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Coated Wire Electrodes Sensitive to Local Anesthetic Cations and Their Application to Potentiometric Determination

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Coated wire electrodes sensitive to procaine(PC), tetracaine(TC), lidocaine(LC), dibucaine(DC), mepivacaine(MC), and bupivacaine(BC), based on ion-pair formation between tetraphenylborate ions and the corresponding local anesthetic cations in a plasticized poly(vinyl chloride) membrane were prepared. The electrodes showed linear responses with Nernstian slopes (55—58 mV/decade) over concentration ranges of 4×10^{-5}—10^{-2}, 4×10^{-5}—10^{-2}, 10^{-4}—10^{-2}, 4×10^{-5}—10^{-2}, 5×10^{-5}—10^{-2}, and 2×10^{-4}—10^{-2} mol dm^{-3} for PC, TC, LC, DC, MC, and BC, respectively. The change in pH did not affect the electrode performance within ranges of 3.2—7.4, 3.2—7.4, 2.1—7.7, 2.5—7.4, 3.5—7.0, and 2.4—5.6 for PC, TC, LC, DC, MC, and BC, respectively. The reproducibility, stability, response time, and selectivity were also investigated. The electrodes were applied satisfactorily to the determination of local anesthetics by potentiometry.

Many local anesthetics in clinical use are basically tertiary amine compounds, and are classified as being of the ester type [procaine (PC), tetracaine (TC), benzocaine etc.] and amide type [lidocaine (LC), dibucaine (DC), mepivacaine (MC), bupivacaine (BC) etc.]. These drugs exist in both positively charged and uncharged forms under normal in vivo conditions.[1] Both species apparently have different biological activities. The establishment of analytical methods for both species will provide useful information concerning the molecular mechanism of local anesthetic action.[2,3]

Although many methods[4—10] (including polarography, spectrophotometry, HPLC, and fluorometry) have been reported for the determination of local anesthetics, these are generally used for measuring the total concentration of local anesthetics. Potentiometry[11—17] using an ion-selective electrode has an advantage in that the electrode enables estimates of free cationic anesthetics. An ion-selective electrode comprising a poly(vinyl chloride) (PVC) membrane is the most suitable type for use as a sensor in the potentiometric determination of organic cations like local anesthetics. So far reported have been a liquid-membrane electrode[12] incorporating PC–tetraphenylborate (TPB^-) and a PVC membrane electrode with a PC–TPB ion pair[11,16] and crown ether[15] used as the sensor elements; these electrodes have complicated structures and electrodes preparation is time consuming. On the other hand, a coated wire electrode based on a PVC membrane can easily be prepared in various shapes and miniaturized. Coated wire electrodes have been used as sensors in the potentiometric titration of PC by K. Vytras et al;[13,14] other local anesthetics were not studied.

This paper discusses the preparation of PVC-membrane coated wire electrodes sensitive to PC, TC, LC, DC, MC, and BC, as well as some of the properties of the resulting electrodes. The concerned electrodes were used successfully as sensors in direct potentiometry and potentiometric titration of local anesthetics.

Experimental

Reagents. All of the reagents used in the preparation of the electrode and solution were of analytical grade. The hydrochloride salts of local anesthetics used were purchased from Sigma Chemical Co., except for lidocaine hydrochloride (Fujisawa Yakuhin Co.). The chemical structures of the local anesthetics used are shown in Fig. 1. Sodium tetraphenylborate (NaTPB) (Aldrich), dioctyl phthalate (DOP) (Tokyo Kasei), PVC (Wako) of high molecular weight, and tetrahydrofuran (THF) (Wako) were used; the THF solvent was dehydrated with molecular sieves (Wako). Ion-pairs of PC–TPB, TC–TPB, LC–TPB, DC–TPB, MC–TPB, and BC–TPB were prepared according to the same method described previously for an ion pair of tetraphenylphosphonium–tetraphenylborate,[18] and were identified by elemental analysis. Water was purified by triple distillation, once from an alkaline potassium permanganate solution.

Construction of Electrode. A coated wire electrode sensitive to local anesthetic cations was prepared by the same method described previously.[18] The copper wire (0.8 mm diameter) was coated with a PVC-membrane of ca. 0.4 mm thickness. The solution used for the PVC-membrane usually included ion pairs, DOP, PVC, and THF with various compositions. The electrode was allowed to stand for at least ten hours in contact with air; it was then conditioned by soaking in 10^{-3} mol dm^{-3} of a local anesthetic drug solution or water prior to measurements.

