

Detection of Environmental Hormones with Electrode Polarity Controlled Method

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It has been noticed recently that there is a possibility of elution of chemical substances from packages of foods. The chemicals act as harmful substances such as environmental hormones for biological bodies at very low concentration. It is therefore necessary to develop a sensing device, which can detect such substances easily and quickly. The purpose of the present study is high sensitive measurement of environmental hormones using electrode polarity controlling method. The responding ability of the polarity-controlled sensor covers various chemicals from electrolytes to nonelectrolytes and from hydrophilic to hydrophobic substances. As a result, we could sensitively detect bisphenol-A at about 50 ppb and dibutyl phthalate at about 3 ppb, and the sensor could distinguish such chemicals from ordinary taste substances.

Keywords: environmental hormone, surface polarity, electrochemical impedance, Pt electrode, sensor

1. Introduction

A variety of chemical substances have a possibility to elute from packages of foods. Such chemicals change the taste of foods and are thought to be harmful to living bodies. In addition, it was revealed that micro-toxins and environmental endocrine disruptors (environmental hormones) have been comprised in foods. The sensors that can detect such harmful substances using simple and quick procedures have not been developed. Thus the technical developments of such a sensing device to detect and to remove such substances in manufacturing process of foods are indispensable and desired strongly.

The purpose of the present study is to develop the high-sensitive technique that can easily and quickly identify chemical substances eluting from packages of foods. The required sensor must have an ability to detect partial structure of chemicals, because chemicals that are micro-toxins and environmental hormones generally have fat-soluble benzene ring in their molecule structures^{(1) (2) (3)}. There are no simple and quick methods to detect such substances in advance. It is of urgent necessity to develop methods to detect them quickly.

In this paper, we describe a sensor using surface polarity controlled method using a simple Pt electrode^{(4) (5)}. With this method, some chemicals considered as environmental hormones can be detected and can be distinguished with high sensitivity. The sensor can be used for the first step method to select a doubtful one from various target chemicals.

2. Materials and Methods

We experimented on the high sensitive detection of environmental hormones using electrode polarity con-

trolled method. This method would try to detect the chemical substances with changes of electrochemical interface impedance depending on interaction between the target chemicals and the electrode surface whose polarization is controlled⁽⁴⁾.

2.1 Measurement system The measurement system is shown in Fig.1. It consists of the potentiostat, the frequency response analyzer (FRA) and a computer. Three electrodes; working electrode (WE), reference electrode (RE) and counter electrode (CE), are connected to the potentiostat and regulated potential from FRA is applied through the potentiostat. The potential difference between WE and RE is controlled by the potentiostat. We used Ag/AgCl in saturated KCl solution for the RE and Pt wire for the CE.

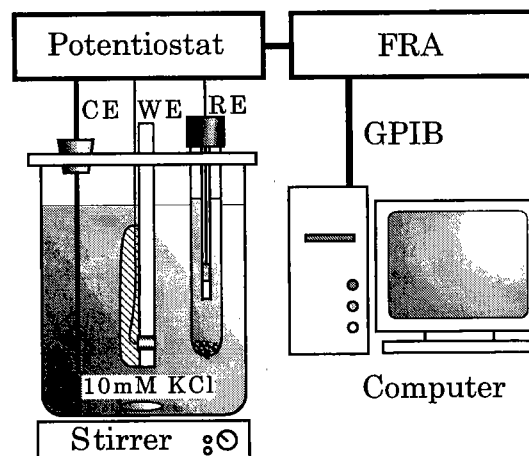


Fig. 1. Measurement system.

2.2 Working electrode We used platinum as the electrode material of the working electrode. Initially, the Pt wire is set in an acrylic board with a hole 5mm in diameter and filled the hole with epoxy resin. The epoxy resin and Pt are polished using sandpaper and a fine alumina polishing suspension until the Pt wire surface become smooth. Before each experimental procedure, we polish the surface with a fine alumina polishing suspension. The roughness of the electrode surface changes the impedance characteristics as mentioned in 2.4.

