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Rapid Communication

Preparation of TPP^+-selective membrane electrodes using polar aprotic solvents

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To embed TPP-TPB complex in PVC membrane, DMA and DMF were the most suitable polar aprotic solvents (among DMA, DMF, DMSO, and THF) that we examined. TPP^+-selective electrodes prepared with TPP-TPB-containing membranes showed linearity from 10^{-7} to 10^{-2} mol L^{-1} TPP^+. The slopes of the TPP^+-selective electrodes were a little lower than the theoretical value, but we think that the electrodes could be practically used to measure TPP^+ concentration. The life spans of the TPP^+-selective electrodes made with the TPP-TPB-containing membranes were longer than that of the TPP^+-selective electrode made with TPB^- containing membrane.

Key words: tetraphenyl phosphonium (TPP^+); polar aprotic solvent; TPP^+-selective membrane electrode; membrane potential

1 Introduction

The difference in electrical potential across the cell membrane of a living cell plays an absolutely essential role in its physiological functions.[1-5] The membrane potential of a large cell like a giant nerve cell can be measured using a microelectrode method, but those of small cells like bacteria and organella like mitochondria cannot be measured using this method. Fluorescent probes[6-9] or radiolabeled probes[10-14] are widely used to measure the membrane potentials of bacteria and mitochondria. Selective electrodes for lipid-soluble cations (unlabeled probes; TPP^+ [15-17] and DDA^+ [18,19]) that are considered to permeate freely to biological membranes have been developed, and the change in the membrane potential of small vesicles can be continuously and easily monitored with these electrodes. DDA^+ requires catalytic amounts of TPB^- to penetrate to biological membrane,[20] but TPP^+ does not.

PVC-based membranes sensitive to DDA^+ and TPP^+ are lipid-soluble cations containing TPB^- as an ion-exchanger. THF is a good solvent for PVC; it dissolved DDA-TPB complex, and the complex was easily embedded in PVC membrane.[19] However, TPP-TPB complex is highly insoluble in THF, one of the polar aprotic solvents, and it was hard to embed that complex in PVC membrane.[15, 16]

We examined whether we could use other polar aprotic solvents (DMA, DMF, and DMSO) to dissolve TPP-TPB complex. As far as we know, TPP-TPB complex was successfully embedded into PVC membrane for the first time. We investigated the properties (response and life span) of TPP^+-selective electrodes made using PVC membrane containing TPP-TPB complex.

2 Materials and Methods

2.1 Materials

The following materials were obtained from the indicated sources: *N*,*N*-dimethylacetamide (DMA), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and tetraphenylphosphonium tetraphenylborate (TPP-TPB) from Wako Pure Chemical Industries, Ltd., Osaka, Japan; polyvinyl chloride (PVC), and dioctyl phthalate (DOP) from Sigma-Aldrich, Co., St. Louis, MO, U.S.A.; tetraphenylphosphonium chloride (TPPCl) from Alfa Aesar, Heysham, United Kingdom; tetraphenylborate sodium (TPBNa) from Dojindo Labs, Kumamoto, Japan; tetrahydrofuran (THF) from Nacalai Tesque, Inc., Kyoto, Japan; agar from Difco Labs., Detroit, U.S.A.

All other chemicals were of reagent grade or better. The water used was 17-Mohm grade.

2.2 Selection of a solvent suitable for membrane preparation

0.1 g of PVC was added to 2 mL of DMA, DMF,

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Abbreviations: TPP^+, tetraphenyl phosphonium; DDA^+, dibenzyl dimethyl ammonium; TPB^-, tetraphenylborate; PVC, polyvinyl chloride; THF, tetrahydrofuran; DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide

DMSO, or THF. 6.58 mg of TPP-TPB was added to 1 mL of DMA, DMF DMSO, or THF. The mixtures were vigorously stirred using a stirrer.

