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Simple Screening Method for Pesticide Residues by Detecting Coexistent Adjuvants Using Potentiometric Measurement

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The screening method for pesticide residues by detecting anionic surfactants used as pesticide adjuvants was examined by membrane potential measurement using a surfactant-sensing membrane composed of tridodecylmethylammonium chloride. A sulfonate anionic surfactant, sodium dodecyl sulfate, was detected at under 10 ppb. In the experiments on pseudopesticides obtained by mixing standard pesticides (i.e., chlorfenapyr, imazalil, and glyphosate) and sodium dodecyl sulfate, our membrane showed no response to the active ingredients of the pesticides but showed a specific response to a coexisting surfactant, indicating the feasibility of our method as a primary screening method.

1. Introduction

Recently, there has been increasing interest in the safety and reliability of foods, and increasing consciousness regarding pesticide residues among general consumers. The standard limits of pesticide residues were set for all types of food in accordance with the Positive List System introduced after the revised Food Sanitation Act was enforced on 29 May 2006. For pesticides without a standard limit, 0.01 ppm (10 ppb) was uniformly set. After this, the distribution and sale of foods containing pesticide residues over standard limits were banned. Therefore, the number of food items to be tested has been continuously increasing yearly and now exceeds 2 million.[(1)] However, the detection and quantification of pesticide residues using government-designated methods require expensive equipment, such as gas chromatography-mass spectrometry (GC-MS) and

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high-performance liquid chromatography-mass spectrometry (HPLC-MS) systems, technical skills, and a considerable amount of time, which makes it difficult to efficiently process a large number of testing targets. With the above background, the development of a detection method based on simple and rapid screening is desired.

There are two main simple methods of detecting pesticide residues: the enzyme-linked immunosorbent assay (ELISA), which targets only approximately 30 types of pesticide, and the cholinesterase inhibition assay, which targets cholinesterase-inhibiting pesticides, such as organophosphates, organochlorines, and carbamates, or approximately 20% of registered pesticides in Japan.

Previously, we studied the detection of pesticide residues in leafy vegetables by membrane potential measurement using lipid/polymer membranes;[2,3] however, the method used still has some problems in terms of measurement sensitivity and stability.

In this study, we examined the validity of a method of detecting pesticide residues targeting anionic surfactants, which are used as pesticide adjuvants, and developed a primary screening method in particular for leafy vegetables and citrus fruits.

In Japan, 799 types of chemical are currently registered as active ingredients (AIs) of pesticides.[4] Pesticides comprise AIs, additives (carriers), and surfactants. AIs show pesticide activities, and carriers carry AIs and facilitate pesticide handling. Carriers and surfactants are called pesticide adjuvants. Surfactants are used to emulsify, disperse, and spread AIs and diluents as well as to increase their solubility. General pesticides contain approximately 0.1–10% surfactants and 2–30% AIs.[5] Pesticides using an anionic surfactant as the adjuvant are considered to account for approximately 70% of registered pesticides.[6,7]