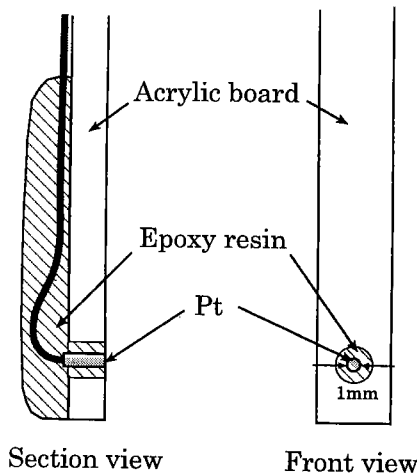


Fig. 2. Working Electrode.

2.3 Measurement procedure The electrode potential was controlled at a certain DC potential value using the potentiostat and a minute sine wave of 0.01 V amplitude from the FRA is superimposed. The electrode potential was swept from -0.8 V or -0.7 V to 0.3 V at 0.05 V steps, and the phase boundary impedance of the electrode was measured. An electrode potential controlled the polarity of the electrode surface and the boundary of scanning potential were determined to avoid an electrochemical reaction of water.

A 10 mM KCl solution was used as a standard solution and the value in the standard solution was considered to be the baseline. The responses to chemicals are the values obtained by the subtraction of the baseline from the measurement value, which is measured after addition of sample solutions.

2.4 Frequency response in a standard solution

Fig.3 shows the frequency dependencies of the electrode impedance of the Pt electrode in a standard solution, 10 mM KCl. In the present experimental conditions, no redox reaction arises, for example generation of hydrogen. An equivalent circuit of the present Pt electrode (WE), which represent the frequency dependency shown in Fig.3, is shown in Fig.4. R_s is mainly a solution resistance, C_{dl} is an electric double layer capacitance of the electrode surface. A parallel resistance r is an interface impedance of the electrode surface and inversely proportional to ω , angular frequency, so r can be written as $r=\alpha/\omega$ where ω is angular frequency and α is constant. In Fig.3, θ , a slope to the real axis, is caused by

roughness of the electrode surface^{(6) (7)}. If the surface is ideal smooth and no redox reaction occur, r becomes infinity and θ seems to be 90 degrees.

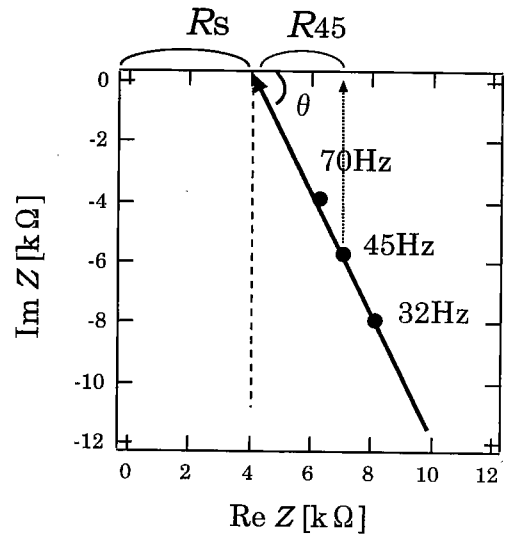


Fig. 3. Vector trace of Pt electrode (WE) impedance in complex plane.

In the present experiment, we measured complex impedance values at three frequency points, 32 Hz, 45 Hz, and 75 Hz with 0.01 V amplitude. Then we regard the value at the point where the trace crosses the real axis, that is $\omega=\infty$, as R_s and R_{45} is estimated from the real part value at 45 Hz. C_{dl} is estimated from imaginary part which changes with $1/\omega$.