2.3 Preparation of PVC membrane including TPP-TPB complex or TPB^-

0.6, 1.2, 2.4, or 3.6 mL of 10^{-2} mol L^{-1} TPP-TPB in DMA or DMF was added to each flat petri dish (ϕ 5.5 cm), then 0.57 mL of DOP was added to the petri dishes, and 3.8 mL of 5 % PVC in THF was finally added followed by thorough stirring to ensure uniformity. The solvent was then slowly evaporated at room temperature. For the sake of simplicity, the membrane prepared with 0.6 mL of 10^{-2} mol L^{-1} TPP-TPB in DMA was called as DMA(0.6) membrane, for example.

0.6, 1.2, 2.4, or 3.6 mL of 10^{-2} mol L^{-1} TPB^- in THF was added to each flat petri dish (ϕ 5.5 cm), then 0.57 mL of DOP was added to the petri dishes, and 3.8 mL of 5 % PVC in THF was finally added followed by thorough stirring to ensure uniformity. The solvent was then slowly evaporated at room temperature. For the sake of simplicity, the membrane prepared with 0.6 mL of 10^{-2} mol L^{-1} TPB^- in THF was called THF(0.6) membrane, for example.

2.4 Construction and properties of TPP^+-selective membrane electrode

A piece of each of the membranes containing TPP-TPB or TPB^- was glued to a PVC tube (inner diameter 0.85 cm) with THF. The PVC tube with the membrane containing TPB^- was soaked overnight in 10^{-2} mol L^{-1} TPP^+ for conditioning before use.

A 10^{-2} mol L^{-1} TPP^+-solution (aqueous) was placed inside the PVC tube as an internal reference solution. This solution and a calomel electrode was bridged with a small polyethylene tube (ϕ 2 mm) filled with saturated KCl agar (a salt bridge). This device was referred to as the TPP^+-selective membrane electrode or TPP^+-electrode. The TPP^+-electrodes prepared with DMA(0.6), (1.2), (2.4), or (3.6) membrane contained 0.93, 1.9, 3.7, or 5.6 μmol of TPP-TPB complex, respectively. The TPP^+-electrodes prepared with DMF (0.6), (1.2), (2.4), or (3.6) membrane contained 0.93, 1.9, 3.7, or 5.6 μmol of TPP-TPB complex, respectively. The TPP^+-electrodes prepared with THF(0.6), (1.2), (2.4), or (3.6) membrane contained 0.93, 1.9, 3.7, or 5.6 μmol of TPB^-, respectively. For the sake of simplicity, the electrodes containing 0.93 μmol TPP-TPB complex were referred to as a DMA(0.93) or DMF(0.93) electrode, depending on the kind of solvent used, and so on. The electrode containing 0.93 μmol TPB^- was referred to as THF(0.93) electrode.

The electromotive force between the TPP^+-electrode and the calomel reference electrode in the sample solution was measured by an electrometer (TR 8651, Takeda Riken, Tokyo, Japan) connected to a pen-writing recorder (R300RV, Rika-Denki, Tokyo, Japan) (Fig. 1).

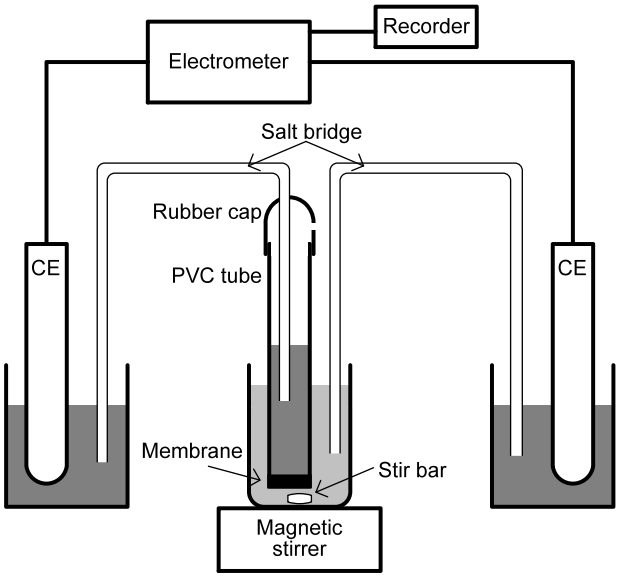

Fig. 1 Construction of TPP^+-electrode and diagram of apparatus for measuring $|\Delta E|$.
CE, calomel electrode.