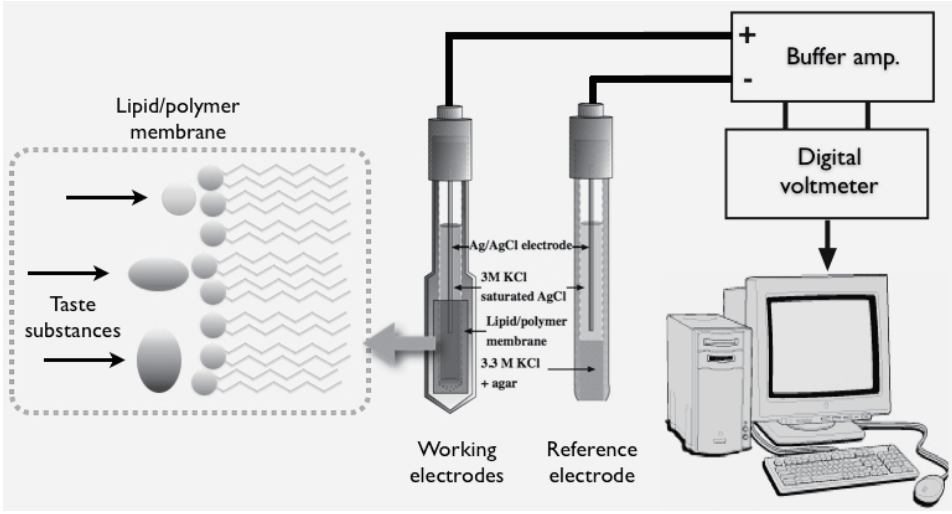
As an analytical method for surfactants, solid-phase extraction high-performance liquid chromatography is designated in the Drinking Water Quality Standards of the Ministry of Health, Labour and Welfare;[8] however, this method is not suitable for on-site screening because it requires expensive equipment and reagents that are difficult to handle. Methylene blue spectrophotometry[9] and potentiometric titration using ion electrodes[10,11] are inadequate because of their insufficient range of detectable concentrations (1–15 ppm) and use of harmful chloroform. Moreover, potentiometric titration is not suitable for the detection of low-concentration surfactants.

In this study, we examined various membrane materials for detecting anionic surfactants at a high sensitivity in a short time using a taste sensing system, in which a membrane was used as the sensing electrode.[12–14] We also carried out an experiment on detecting surfactants in pseudopesticides using the sensor with the membranes that we fabricated.

2. Experimental Methods

Figure 1(a) shows a photograph of TS-5000Z (Intelligent Sensor Technology, Inc.), the taste sensing system used in this experiment. Figure 1(b) shows a schematic of the sensing electrode. The functional membranes fabricated by synthesizing lipids and polymers are used to sense taste substances, and the interactions of the membranes with tastants are converted into electrical signals by membrane potential measurement and loaded onto a computer to distinguish between and identify different tastes.[13,14]

(a)



(b)

Fig. 1. (a) Taste Sensing System TS-5000Z. (b) Schematic of sensing section.

2.1 *Measurement procedures*

A membrane is rinsed in the first cleaning solution (30% ethanol + 100 mM KCl + 10 mM KOH) for 120 s, and then rinsed in the second and third cleaning solutions (30 mM KCl + 0.3 mM acidum tartaricum) for 60 s each. The potential of the reference solution (V_r) is measured. The measurement of V_r is repeated until the difference between the initial V_r and V_r measured 30 s after the previous measurement falls within 0.5 mV.

The potential of a sample (V_s) is measured after the measurement of V_r is stabilized. The target potential is the subtraction of V_s from V_r. This measurement is repeated five times, and the average potential is calculated.

2.2 *Fabrication of surfactant-sensing membrane*

1. A quaternary ammonium salt (NR_4^+) and 1 ml of plasticizer (2-nitrophenyl octyl ether) are added to 5 ml of tetrahydrofuran (THF), which is stirred on a magnetic stirrer for 30 min.
2. An additional 5 ml of THF is added and the mixture is stirred for 30 min.
3. Eight hundred milligrams of polyvinyl chloride (PVC) is added, and the mixture is stirred for 1 h.
4. The obtained mixture is poured onto a petri dish (90 mm diameter) and dried in a draft chamber for 3 days to volatilize THF.

The thus-obtained membrane is approximately 200 μm thick. The membrane is immersed into the solution composed of 30 mM KCl + 0.3 mM acidum tartaricum for 48 h as a preconditioning process.

The names and molecular formulae of the membrane materials and anionic surfactant used in this experiment are summarized in Tables 1–3. Also, the names of the standard pesticides used are summarized in Table 4.

Table 1
Quaternary ammonium salts (classified by side-chain structure).

