

Charged Impurities of Plasticizer Used for Ion-Selective Electrode and Taste Sensor

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Receptor membranes used in ion-selective electrodes and a recently developed taste sensor are made from polyvinyl chloride (PVC) and plasticizer mixed with some ion carriers or lipids. A popular plasticizer material is dioctyl phenylphosphonate (DOPP). It is known that the membrane made of PVC and DOPP shows selectivity to cations; however, its mechanism has not been clarified because this membrane should have no negative electric charge to cause cation selectivity. In this study, identification of the charged impurity, phenylphosphonic acid mono-octyl ester, from commercially available DOPP was made using frit fast atom bombardment (FRIT-FAB). Response characteristics of three kinds of PVC membranes containing different plasticizers (DOPP, 2-nitrophenyl octyl ether, 1-tetradecanol) to KCl were also shown to be much different. These results will contribute to the fabrication of receptor membranes.

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1. Introduction

A multichannel taste sensor has a transducer made of lipid/polymer membranes, which consist of lipids, a plasticizer and polyvinyl chloride (PVC).⁽¹⁾ This taste sensor has been applied to many kinds of foodstuffs such as beer,^(2,3) coffee,⁽⁴⁾ mineral water,⁽⁵⁾ sake,⁽⁶⁾ milk⁽⁷⁾ and soybean paste (miso).⁽⁸⁾ The durability of the membrane is very high, lasting over 6 months.

This kind of polymer membrane has been used as a receptor membrane in an ion-selective electrode.^(9,10) Dioctyl phenylphosphonate (DOPP) is used as a popular plasticizer material mixed with PVC. However, it has been pointed out that the membrane made of DOPP and PVC shows cationic selectivity.⁽¹¹⁻¹³⁾ Usually, sensitivity to cations occurs in the case of negatively charged membranes. Thus, the above cationic selectivity is very peculiar because DOPP and PVC should have no electric charge. A definite conclusion has not been obtained yet, as previously discussed in detail,⁽¹¹⁾ although the presence of negatively charged impurities in commercially available DOPP or sulfonate groups in PVC, which originate in a persulfate initiator, has been suggested.

In previous papers,⁽¹⁴⁻¹⁶⁾ theoretical consideration was made for the mechanism of responses of PVC membranes and lipid/polymer membranes to taste substances. As a result, quantitative explanations were given for observed data; hence, the roles of both hydrophobic and hydrophilic (i.e., electrostatic) interactions between the membranes and taste substances were clarified. While the theory is based on the descriptions of surface electric potential and diffusion potential within the membrane, one important assumption is the presence of negatively charged impurities to explain the response of the PVC membrane to NaCl. Furthermore, a suitable mixture ratio of lipids is required to obtain the desired responses to taste substances using hybrid membranes composed of two lipid species.⁽¹⁴⁾ This is because the response characteristics are seriously affected by the sign of the electric charge, which can be changed by negatively charged impurities.

The purpose of the present study is to detect and identify negatively charged impurities presumably contained in commercially available DOPP. Reliable detection is indispensable, because the presence of negatively charged impurities may affect the response characteristics of the taste sensor and ion-selective electrodes. If we can quantify the amount of impurities, we can facilitate fabrication of the receptor membranes. As a result, we succeeded in detecting phenylphosphonic acid mono-octyl ester using FRIT-FAB LC/MS. In addition, the response characteristics of PVC membranes containing different plasticizers to KCl were found to differ greatly.

2. Materials and Methods

2.1 Materials

Dioctyl phenylphosphonate (DOPP) was purchased from Dojindo Laboratories (Kumamoto, Japan) and Aldrich Chemical Co. (Milwaukee, WI, USA); and 2-nitrophenyl octyl ether (NPOE) and 1-tetradecanol (TDA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) solvents and other reagents were of the highest purity commercially available and were used without

further purification.