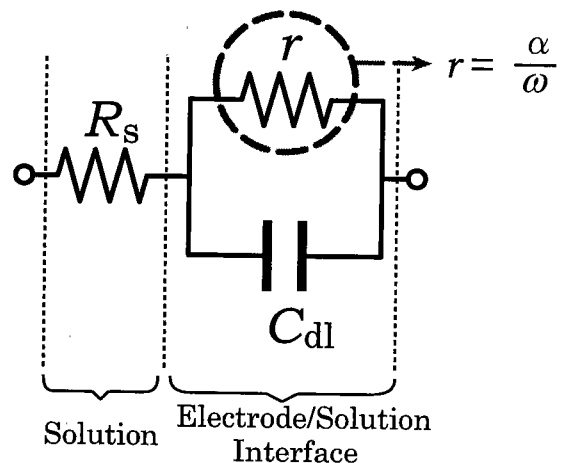


Fig. 4. Equivalent circuit of Pt electrode (WE).

2.5 Potential profile for a standard solution

The electrode potential dependencies of R_{45} and C_{dl} in the standard solution are shown in Fig.5. R_{45} and C_{dl} depend on the electrode potential. It is considered that the dependencies are caused by the adsorption of hydrogen and oxygen to the Pt electrode(WE) surface and the formation of an oxidation layer on the surface. Point of zero charge (PZC) is located around -0.1 V. This is an electrode potential point where the rate of change of R_{45} and C_{dl} are maximum. In Pt electrode (WE),

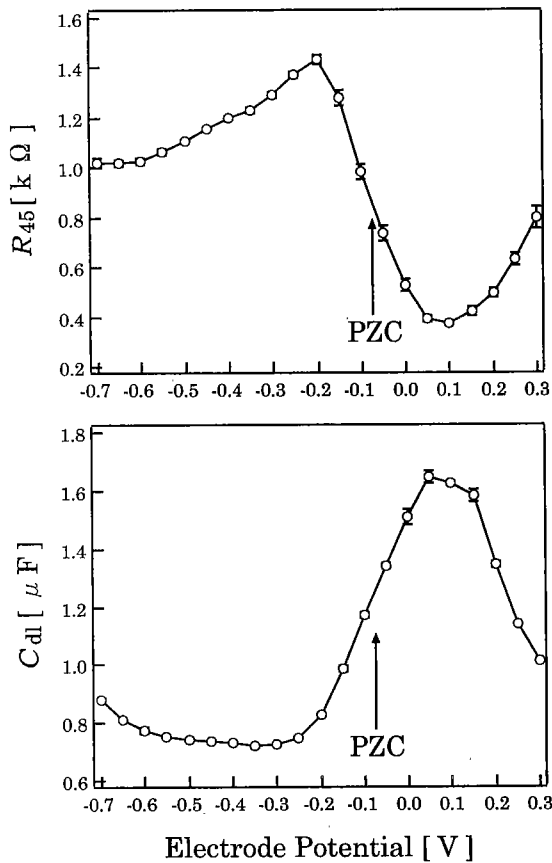


Fig. 5. Potential profile for a standard solution. Vertical bars are standard deviations.

there is not only the surface polarization of electrode but also various states of surface, so we could expect various interactions with the chemicals in the solution.

3. Results

3.1 Responses to basic taste substances Potential profiles for typical taste substances are shown in Fig. 6.

We can summarize the character of the patterns of the potential profiles as following;

- NaCl, salty taste, has concave in the -0.2 V and had a gentle peak in the 0.1 V area.
- HCl, sour taste, has a peak in the -0.3 V area.
- Sucrose, sweet taste, increases R_{45} as the electrode potential increased.
- Quinine-HCl, bitter taste, has a peak in the -0.65 V area.
- MSG, umami taste, has a peak in the 0.1 V area.
- Capsaicin, pungent taste, has a peak in the -0.65 V area.