Name	Molecular formula	CH_3
Tetradodecylammonium bromide (TDAB)	$[CH_3(CH_2)_{10}CH_2]_4NBr$	$CH_3\times0$
Tridodecylmethylammonium chloride (TDAC)	$[CH_3(CH_2)_{11}]_3NCH_3Cl$	$CH_3\times1$
Didodecyldimethylammonium bromide (DDAB)	$[CH_3(CH_2)_{11}]_2N(CH_3)_2Br$	$CH_3\times2$
Dodecyltrimethylammonium bromide (DTAB)	$CH_3(CH_2)_{11}N(CH_3)_3Br$	$CH_3\times3$

Table 2
Anionic surfactant.

Name	Structural formula
Sodium dodecyl sulfate (SDS)	$CH_3(CH_2)_{11}OSO_3Na$

Table 3
Quaternary ammonium salts (classified by number of carbons in side chain).

Abbr.: Name	Structural formura
C7: Tetraheptylammonium bromide	$[CH_3(CH_2)_6]_4NBr$
C8: Tetraoctylammonium bromide	$[CH_3(CH_2)_7]_4NBr$
C10: Tetradecylammonium bromide	$[CH_3(CH_2)_9]_4NBr$
C16: Tetrahexadecylammonium bromide	$[CH_3(CH_2)_{15}]_4NBr$

Table 4
Standard pesticides.

Name	Purpose/Type	Affinity
Chlorfenapyr	Insecticide/Organochlorine	Hydrophobic
Imazalil	Bactericide/Imidazole	Hydrophobic
Glyphosate	Herbicide/Amino acid	Hydrophilic

Polyvinyl chloride (PVC), sodium dodecyl sulfate (SDS), tetraheptylammonium bromide (C7), tetraoctylammonium bromide (C8), dodecyltrimethylammonium bromide (DTAB), chlorfenapyr, imazalil, and glyphosate were obtained from Wako Chemical Industry Co., Ltd., Tokyo, Japan. Tetradodecylammonium bromide (TDAB), tridodecylmethylammonium chloride (TDAC), tetrahexadecylammonium bromide (C16), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich, USA. Tetradecylammonium bromide (C10) was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. 2-Nitrophenyl octyl ether was purchased from Dojindo Laboratories, Kumamoto, Japan. All other chemicals used were of reagent grade.

3. Results and Discussion

3.1 *Change in membrane potential due to difference in side-chain structure between quaternary ammonium salts*

The interaction between cationic quaternary ammonium salts (NR_4^+) and anionic surfactants and the length and number of alkyl side chains of NR_4^+ strongly affect the surface tension of anionic surfactants.[15,16] Therefore, we used NR_4^+s as membrane-forming materials and examined the sensitivity and detection ability of the membranes for anionic surfactants with counterions.

For the NR_4^+s shown in Table 1, their structures and electric responses (membrane potentials) in sodium dodecyl sulfate solution, which is a typical surfactant of sulfate type, were measured following the procedures described in § 2.1.

Figure 2 shows the result of optimizing the amount of added tridodecylmethylammonium chloride (TDAC). It shows the dependence of membrane potential on SDS concentration and the highest sensitivity with the membrane fabricated using 0.02 mM TDAC. In our previous study, similar types of response profile were obtained from hydrophobic compounds, namely, adsorptive components on a membrane surface.[17]

The following maximum membrane potentials (i.e., maximum sensitivity) were obtained: −42.6 mV for 0.04 mM tetradodecylammonium bromide (TDAB), −49.5 mV for 0.02 mM tridodecylmethylammonium chloride (TDAC), −39.5 mV for 0.06 mM didodecyldimethylammonium bromide (DDAB), and −14.0 mV for 0.10 mM dodecyltrimethylammonium bromide (DTAB). Figure 3 summarizes the relationship between side-chain structure (number of methyl groups) and membrane potential in 100 ppb SDS solution. It is reported that the number of methyl groups correlates with surface tension, suggesting that some interaction exists on the membrane surface.[15]