2.2 HPLC separation of the impurities of DOPP

Separation of the impurities of DOPP was performed on a HP-1090L high-performance liquid chromatograph. DOPP was diluted ten times with methanol and 5 μl of the diluted sample was applied to an Intertosil ODS-2 (4.6 mm ID \times 250 mm) column (GL Science, Tokyo, Japan). The column was eluted with a linear gradient of 10 mM ammonium acetate/acetonitrile (from 40 to 100% v/v in 20 min) in 10 mM ammonium acetate/H₂O. The flow rate was 0.8 ml min⁻¹. The column temperature was maintained at 40°C using a column oven. Before being passed on to the LC/MS interface, the eluted solvent was led through a UV detector set at 254 nm.

2.3 Mass spectrometer and LC/MS interface

The mass spectrometer employed was a JMS-LX2000 double-focusing model interfaced to a JMA-DA7000 data system (JEOL, Tokyo, Japan). Xenon atoms with 4 keV of energy were used for the bombardment. The scanned mass range was m/z 50 ~ 1000 at 3 kV accelerating voltage and the scan speed was 4 s. The frit fast atom bombardment (FRIT-FAB) system was used as the LC/MS interface. The interface was made of fused-silica capillary tubing, 50 μm I.D. and 0.15 mm O.D., and a stainless-steel frit (2 μm porosity) was attached to the cross section of the capillary tubing. Glycerol (1%) in methanol was added by means of a post-column at a flow rate of 0.2 ml min⁻¹. Glycerol served as the matrix of FAB ionization. The effluent that reached the porous frit was subjected to bombardment by the xenon beam.

2.4 Membranes

A membrane was made by impregnating DOPP (Aldrich) into the pores of a cellulose nitrate filter.^(15,17) Three other kinds of membranes were fabricated by mixing 800 mg PVC with 1.0 ml DOPP (Aldrich), 1.0 ml NPOE or 300 mg TDA in a manner similar to previous works.⁽¹⁻³⁾ The membrane potential, i.e., the electric potential difference across the membrane, was measured by taking the outer aqueous phase as the origin of the electric potential, as shown in Fig. 1. Changes in the membrane potentials with the addition of KCl to the outer aqueous phase were measured using one pair of electrodes containing Ag/AgCl wire surrounded by saturated KCl and agar at room temperature. The output was converted to a digital code using a digital voltmeter and was recorded using a computer.

3. Results and Discussion

Figure 2 shows the electric potential of the membrane composed of a cellulose nitrate filter, whose pores are filled with DOPP, in response to KCl. The response is positive; this result is similar to the previous result obtained using NaCl.⁽¹⁵⁾ The response to KCl is smaller than that to NaCl; it seems that the difference in activity coefficient between K⁺ and Na⁺ is reflected by this response. More systematic measurements for other ions are necessary to confirm it. The positive response implies that the membrane is negatively

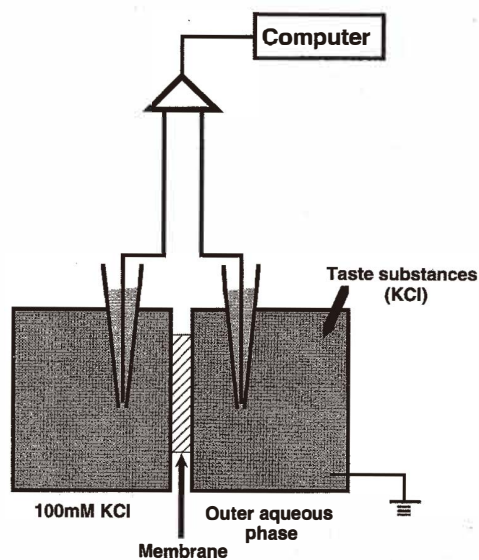


Fig. 1. Experimental setup for measuring the membrane potential.

