other hand, nitrophenyloctyl ether (NPOE) and nitrophenyl phenyl ether (NPPE), also reportedly electron-deficient plasticizers, are frequently used as plasticizers for cation-selective electrodes [21–24], as well as for anion-selective electrodes [24–26]. Moreover, DOP, said to be electron-donating [19] and useful for providing specificity to cations alone, was used by Shoukry [27] to plasticize TPB- and hexadecylpyridinium-selective electrodes.

From the above results, we selected PT as counter ion and the 1:1 mixture of DOP–NB as the plasticizer to prepare and characterize the local anaesthetic-selective electrodes in subsequent studies.

Effect of pH

The influence of pH on the potential of the electrodes was investigated by measuring the e.m.f. of the cell at 10^{-3} M local anaesthetic. The pH values of the cell were adjusted by adding small volumes of concentrated sodium hydroxide solution (10 M) to the initial solution of 0.1 M acetic acid. The results shown in Fig. 2 indicate that the electrodes can function effectively in pH ranges 2.7–6.3, 2.6–6.7 and 2.8–7.5 for the TC, LD and PC electrodes, respectively. The gradual decrease in potential observed at high pH values is due to the

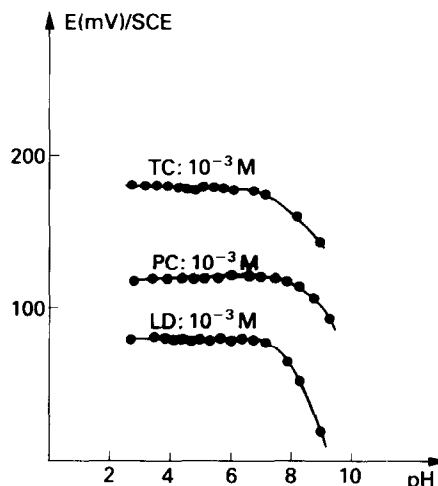


Figure 2
Effect of pH on the response of the local anaesthetic-selective electrodes: TC, 10^{-3} M; PC, 10^{-3} M; and LD, 10^{-3} M.

gradual decrease in the protonated forms of the drugs.

The potential–pH curves can be used to calculate the dissociation constants, K_a , of the three drugs. Since pK_a is equal to the pH value at which the initial concentration of the protonated local-anaesthetic compound is halved, the pH at which the potential of the respective electrode decreases by $S \log 2$ (mV) (S = electrode slope) provides pK_a [28]. The pK_a values of TC, LD and PC were found to be 8.2 ± 0.1 , 7.9 ± 0.1 and 8.9 ± 0.1 , respectively, for the three compounds. These values are in good agreement with the literature values of 8.39, 7.86 and 8.7, respectively [29].

Selectivity of electrodes

The response of the present electrodes toward different substances was determined, and the selectivity coefficients, $K_{A,B}^{pot}$, were used to evaluate their interferences. The $K_{A,B}^{pot}$ values given in Table 3 were evaluated by the standard mixed-solution method [30]. Calibration curves were established by measuring the potential for mixed solutions, each containing 10^{-2} M foreign ion and variable local anaesthetic concentrations in the range 10^{-5} – 10^{-2} M. The $K_{A,B}^{pot}$ values were calculated from the graphs using the following equation:

$$K_{A,B}^{pot} = c_A / (c_B)^{z_A/z_B},$$

where c_A is the concentration of the primary ion, of charge z_A , and c_B is the concentration of the interfering ion, of charge z_B .

Table 3
Selectivity coefficients for the local anaesthetic-selective electrodes

Interferent, B	Selectivity coefficient ($K_{A,B}^{pot}$)		
	Tetracaine electrode	Lidocaine electrode	Procaine electrode
Tetracaine	—	1.4	21
Lidocaine	1.3×10^{-2}	—	7.9×10^{-1}
Procaine	1.3×10^{-2}	2.2×10^{-1}	—
Phenazone	1.0×10^{-4}	1.2×10^{-2}	4.0×10^{-2}
Ephedrine	1.1×10^{-3}	5.0×10^{-2}	6.0×10^{-2}
Urea	1.2×10^{-4}	3.1×10^{-3}	1.0×10^{-3}
Glycine	1.9×10^{-4}	2.2×10^{-3}	1.4×10^{-3}
Caffeine	2.7×10^{-4}	5.4×10^{-3}	1.9×10^{-3}
L-Histidine	2.1×10^{-4}	2.7×10^{-3}	1.6×10^{-3}
Ascorbic acid	1.1×10^{-4}	3.2×10^{-3}	1.5×10^{-3}
Citric acid	5.6×10^{-5}	3.5×10^{-3}	1.9×10^{-3}
Lactic acid	7.0×10^{-5}	4.2×10^{-2}	2.0×10^{-3}
Na	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}
K	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}