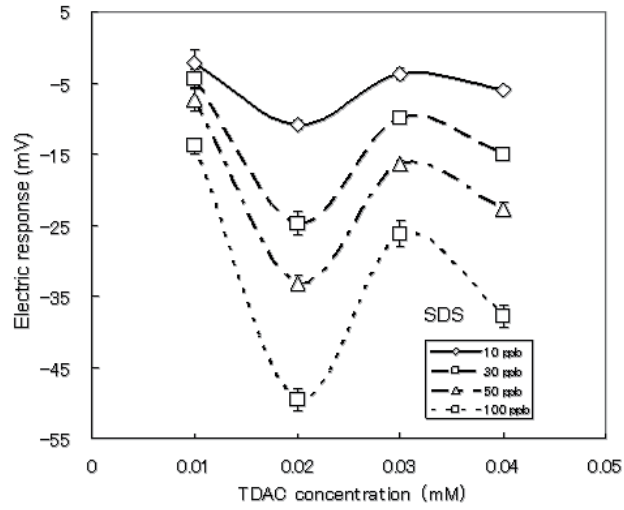


Fig. 2. Relationship between TDAC concentration and membrane potential for each SDS concentration. The TDAC concentration is expressed in molar concentration of the TDAC in 10 ml of THF during the fabrication of the membrane.

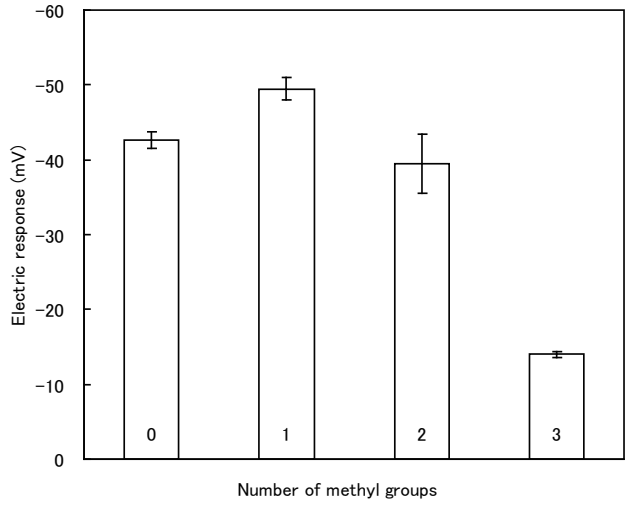


Fig. 3. Relationship between number of methyl groups in quaternary ammonium salt side chains and membrane potential.

3.2 *Change in membrane potential due to difference in alkyl side-chain length between quaternary ammonium salts*

Similarly, the membrane potential in the SDS solution was measured for NR_4^+s with different numbers of alkyl side-chain carbons (C7, C8, C10, C12, and C16) by changing the SDS concentration. As shown in Fig. 4, the trends of the response can be classified into two groups: one is for C7, C8, and C12, and the other is for C10 and C16, all of which exhibited a profile similar to the result shown in Fig. 2 and the maximum membrane potential was taken as the plotted points in Fig. 4. The reason for this is considered to be the compatibility between the NR_4^+ and the membrane substrates, such as PVC and plasticizer, requiring further detailed discussion.

We used a C12 membrane in the subsequent experiments considering the durability of the membranes, for example, the elusion of lipid from the membrane; the experimental results revealed that the C7 and C8 membranes also showed high sensitivities.

3.3 *Calibration curve of sodium dodecyl sulfate*

Surfactants were prepared with SDS concentrations of 10, 30, and 50 ppb, and the potential of a TDAC membrane was measured. Figure 5 shows a calibration line obtained by the regression analysis of the measurement result. The correlation coefficient R^2 was 0.994, and the detection limit of the calibration line was 7.1 ppb.