Potential Measurement. The electrochemical system was as follows: A local anesthetic coated wire electrode/test solution/1 mol dm^{-3} sodium nitrate agar-bridge/saturated potassium chloride/Ag-AgCl electrode.

The electromotive force (EMF) was measured at 25 °C with a multi-ion monitor (Yamashita Giken) in a constantly stirred solution. The temperature of the test solution was controlled by circulating water from a thermostatic bath (Komatu Yamato) through a double-wall beaker.

Calibration curves of the EMF vs. the logarithm of the anesthetic concentration were made as follows: The EMF values were recorded after suitable increments of standard local anesthetic solution was added to 50 cm^3 of water, so as to cover the concentration range 10^{-6}—10^{-2} mol dm^{-3}. The

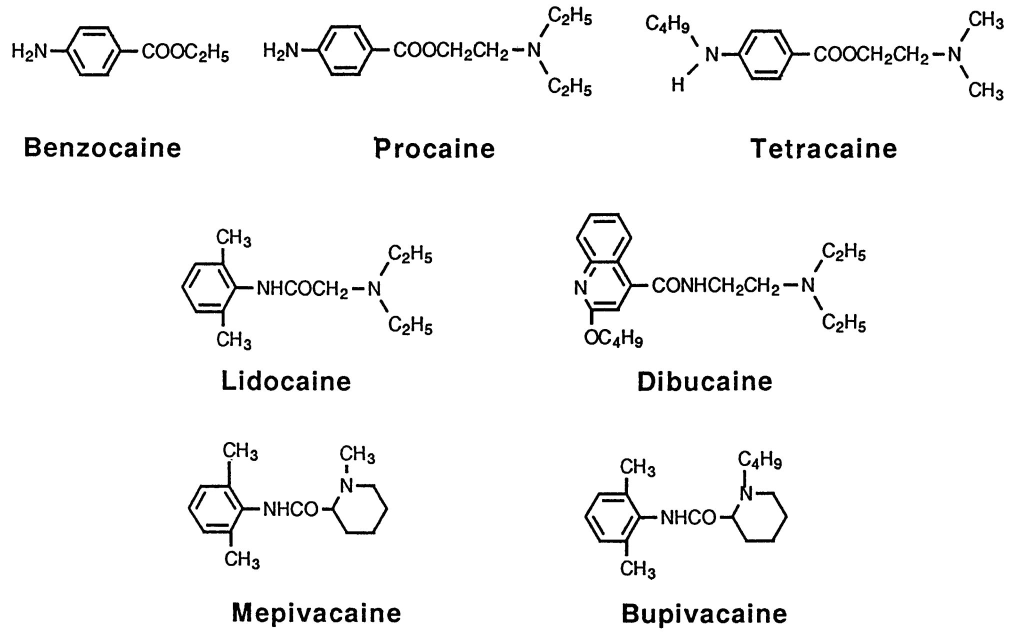

Fig. 1. Chemical structure of local anesthetics.

local anesthetic coated wire electrode and the sodium nitrate agar-bridge connected to a reference electrode was immersed in each test solution; the EMF values were recorded after each addition.

Potentiometric Titration. The anesthetic solutions for potentiometric titration were prepared in three ways. Ten cm^3 of test solution was transferred into a 100 cm^3 beaker; distilled water was added until obtaining a total volume of 50 cm^3. The resulting solution was titrated with 10^{-2} mol dm^{-3} standard NaTPB solution using the respective electrode as the sensor.

Results and Discussion

Response of the Electrode. Since the potential response of the coated wire electrode is considered to be similar to that of a liquid-membrane electrode,[19,20] the membrane potential of the electrodes depends on the ion-exchange process at the membrane/solution interface, the mobilities of anesthetic cations in the membrane, and the potentials at the membrane/electrode copper interface. Provided that both the potential at the copper/PVC membrane interface and the diffusion potential in the PVC membrane are constant, and the anesthetic cations (A^+) and TPB^- (X^-) in the membrane are in chemical equilibrium with the anesthetic–TPB ion pairs (AX) in the membrane, the membrane potential of the electrode (E_M) can be represented by