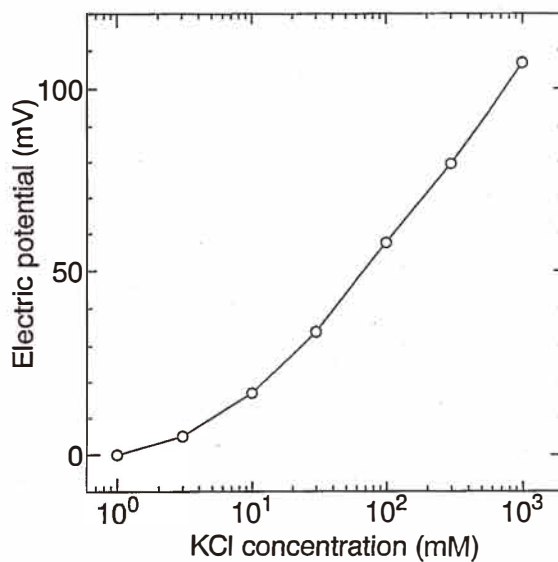


Fig. 2. Response of the DOPP-adsorbed membrane to KCl. The membrane potential was measured by taking the solution, to which KCl was added, as the origin. The other side of the membrane, i.e., inner solution, was a 100 mM KCl solution. The change from the initial 1 mM KCl is shown.

charged such that it responds to cations. This result was interpreted to originate in negatively charged impurities contained in commercially available DOPP.⁽¹⁵⁾

Negatively charged impurities of each DOPP (purchased from different manufacturers) were determined by the FRIT-FAB LC/MS system. Figures 3 and 4 show the UV chromatogram (a), the total ion chromatogram (b), and the mass chromatograms (c) of DOPP samples from Dojindo and Aldrich, respectively. We can see a good correspon-

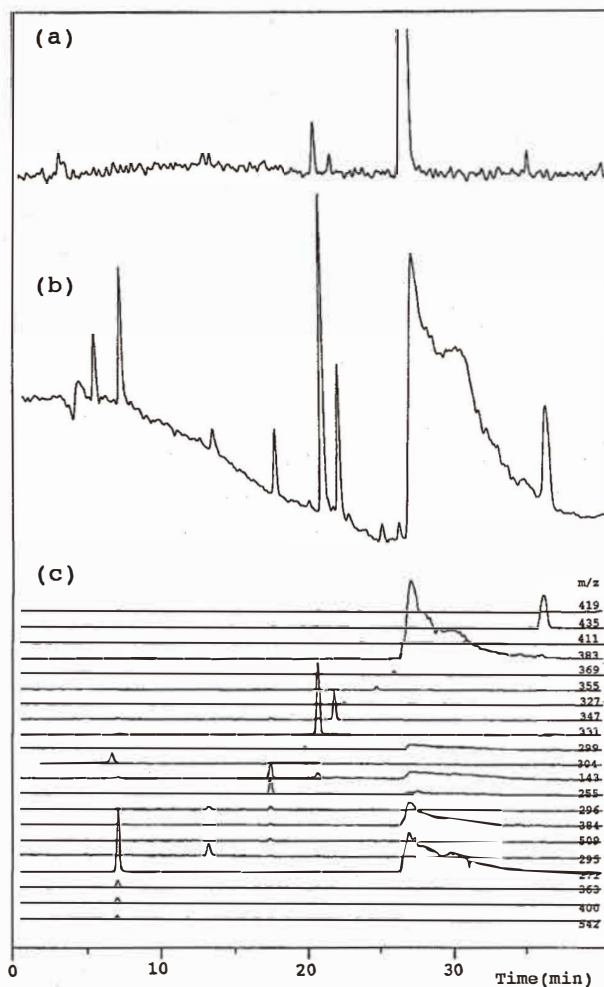


Fig. 3. UV chromatogram (a), total ion chromatogram (b) and mass chromatograms (c) of DOPP purchased from Dojindo.