We requested QSAI Analysis and Research Center Co., Ltd., approved by the Ministry of Health, Labour and Welfare, to perform a survey on pesticide residues

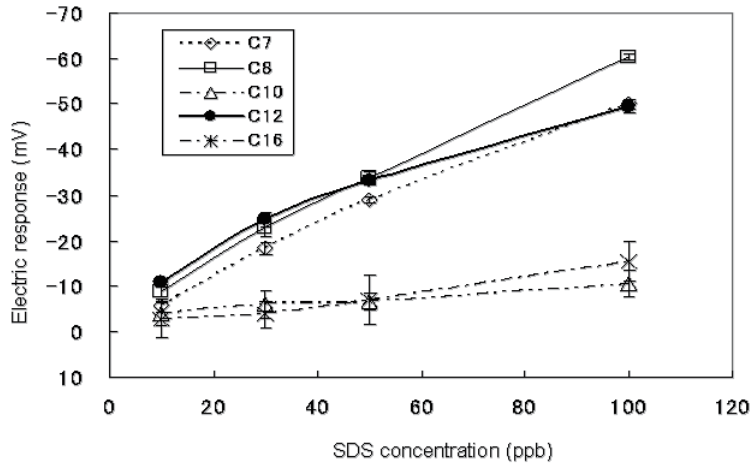


Fig. 4. Relationship between sodium dodecyl sulfate concentration and membrane potential for different quaternary ammonium salt side-chain lengths.

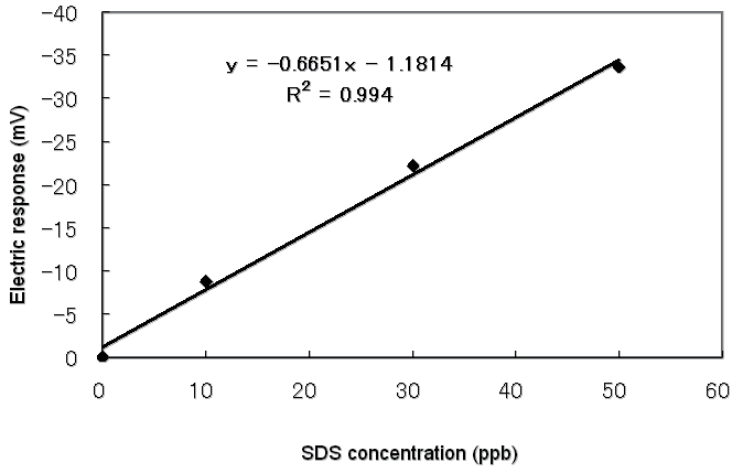


Fig. 5. Calibration line of sodium dodecyl sulfate concentration measurement (0.02 mM TDAC).

in a leafy vegetable and were informed that two types of pesticide (flufenoxuron and cypermethrin, 0.01 and 0.02 ppm, respectively) and anionic surfactants (equivalent to 0.9 ppm) were detected in the leafy vegetable, Komatsuna (*Brassica rapa* var. *peruviridis*) (data not shown). This suggests that the method of potentiometric measurement with NR_4^+ membranes is sensitive enough to detect the surfactant on leafy vegetables.

3.4 *Measurement of membrane potential with respect to sodium dodecyl sulfate in pseudopeticides*

The membrane potential of the surfactant-sensing membrane with 0.02 mM TDAC, which exhibited the highest sensitivity, with respect to SDS in pseudopesticides was measured. The three standard pesticides in Table 4, i.e., 1) chlorfenapyr, 2) imazalil, and 3) glyphosate and mixtures of each of these pesticides and SDS with the same concentration as that of each standard pesticide were prepared using reference solutions to obtain pseudopesticides with SDS concentrations of 10, 30, 50, and 100 ppb. The results are presented in Figs. 6 and 7. As shown by these results, the TDAC membrane responded to the coexisting SDS at a high sensitivity, and the membrane potential depended on the SDS concentration. In contrast, the surfactant-sensing membrane showed little response to the standard pesticides and no dependence of the membrane potential on SDS concentration was confirmed. These results indicate that the membrane selectively responds to SDS.