$$\begin{aligned} E_M &= \text{Const} + \frac{RT}{Z_A F} \ln \frac{a_{A^+}}{\bar{C}_{A^+}} \\ &= \text{Const} + \frac{RT}{Z_A F} \ln a_{A^+} + \frac{RT}{Z_A F} \ln K_{AX} \cdot \bar{C}_{X^-} \\ &\quad - \frac{RT}{Z_A F} \ln \bar{C}_{AX}, \end{aligned} \tag{1}$$

where a_{A^+} is the activity of the anesthetic cation in the test solution, and Z_A is the charge of anesthetic cation. $\bar{C}_{A^+}$, $\bar{C}_{X^-}$ and $\bar{C}_{AX}$ represent the concentrations of the anesthetic cation, TPB^- and ion pairs in the membrane, respectively. K_{AX} is the association constant of the ion pair. F, R, and T are the Faraday constant, gas con-

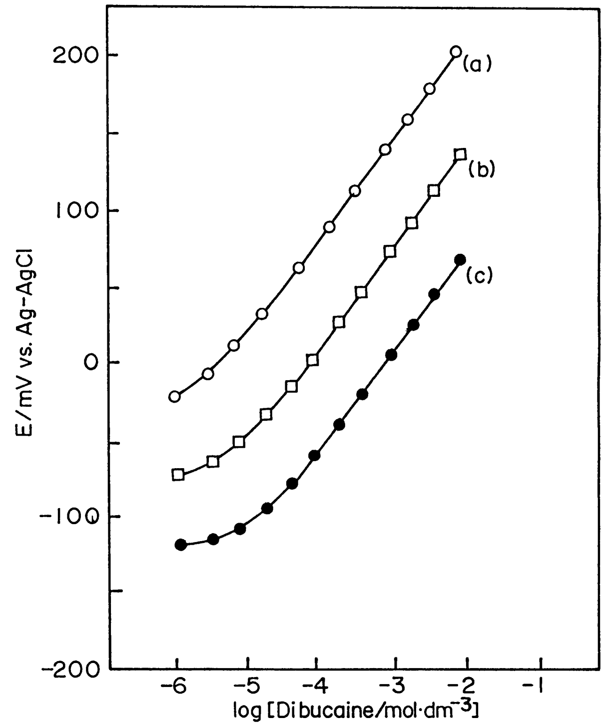

Fig. 2. Effect of the number of ion pairs in a PVC membrane on the potential of a dibucaine electrode. (a) Ion pair 1 mg+PVC 110 mg+DOP 100 mg, (b) Ion pair 5 mg+PVC 110 mg+DOP 100 mg, (c) Ion pair 50 mg+PVC 110 mg+DOP 100 mg.

stant and absolute temperature, respectively.

Equation 1 means that the higher is the concentration of the anesthetic–TPB ion pairs in PVC membrane, the lower is the membrane potential. Figure 2 shows the response curve for a dibucaine electrode containing various amounts of the dibucaine–TPB ion pairs in the PVC membrane. The potential response curve shifts to the negative side with increasing number of ion pairs in the PVC membrane, as expected from Eq. 1. Consequently, the membrane potential was defined as the activity of the anesthetic cations in solution in the case of a constant concentration of anesthetic–TPB ion pairs in the PVC membrane.

If interfering ions (B^+) exist, when the concentration of anesthetic–TPB ion pairs in the PVC membrane is kept constant, the membrane potential of the electrode is empirically given by

$$E_M = \text{Const} + \frac{RT}{Z_A F} \ln(a_{A^+} + K_{AB} \cdot a_{B^+}{}^{\frac{Z_A}{Z_B}}), \qquad (2)$$

where K_{AB} is the selectivity coefficient of the B^+ ions for the anesthetic electrode, and a_{B^+} and Z_B are the activity and charge of a B^+ ion.