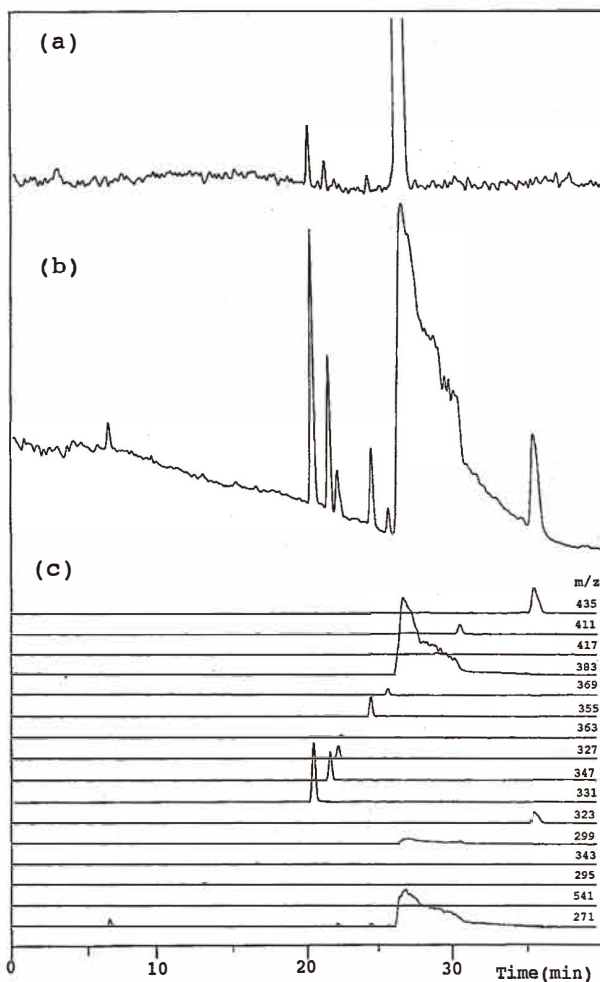


Fig. 4. UV chromatogram (a), total ion chromatogram (b) and mass chromatograms (c) of DOPP purchased from Aldrich.

dence of the main peak in all the chromatograms in Figs. 3 and 4. Since the data of the DOPP sample from Aldrich are similar to those of the DOPP sample from Dojindo, the following data from Aldrich were abbreviated. The mass chromatogram shows selected ion monitoring of DOPP, where the signals correspond to protonated molecular ions.

Figure 5(a) shows the positive FAB mass spectrum of DOPP components detected at a retention time of 26.65 min. The signal at m/z 383 corresponds to a protonated molecular ion (DOPP), while those at m/z 271 and 159 correspond to fragment ions. Judging by the