Using these profile patterns, we can get the characteristic potential profile for each of the taste substances. Furthermore the similar pattern appeared for the chemicals which have similar taste, so it seems that R_{45} has enough information about taste. It should be noticed that we could detect the sweet and the pungent substances. These substances are difficult to measure using the multichannel lipid membrane taste sensor^{(8) (9)}, so

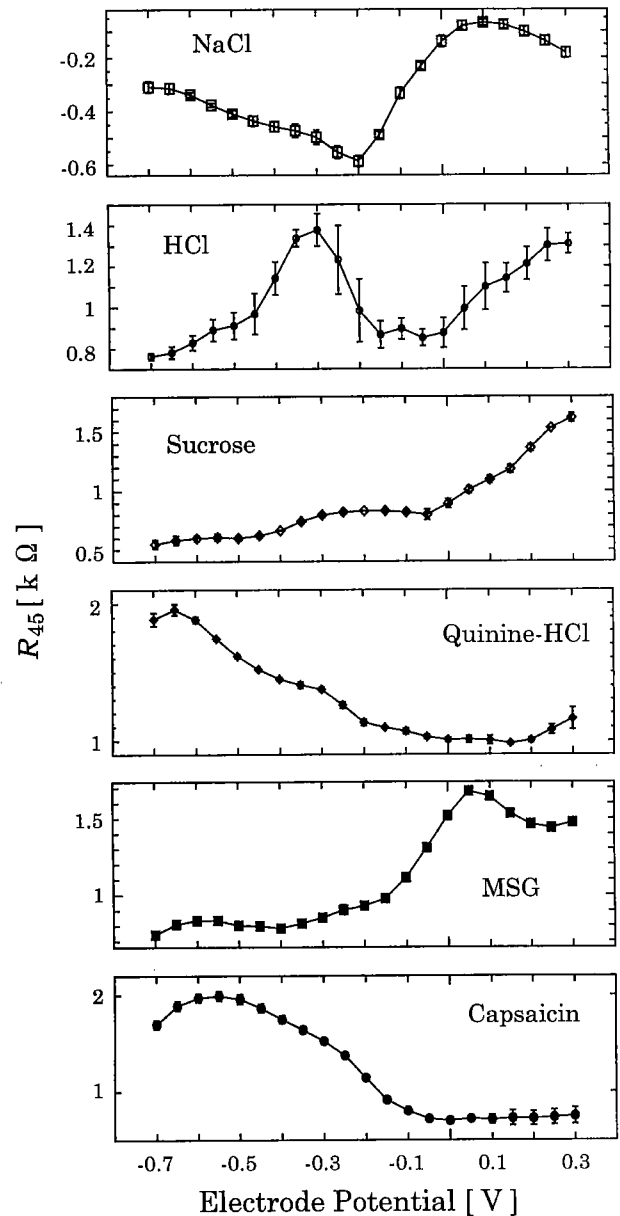


Fig. 6. Potential profiles of electrode impedance to taste substances. Vertical bars are standard deviations.

this method is expected to make up for the taste sensor with high taste detecting ability.

This method can detect nonelectrolytes such as capsaicin and sucrose with the same magnitude as electrolytes that can be easily detected using electrochemical methods such as potentiometry or amperometry. Obtained potential profiles due to taste substances are different from each other and the profiles consist of many peaks; each peak corresponds to adsorption and interaction with the electrode surface at different electrode potential, i.e., electrical polarity of the electrode surface.

Capsaicin and quinine-HCl have the same peak at about -0.6 V. Similar potential profile patterns can be obtained for caffeine, L-tryptophan and piperine. The peak caused by a common partial structure of the

molecules. In this case, the common partial structure of the taste substances is benzene ring. It is confirmed that peaks in potential profiles depend on partial structure of molecules of other various taste substances.

3.2 Responses to environmental hormones

We measured a bisphenol-A (BPA), one of the environmental hormones considered as a chemical that may elute from food packages⁽¹⁰⁾. Fig.7 shows potential profile for BPA. The potential profiles had a peak around the -0.65 V electrode potential area, and had a slight peak in the 0 V area too. Such characteristic of the profile pattern was identified at concentrations above 0.2 μM . Concentration of 0.2 μM BPA is 45.6 ppb, so it was proved that the electrode polarity controlled method can detect BPA with high sensitivity. Comparing with potential profile by taste substances in Fig.6, capsaicin, pungent taste substance and quinine-HCl, bitter taste, both hydrophobic substances possessing the benzene ring, had a peak in the -0.65 V area. BPA has the same peak, common so it is implied that this peak seems to derive from the benzene ring in the molecules. BPA potential profiles also resemble sucrose and ethanol (date not shown) in positive potential area, because of hydroxyl group properties of BPA and sucrose^{(5) (11)}.