Composition of Electrode Membrane. Coated wire electrodes sensitive to local anesthetics with various membrane compositions were prepared, and the effect of the membrane composition on both the response time of the electrode and the slope of the EMF vs. concentration curve was investigated. A suitable PVC-membrane composition is shown in Table 1. The best results were obtained with an electrode membrane prepared from mixtures of local anesthetic–TPB ion pairs (5 mg), DOP (100 mg), PVC (110 mg) and 1.5 cm^3 of THF. Typical calibration curves of PC, TC, LC, DC, MC, and BC are shown in Fig. 3. The usable concentration range determined by the linear region of the calibration curves was 4×10^{-5}—10^{-2}, 4×10^{-5}—10^{-2}, 10^{-4}—10^{-2}, 4×10^{-5}—10^{-2}, 5×10^{-5}—10^{-2}, and 2×10^{-4}—10^{-2} mol dm^{-3} for PC, TC, LC, DC, MC, and BC, respectively. The response of the concerned electrodes was Nernstian (57.7, 56.6, 55.9, 58.6, 54.8, and 58.2 mV/decade for PC, TC, LC, DC, MC, and BC, respectively) and the standard relative deviation (%) of the slopes was less than 1%. The electrode can be used to determine the concentration of local anesthetic cations over a period of one month. When the contents of DOP (<50 mg) and ion-pairs (<1 mg) decreased, the electrode potential became unstable because of the high resistance of the membrane. A large excess of DOP and ion pairs in the membrane caused the surface of the copper wire to gradually become black, thus shortening the electrode lifetime and decreasing the slope of the potential curves.

Response Time and Reproducibility. The response time of the local anesthetic electrode was less than 30 seconds for a local anesthetic concentration range of 10^{-2} to 10^{-5} mol dm^{-3} and 2 min for 10^{-6} mol dm^{-3}. The reproducibility of the electrode potential was checked by either increasing or decreasing the local anesthetic concentration. The fluctuation of the electrode potentials was within a 1% standard relative deviation, with a concentration range from 10^{-4} to 10^{-2} mol dm^{-3} or from 10^{-2} to 10^{-4} mol dm^{-3}. Even after a

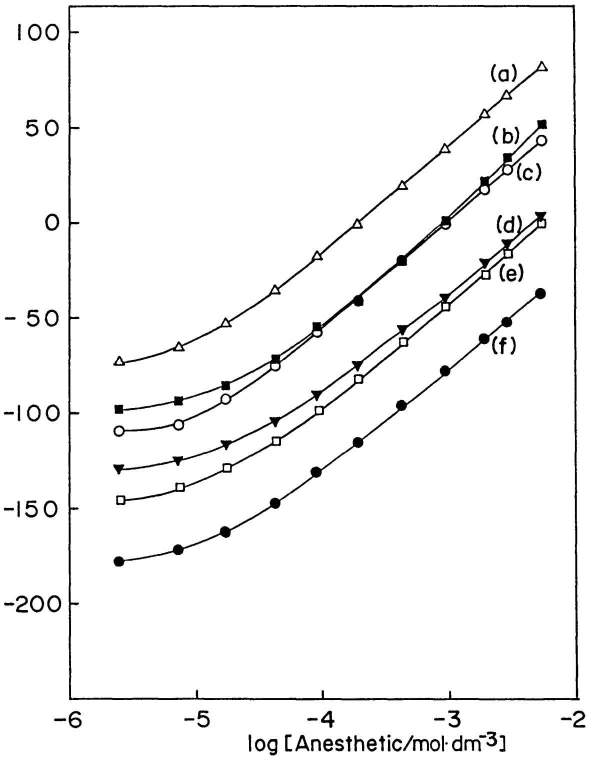

Fig. 3. Relationship between the local anesthetic concentration and the electrode potential: (a) Dibucaine; (b) Bupivacaine; (c) Tetracaine; (d) Lidocaine; (e) Mepivacaine; (f) Procaine.

Table 1. Suitable Composition of a PVC Membrane

Electrode	Ion-pair	DOP	PVC	THF	Slope	
	mg	mg	mg	ml	(mV/decade)	S.R.D (%)
Procaine	1-12 (5)	100	110	1.5	57.7	0.5
Tetracaine	1-10 (5)	100	110	1.5	56.6	0.5
Dibucaine	1-25 (5)	100	110	1.5	58.6	0.3
Lidocaine	1-25 (5)	100	110	1.5	55.9	0.9
Mepivacaine	1-10 (5)	100	110	1.5	56.8	0.6
Bupivacaine	1-25 (5)	100	110	1.5	58.2	0.5

S.R.D; Standard relative deviation. ( ): Suitable amount.