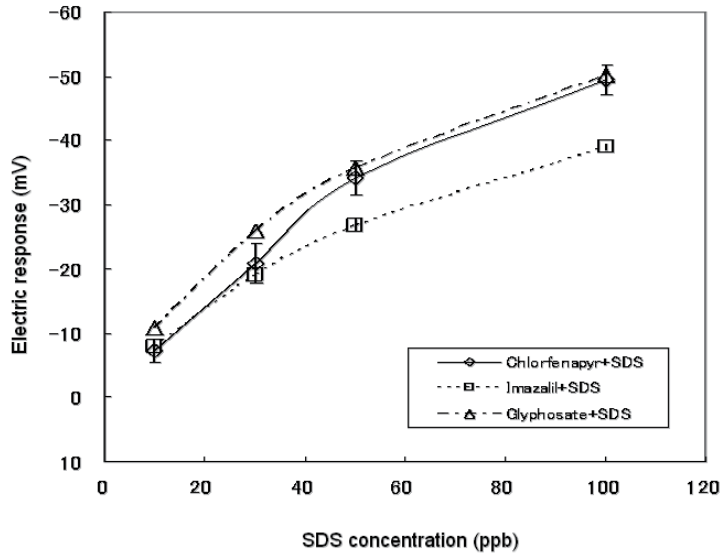


Fig. 6. Relationship between sodium dodecyl sulfate concentration of pseudopesticides and membrane potential. Mixtures of each of the pesticides and SDS with the same concentration as that of each standard pesticide were prepared using reference solutions to obtain pseudopesticides with SDS concentrations of 10, 30, 50, and 100 ppb.

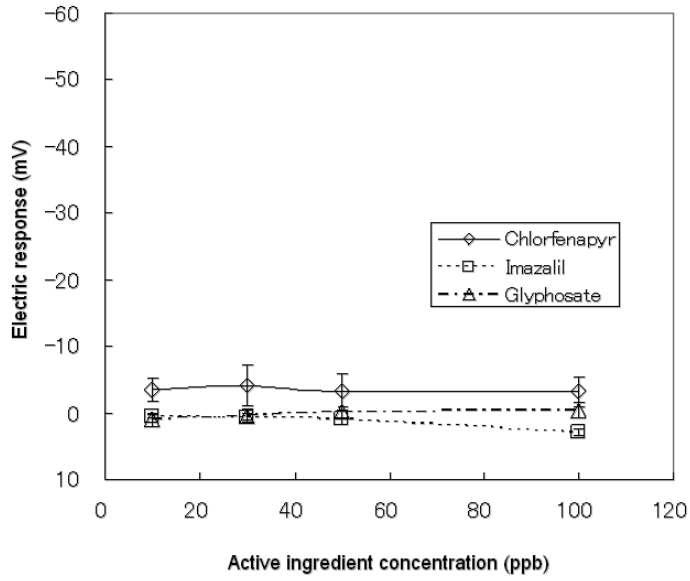


Fig. 7. Relationship between AI concentration of pseudopesticides and membrane potential (for solutions obtained by dissolving only standard pesticides in reference solution).

4. Conclusions

We studied a screening method using an NR_4^+ membrane as the sensing electrode for detecting anionic surfactants at a high sensitivity. To determine the effects of the type of NR_4^+ on membrane potential, membrane composition and the structure of NR_4^+ (the numbers of methyl groups and the alkyl chain length) were investigated. TDAC, with three long alkyl chains C12, exhibited the highest sensitivity. Also, C7 and C8, which have shorter alkyl chain lengths, exhibited high sensitivities. These results suggest that membrane potential is markedly affected by membrane composition and the suitability of the structure of the alkyl group that leads to the compatibility with the membrane substrates, such as PVC and plasticizer.

SDS in pseudopesticides was detected at a high sensitivity (at under 10 ppb) using our membrane with 0.02 mM TDAC, indicating the feasibility of our primary screening method for pesticide residues.

There are various types of anionic surfactant, and their adsorption mechanism is related to various phenomena, such as ion exchange, ion pair formation, acid-base interaction, and hydrophobic interaction. By further discussing membrane compositions in detail, we will increase the detectable range and improve the sensitivity of membranes.

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