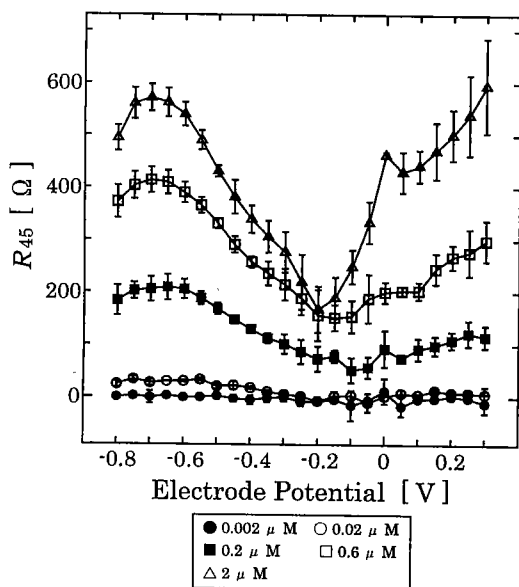


Fig. 7. Potential profile for bisphenol-A. Vertical bars are standard deviations.

Results for dibutyl phthalate (DBP) are shown in Fig.8. DBP is one of the environmental hormones, and is used as a plasticizer for various polymer products. We display a typical measurement result in order to abstract the character of the potential profile. The response to DBP was less reproducible than BPA as shown in Fig.8, because DBP does not stably exist in water solution. DBP can be detected more sensitively than BPA. Threshold concentration for DBP response is under 0.01 μM (2.78 ppb). In Fig.8, it is found that potential profiles for DBP had a peak in the -0.65 V area that seems to be brought about by benzene ring. As well as BPA, it had a peak in the 0 V area while

the peak could not be seen in the potential profiles capsaicin and quinine-HCl as shown in Fig.6. So, it is concluded that BPA and DBP have similar potential profile. With these results it can be confirmed that there is a possibility of grouping chemicals using sensor output patterns. This result is important in environmental hormone detection because numerous candidate chemicals for environmental endocrine disruptors exist in our circumstance and the sensor must detect the categorized chemicals with near chemical characteristics and same partial structure in the molecules. At the present, however, peak around 0 V cannot be discussed in relation to molecular structure of chemicals because only a few similar pattern can be obtained from sample substances.

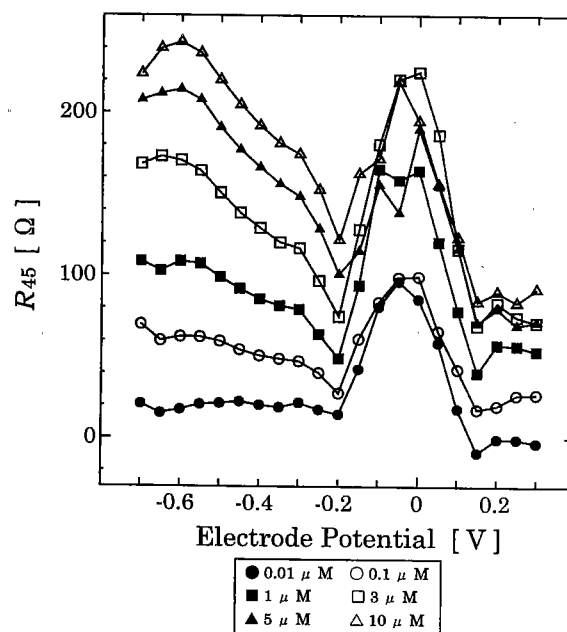


Fig. 8. Potential profile for dibutyl phthalate.