Table 2. Effect of the Ionic Strength on the Performance Characteristic of a Procaine Electrode

Ionic strength[a)]	Slope	Intercept at log[PC]=0.0	Usable log[PC] range	Correlation coefficient
0.0	56.3	76.4	−4.8—−2.2	0.9999
0.06	56.2	70.8	−4.5—−2.2	0.9998
0.107	56.3	70.5	−4.5—−2.2	0.9999
0.167	55.9	69.1	−4.2—−2.2	0.9999

a) NaCl added.

continuous measurement of the electrode potential over a period of eight hours, the change of the electrode potential was within 3 mV.

Effect of Ionic Strength. The performance characteristics of the PC electrode at various ionic strengths were examined by adding sodium chloride as the supporting electrolyte. The results are given in Table 2. The slope was almost independent of the ionic strength. The usable range of the calibration curve became slightly narrow.

Effect of pH. The effect of the pH on the electrode potential was examined for 10^{-3} mol dm^{-3} test solutions of local anesthetics, except for dibucaine (10^{-4} mol dm^{-3}). Figure 4 shows the variation in the electrode potential with changes in the pH by the addition of 10^{-1} mol dm^{-3} hydrochloric acid and/or sodium hydroxide solutions which included a corresponding local anesthetic of 10^{-3} mol dm^{-3}. The results indicate that the change in the pH did not affect the potential reading within the pH ranges 3.2—7.4, 3.2—7.4, 2.1—7.7, 2.5—7.4, 3.5—7.0, and 2.4—5.6 for PC, TC, LC, DC, MC, and BC, respectively. In acidic media of lower pH than these ranges, the electrode potential decreased as the pH value decreased. This may be attributed to a disappearance of the protonated local anesthetic, since they transformed to a diprotonated species.[11)] The negative shift of the electrode potential at pH higher than about 7.5[11)] may be due to a decrease in the concentration of the protonated species as a result of the precipitation of uncharged species of the local anesthetic.[11,21)] In the case of the BC electrode, positive shifts of the electrode potential were observed at pH higher than 5.6. This phenomenon needs further clarification.

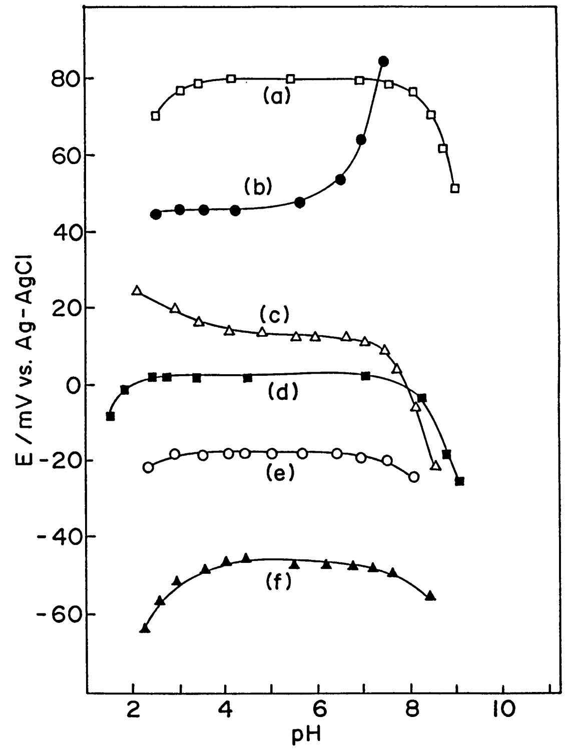

Fig. 4. Effect of pH on the response of a local-anesthetic coated wire electrode: (a) Tetracaine (9.92×10^{-4} mol dm^{-3}); (b) Bupivacaine (9.92×10^{-4} mol dm^{-3}); (c) Mepivacaine (9.92×10^{-4} mol dm^{-3}); (d) Lidocaine (9.92×10^{-4} mol dm^{-3}); (e) Dibucaine (9.98×10^{-5} mol dm^{-3}); (f) Procaine (10^{-3} mol dm^{-3}).