The data presented show that the three electrodes exhibit very small interferences from many organic and the alkali metal ions, notably from phenazone, ephedrine, ascorbic acid and lactic acid, which are frequently present in TC, LD and PC preparations. Cation molecules of similar structure (i.e. the other local anaesthetics) interfere with LD and PC, but only to a small degree with TC, whose electrode exhibits high selectivity. While the relative non-selectivity of the LD and PC electrodes would preclude their application in mixtures of local anaesthetics, they are useful in monitoring pharmaceutical preparations of the individual local anaesthetics (see below). Concerning the TC electrode, the lower values of all its selectivity coefficients compared with those of LD and PC electrodes should be noted (Table 3). The poorer selectivity of these two last electrodes may be due to the fact that the ion-pair complexes of LD and PC with phosphotungstate are less lipophylic than that formed by TC. In fact, the selectivity coefficients are related to the partition coefficients [4, 31–33] and in some cases to the stability constant of the complexes involved [31].

Response time

The response times of the three E/VAC electrodes were fast, being nearly instantaneous in the 10^{-2} – 10^{-4} M range, demonstrating that the interface process attains equilibrium very rapidly. In more dilute solutions, 50–60 s were necessary to reach stable response. These electrodes exhibited good day-to-day stability and reproducibility in the e.m.f. readings in their linear ranges. The

slopes of the calibration curves, as well as the usable range, did not change over 4 months for the TC electrode, 2 months for the LD electrode and 1 month for the PC electrode. These electrodes cannot be used under unfavourable extreme solution conditions, i.e. in highly alkaline solutions.

Analytical application

The electrodes proved to be useful for the determination of the drugs present in pharmaceutical preparations. Direct potentiometry was applied without any prior separation or extraction. Different aliquots of local anaesthetic present in solution were added to 10 ml of 0.1 M acetate buffer. The weight of aliquot added was chosen in a way to give a final concentration of local anaesthetic in the range of the concentrations used to calibrate the electrode. The results of these assays are listed in Tables 4–6. Average recoveries of 99.7, 101.0 and 99.9% with mean relative standard deviations of 1.1, 1.3 and 0.46% for the determination of TC, LD and PC, respectively, demonstrate that the electrodes are suitable for application in pharmaceutical analysis.

Conclusion

The direct potentiometry procedure with the three E/VAC membrane electrodes is rapid, simple, sensitive, precise and inexpensive for the determination of some local anaesthetic compounds in pharmaceutical preparations, without prior treatment, i.e. by direct dissolution in 0.1 M acetate buffer at pH 4.6. From the above discussions, we conclude that

Table 4
Assay of tetracaine in pharmaceutical preparation*

Compound	Weight sample added (g)	Principal ingredient weight (mg TC)	Found† (mg)	Accuracy (%)	RSD (%)
Otosan (10 mg g ⁻¹)	0.025	0.25	0.248	99.2	0.40
	0.061	0.61	0.603	98.9	1.8
	0.069	0.69	0.693	100.4	1.3
	1.110	1.10	1.096	99.6	0.48
	1.180	1.18	1.186	100.5	1.3
Mean				99.7	1.1

* Calibration between 10⁻⁵ and 10⁻⁴ M; slope, 59.2 mV dec⁻¹.

† Average of three determinations.

Table 5
Assay of lidocaine in pharmaceutical preparation*

Compound	Volume sample added (ml)	Principal ingredient weight (mg LD)	Found† (mg)	Accuracy (%)	RSD (%)
Xylocaine 2% 20 mg 10 ml ⁻¹	1.000	2.00	2.04	102	0.75
	0.500	1.00	1.02	102	3.0
	0.250	0.500	0.495	99.0	0.51
	0.125	0.250	0.253	101.2	0.82
Mean				101.0	1.3

* Calibration between 10⁻³ and 10⁻⁴ M; slope, 61 mV dec⁻¹.

† Average of three determinations.

Table 6
Assay of procaine in pharmaceutical preparation*

Compound	Weight sample added (g)	Principal ingredient weight (mg PC)	Found† (mg)	Accuracy (%)	RSD (%)
Tymalgine (50 mg g ⁻¹)	0.025	1.25	1.253	100.2	0.62
	0.027	1.35	1.347	99.8	0.24
	0.029	1.45	1.434	99.9	0.21
	0.041	2.05	2.043	99.7	0.75
Mean				99.9	0.46

* Calibration between 10⁻³ and 10⁻⁴ M; slope, 59.5 mV dec⁻¹.

† Average of three determinations.

the E/VAC copolymer, like PVC and some other polymers, is suitable for the construction of polymeric electrodes.

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