4. Discussion

The eluted chemicals have a possibility to change taste of foods. We measured BPA with multichannel taste sensor to make sure the detecting ability of BPA in the sense of taste. The taste sensor uses lipid biomembranes as multiple receptors, and has what is called global selectivity. The multichannel lipid membrane sensor has been successfully used in detection of various taste substances and controlling food production^{(8) (9)}. The measurements were taken using a multichannel taste sensing system (SA401, Anritsu).

The taste sensor responded to BPA at about 2 μM (Fig.9). Although the responses of positively charged membranes are large, they have a large experimental deviation, so it seems that it is difficult to distinguish BPA from other chemicals such as taste substances.

The multichannel lipid membrane taste sensor is sensitive to electrolytes because the sensor measures the membrane potential. However, it cannot detect electrically neutral substances, which are often seen in sweet and bitter substances, so it seems to get no advices

about many environmental hormones. Because BPA is also electrically neutral substance with low electrical polarity, it is supposed that BPA makes no noticeable effect to the membrane potential.

The surface polarity-controlled sensor can respond to variety of chemicals both hydrophobic and hydrophilic, so the sensing technique can be utilized for the eluting environmental hormones. As a result, the sensor can detect the target chemicals with high sensitivity. However, there exist variety of taste substances in real application, then it is difficult to detect dilute environmental hormones in such a dense solution and to identify the chemical candidate clearly. The present sensor will be utilized for the purpose of the first step assay to detect doubtful and uncertain food samples. To determine a kind of chemicals, HPLC or gas chromatography will be used after screening through a sensor technique with simple and quick procedure as the proposed detecting method here.

For interfering substances eluting from sample which become an adhesive agent to working electrode, more sensitive and reproducible sensor is expected with wide dynamic range of responses to target chemicals. The stability and durability of the present electrode is sufficiently high, so pretreatments such as filtration of samples should be adopted to eliminate the interference of coexisting chemicals. In the present experiment, we measured only two samples, so we will measure other substances which may coexist in target samples and compare with the potential profile pattern.

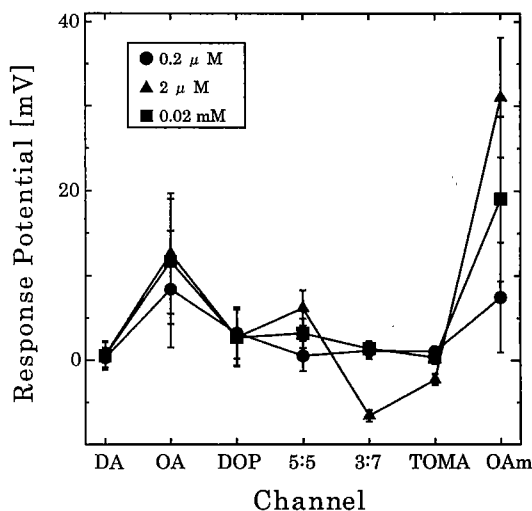


Fig. 9. Response potential for bisphenol-A. Vertical bars are standard deviations. Horizontal axis is lipid materials of the membrane constituent. DA : decyl alcohol, OA : oleic acid, DOP : dioctyl phosphate, 5:5 : the ratio of mole DOP:TOMA=5:5, 3:7 : the ratio of mole DOP:TOMA=3:7, TOMA : tri-octylmethylammonium chloride, OAm : oleyl amine.

5. Conclusion

The present study is tried to detect environmental hormones easily with high sensitivity by changing electrical polarity of the Pt electrode. Results were advanced to detect, in ppb order, chemical substances

considered as environmental hormones and to have concentration-dependent responses by electrical polarities. The results therefore implies that quantification and identification of environmental hormones are possible.

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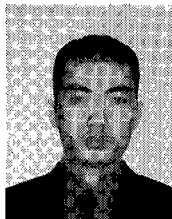
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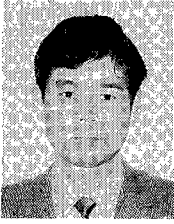
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