Selectivity of Electrodes. The selectivity of a membrane electrode based on ion-pair formation depends on the selectivity of ion-exchange at the membrane-test solution interface, the mobilities of the respective ions in the membrane, and hydrophobic interactions between the anesthetic cation and the organic membrane. The influence of some inorganic and organic cations, as well as the various substances contained in a tablet or injection solution of the local anesthetic drug, on the electrodes were investigated. The selectivity coefficients determined by a separate solution method[22,23)] using 10^{-2} mol dm^{-3} of local anesthetic and interferent solution are summarized in Table 3. None of the investigated species (in Table 3) were found to interfere, as shown by the very small values of the selectivity coefficients, except for atropine, to the PC electrode. It is known that an atropine reacts with TPB$^-$ to form an atropine–TPB ion pair.[24)] The low selectivity of the PC electrode toward atropine is attributable to the partial ion-exchange of PC in a PC–TPB ion pair with atropine. The selectivity coefficients of each electrode toward other anesthetic cations was determined (summarized in Table 4). None of the electrodes were interfered with benzocaine. The DC electrode was found to have the most significant selectivity toward other local anesthetics, and the selectivity coefficients decreased in the order TC=BC>MC=LC>PC>benzocaine. The PC electrode has the lowest selectivity toward other local anesthetics. The PC electrode can therefore be used to measure the PC, TC, LC, DC, and BC concentration, as is shown in Fig. 5, even though its response is very slow. The difference in the hydrophobic property of local anesthetic molecules seems to be responsible for the difference in the electrode potential and the selectivity

Table 3. Selectivity Coefficient of Procaine, Tetracaine, Lidocaine, Dibucaine, Mepivacaine, and Bupivacaine Electrodes

Interferent	Procaine	Tetracaine	Lidocaine	Dibucaine	Mepivacaine	Bupivacaine
Sodium nitrate	−3.25	−2.98	−3.22	−2.50	−2.64	−2.76
Sodium chloride	−4.16	−4.05	−3.56	−3.62	−3.11	−3.43
Potassium nitrate	−3.21	−2.79	−2.89	−3.58	−2.39	−3.29
Disodium hydrogenphosphate	−3.36	−3.04	−4.05	−4.48	−2.93	−3.28
Calcium chloride	−4.49	−3.02	−4.40	−4.62	−3.45	−3.62
Leucine	−3.32	−2.80	−3.14	−3.91	−3.13	−2.35
Nicotinic acid	−3.34	−3.78	−2.19	−3.91	−2.39	−2.35
Atropine	−0.85	−3.20	−1.45	−3.16	−2.48	−2.98
Piperazine	−3.15	−1.91	−5.33	−4.05	−4.11	−4.95
Urea	−3.18	−3.93	−4.28	−4.26	−3.14	−2.98
Benzoic acid	−3.23	−3.41	−4.04	−4.00	−2.60	−2.83
Triethanolamine	−3.13	−3.14	−3.65	−3.80	−2.52	−2.48
Caffeine	−3.26	−3.01	−3.52	−4.10	−2.65	−2.27

Table 4. Selectivity Coefficient of Procaine, Tetracaine, Lidocaine, Dibucaine, Mepivacaine, and Bupivacaine Electrodes

Interferent	Procaine	Tetracaine	Lidocaine	Dibucaine	Mepivacaine	Bupivacaine
Benzocaine	−1.03	−2.28	−1.79	−3.40	−1.26	−1.98
Procaine	—	−1.49	−0.85	−2.59	−0.17	−1.40
Tetracaine	1.59	—	1.05	−0.92	1.10	0.01
Lidocaine	0.34	−1.05	—	−2.09	0.05	−1.28
Dibucaine	2.50	0.85	1.97	—	1.74	0.77
Mepivacaine	0.22	−1.36	0.05	−1.99	—	−1.46
Bupivacaine	1.59	−1.28	1.30	−0.78	1.50	—

coefficients among the anesthetics appearing in Fig. 5 and Table 4. The distribution coefficients between 1-octanol and a buffer solution (pH 7.4), which reflect the hydrophobicity of anesthetic molecules, are known to be 1.7 for PC, 221 for TC, 43 for LC, 21 for MC, and 346 for BC.[21] The hydrophobicity of anesthetic molecules was closely related to the binding affinity of anesthetics with protein.[1] The binding affinity increased in the order PC<LC<MC<TC<BC. The larger is the distribution coefficient of the anesthetic, the higher is the electrode potential and selectivity to other anesthetics. As can be seen from Table 4, the high selectivity of the DC electrode toward other anesthetics accurately reflects differences in the distribution constant or ion-exchange ability between anesthetics. The difference in the selectivity of each electrode toward various local anesthetic can therefore be closely related to the hydrophobicity of local anesthetic molecules and the stability of anesthetic–TPB ion pair.