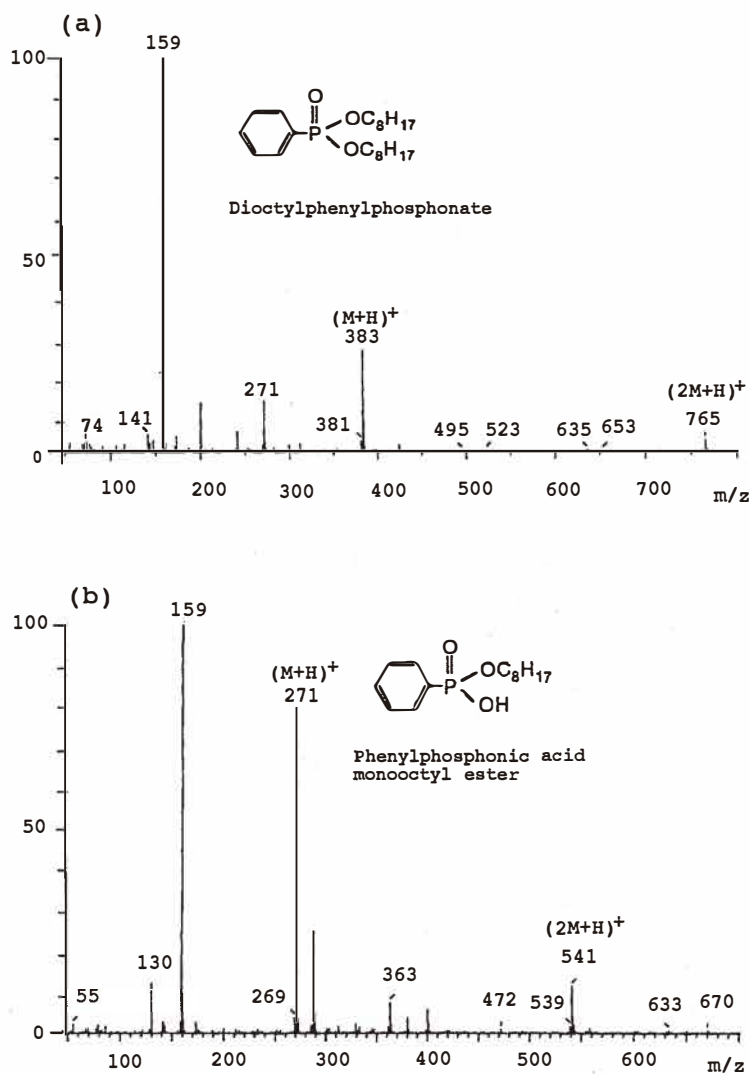


Fig. 5. Positive-FAB mass spectra of (a) DOPP detected at a retention time of 26.65 min and (b) phenylphosphonic acid monoethyl ester detected at a retention time of 6.65 min.

mass difference between the molecular ion and the fragment ion, these fragment ions are due to the loss of one or two octyl groups from DOPP. Figure 6 shows the mass chromatograms of the ions at m/z 271 and 159. They indicate the presence of an octyl phenylphosphonic acid unit and a phenylphosphonic acid unit, respectively. The two ions

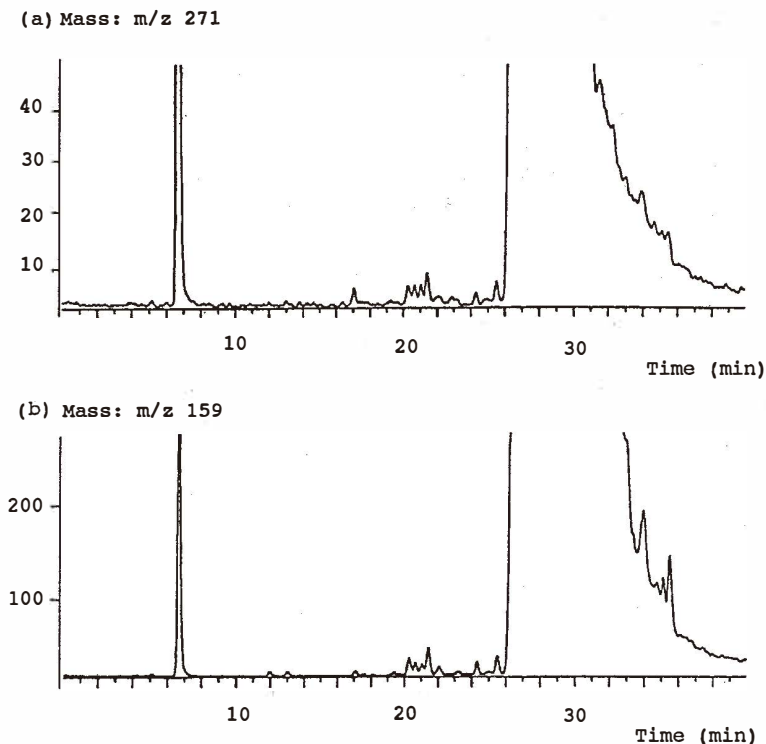


Fig. 6. Mass chromatograms of the ions at m/z 271 (a) and m/z 159 (b).

were detected at both retention times of 6.65 min and 26.65 min. The ion detected at 26.65 min was confirmed to be the fragment ion of DOPP, while that detected at 6.65 min was suggested not to originate from DOPP. Figure 5(b) shows the FAB mass spectrum of the ion detected at 6.65 min. It was recognized that the ion at m/z 271 was the protonated molecular ion and that at m/z 159 was the fragment ion. Therefore, it is clear that the ion at m/z 271 detected at a retention time of 6.65 min is phenylphosphonic acid monoethyl ester, not the fragment ion of DOPP.