We kept the DMA(5.6) and DMF(5.6) electrodes in purified water and the THF(3.7) electrode in 10^{-2} mol L^{-1} TPP^+ to examine the life spans of these electrodes. We washed the electrodes with purified water and removed the water attached to the electrodes before measurement.

3 Results and Discussion

3.1 Selection of a solvent suitable for membrane preparation

We tried to dissolve TPP-TPB complex in DMA, DMF, DMSO, and THF. We used THF to reconfirm that the complex was insoluble in THF. The complex could be dissolved to 10^{-2} mol L^{-1} in DMA and DMF, but it was insoluble at 10^{-1} mol L^{-1} in DMA and DMF by visual observation. The complex was insoluble in DMSO and THF by visual observation. PVC was insoluble in DMSO by visual observation, but dissolved in THF, DMA, and DMF. 5 % PVC in THF, DMA, and DMF were placed in petri dishes and were allowed to stand overnight at room temperature. THF completely evaporated and a thin flat transparent PVC membrane formed. Evaporation of DMA and DMF were incomplete, with a cloudy PVC membrane and a little solvent remaining in the petri dishes. Thus, we used DMA or DMF to dissolve TPP-TPB complex and THF was used as the solvent for PVC.

3.2 Properties of the electrodes

The electrical potential of a TPP^+-electrode is given by the Nernst equation,

$$E = E^{\circ} + \frac{RT}{F}\ln\left[\mathrm{TPP}^{+}\right] \quad (1)$$

where E°, R, T, F, and [TPP^+] are the standard potential, the gas constant (8.32 J mol^{-1} K^{-1}), the temperature of the system in kelvins (K), the Faraday constant (9.65 × 10^4 C mol^{-1}), and the concentration of TPP^+, respectively. The difference of electrical potential, $|\Delta E|$ between E_1 at $[TPP^+]_1$ and E_2 at $[TPP^+]_2$ is given by the following equation,

$$|\Delta E| = \left|\frac{2.303RT}{F}\log\frac{\left[\mathrm{TPP}^{+}\right]_1}{\left[\mathrm{TPP}^{+}\right]_2}\right| \quad (2)$$

If $[TPP^+]_1$ is $10[TPP^+]_2$ in Eq. (2), $|\Delta E|$ is 58 mV at 20 °C.

Fig. 2 shows the response of each TPP^+-electrode from 10^{-8} to 10^{-2} mol L^{-1} TPP^+ (aqueous solution). The properties of each TPP^+-electrode from these data are summarized in Table 1. The theoretical value of the slope was 58 mV between 20 and 22 °C. The slopes of DMA(*) and DMF(*) electrodes increased with increasing amount of TPP-TPB complex (ion-exchanger), and were almost constant for the electrodes with more than 3.7 μmol of TPP-TPB complex. The slopes of DMA(3.7), DMA(5.6), DMF(3.7), and DMF(5.6) electrodes were about 50 mV and were a little lower than the theoretical value (58 mV). However, DMA(3.7), DMA(5.6), DMF(3.7), and DMF(5.6) electrodes could be used to measure the TPP^+ concentration. The slopes of THF(0.93), THF(1.9), and THF(3.7) electrodes were close to the theoretical value regardless of the TPB^- amount included in the membrane. The slope of THF(5.6) electrode was somewhat higher than the theoretical value. It is unknown at present why the slopes of THF(*) electrodes did not increase with increasing amount of TPB^- in the PVC membrane. This will be the subject of a future study.