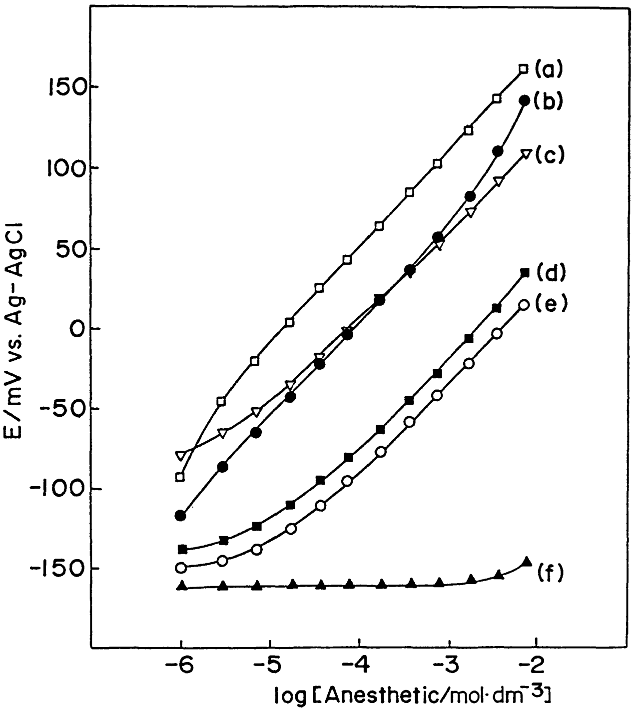

Fig. 5. Relationship between the electrode potential of a PC electrode and the local-anesthetic concentration: (a) Dibucaine; (b) Bupivacaine; (c) Tetracaine; (d) Lidocaine; (e) Procaine; (f) Benzocaine.

Analytical Application. It was proved by both potentiometric titration and direct potentiometry that PC, TC, and DC electrodes are useful sensors for the potentiometric determination of local anesthetic drugs in either pure forms or in pharmaceutical preparation. The results are shown in Tables 5 and 6. Potentiometric titration, which offers higher precision and accuracy than does a direct method, was applied to determining the purity of DC, PC, and TC reagents. PC, TC, and DC were quantitatively analyzed at a standard relative

Table 5. Potentiometric Titration of Procaine, Tetracaine, and Dibucaine

Sample	Purity (%)	S.R.D (%)
Procaine	99.18	0.2
Tetracaine	100.4	0.1
Dibucaine	98.53	0.5

a) S.R.D: Standard relative deviation; Sample: 2×10^{-3} mol dm^{-3}, 50 cm^3; Titrant: 10^{-2} mol dm^{-3} Sodium tetraphenylborate.

Table 6. Determination of Procaine in an Aqueous Injectable Solution and a Tablet

Sample	Taken (mg)	Titration$^{a)}$		Direct method
		Found (mg)	S.R.D (%)	Found (mg)
I	100.3	100.2	0.4	104.7
II	100.5	100.5	0.4	101.9
III	100.3	100.6	0.3	101.5

I: 100 mg of procaine · HCl+6 mg of benzoic acid+5 mg of potassium disulfite+1 mg of disodium hydrogenphosphate+distilled water to 50 cm^3.
II: 100 mg of procaine · HCl+64 mg of glutamic acid+6 mg of benzoic acid+24 mg of potassium hydroxide+5 mg of potassium disulfite+0.5 mg of disodium hydrogenphosphate+distilled water to 50 cm^3.
III: 100 mg of procaine · HCl+6 mg of benzoic acid+5 mg of potassium disulfite+0.5 mg of disodium hydrogenphosphate+30 mg of vitamin B_6+200 mg of meso-inositol+distilled water to 50 cm^3.
a): Sample volume: 5 cm^3; titrant: 10^{-2} mol dm^{-3} sodium tetraphenylborate.

deviation (%) less than 0.5% by potentiometric titration using the respective electrode as the sensor, and NaTPB as a titrant (Table 5). The determination of PC in a tablet (sample I) and two aqueous injection solutions (samples II and III)[12] was carried out by potentiometric analysis. The preparation of pharmaceutical solutions and the results of analysis are shown in Table 6. It was found that PC in pharmaceutical drugs can be determined with good precision without the influence of added ingredients.

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