On the other hand, according to above results the ion at m/z 159 detected at retention times of 6.65 min and 26.65 min was the fragment ion of phenylphosphonic acid monoethyl ester and of DOPP, respectively. Spectral analysis of all the peaks in the mass chromatogram of the ion at m/z 159 revealed that a molecular ion of m/z 159 was not present in DOPP. This means that phenylphosphonic acid does not exist as one of the negatively charged impurities of DOPP. All the other molecular ions detected in DOPP (Fig. 3) were confirmed to have no negative charge by spectral analysis. Consequently, we confirmed that phenylphosphonic acid monoethyl ester is the only negatively charged impurity of

DOPP by means of the FRIT-FAB LC/MS method. The molar content estimated from the integration list (data not shown) of the total ion chromatogram was 2.44% (Dojindo) and 0.61% (Aldrich). This result agrees fairly well with the theoretically estimated value of 1%, as previously reported.^(15,16) The observed response electric potentials for NaCl and quinine can be explained quantitatively in the presence of almost fully dissociated H^+ from the impurity of commercial DOPP.

Figure 7 shows responses of three membranes made of PVC and plasticizers to KCl. It can be seen that the PVC membranes containing DOPP or TDA respond positively with increasing KCl concentration, whereas the membrane containing NPOE responds negatively. This result suggests that commercial DOPP and TDA contain negatively charged impurities, whereas positively charged impurities are contained in NPOE. In addition to plausible impurities, the hydrophobicity of plasticizers, represented by the dielectric constant, may also affect the response. These two possibilities may provide an explanation of the experimental result in which the response of the PVC membranes to ionic surfactants is affected by the included plasticizer.⁽¹⁸⁾

In this study, definite evidence of the presence of a negatively charged impurity, phenylphosphonic acid mono-octyl ester, was obtained using FRIT-FAB LC/MS. The effect of impurities on the property of ion-selective electrodes may be small, because a host molecule is generally mixed in the polymer membrane and receives an ion. The effect may appear in the form of small variations in the response characteristics of one electrode from another, which can be removed by an adequate calibration procedure. In the case of the

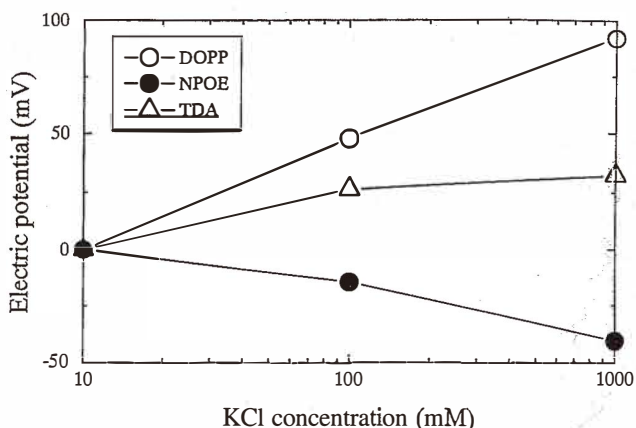


Fig. 7. Responses of PVC membranes containing DOPP ($-\circ-$), NPOE ($-\bullet-$), TDA ($-\triangle-$) to KCl. The membrane potential is taken as relative to 10 mM KCl as the initial value. The initial membrane potentials were -148 , 24 and -51 mV, respectively, for the membranes containing DOPP, NPOE and TDA. The inner solution was a 3.3 M KCl solution. The above values of membrane potential also suggest that the two membranes containing DOPP and TDA are significantly different from the membrane containing NPOE.

taste sensor, the effect of impurities may appear largely in electrically neutral lipid/polymer membranes, *e.g.*, composed of the equimolar dioctyl phosphate and trioctyl methylammonium chloride. Both factors of hydrophilicity and hydrophobicity of the taste sensor membrane are very important; most salty substances are hydrophilic electrolytes, while bitter substances show hydrophobicity to some extent, as discussed concerning the reception mechanism of effects of hydrophilicity and hydrophobicity on the response potential.^(14,15,19) Therefore, it is favorable to grasp the situation of electric charge of the materials used in making lipid/polymer membranes of the taste sensor. The present result will contribute to the fabrication of receptor membranes of the taste sensor and ion-selective electrodes.

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