We examined the life spans of DMA(5.6), DMF(5.6), and THF(3.7) electrodes. Fig. 3 shows the change over time for the slopes of these electrodes. For this examination we used THF(3.7) electrode having a slope close to that of DMA(5.6), and DMF(5.6) electrode instead of

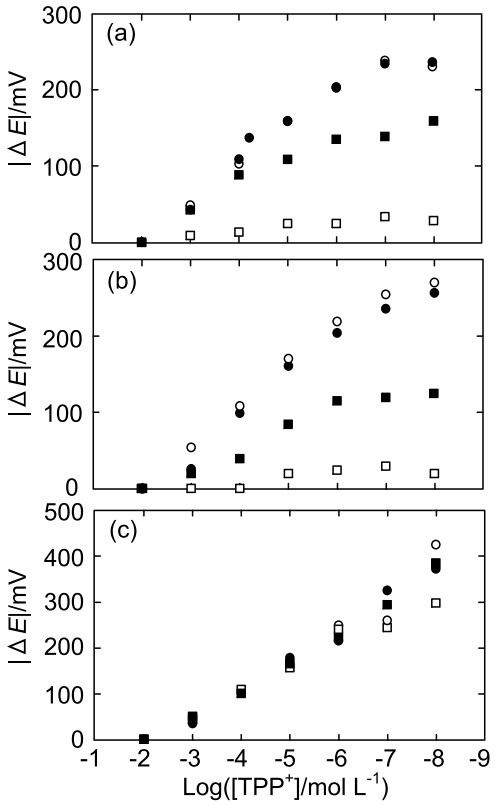

Fig. 2 Response of the TPP^+-electrodes to TPP^+ solutions (aqueous) of varying concentration.
(a) □, DMA(0.93) electrode; ■, DMA(1.9) electrode; ○, DMA(3.7) electrode; ●, DMA(5.6) electrode.
(b) □, DMF(0.93) electrode; ■, DMF(1.9) electrode; ○, DMF(3.7) electrode; ●, DMF(5.6) electrode.
(c) □, THF(0.93) electrode; ■, THF(1.9) electrode; ○, THF(3.7) electrode; ●, THF(5.6) electrode.

Table 1 Properties of the TPP^+-electrodes.

Electrode	Temp/°C	Linear range/mol L^{-1}	Slope/mV	
			Experimental	Theoretical
DMA(0.93)	21	10^{-5} - 10^{-2}	8	58
DMA(1.9)	22	10^{-6} - 10^{-2}	34	58
DMA(3.7)	21	10^{-7} - 10^{-2}	48	58
DMA(5.6)	21	10^{-7} - 10^{-2}	49	58
DMF(0.93)	21	10^{-7} - 10^{-2}	6	58
DMF(1.9)	22	10^{-6} - 10^{-2}	30	58
DMF(3.7)	21	10^{-7} - 10^{-2}	52	58
DMF(5.6)	21	10^{-7} - 10^{-2}	51	58
THF(0.93)	21	10^{-6} - 10^{-2}	61	58
THF(1.9)	20	10^{-7} - 10^{-2}	58	58
THF(3.7)	21	10^{-7} - 10^{-2}	54	58
THF(5.6)	21	10^{-7} - 10^{-2}	66	58

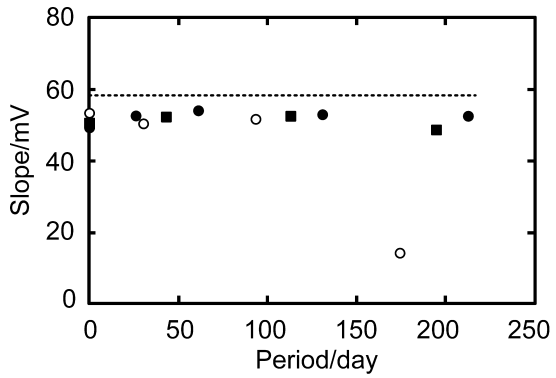

Fig. 3 Life span of TPP^+-electrodes.
●, DMA(5.6) electrode; ■, DMF(5.6) electrode; ○, THF(3.7) electrode.
The dashed line is a theoretical value.

THF(5.6) electrode. The slopes of DMA(5.6) and DMF(5.6) electrodes were constant for about 200 days after preparation, but the slope of THF(3.7) electrode was reduced by about 55 % at 175 days after preparation.

We consider that DMA(5.6) and DMF(5.6) electrodes can be used as a device for measuring membrane potential in biological systems, in place of the TPP^+-electrodes[21, 22] used in recent years. However, we must also examine substances that may interfere with these electrodes, depending on the biological system where the electrodes are used, and selectivity coefficients[23] must be evaluated for these electrodes in biological systems.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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