Sensors and Materials, Vol. 13, No. 3 (2001) 179–187
MYU Tokyo

S & M 0441

Development of Technology for Separating and Identifying Bitter Substances

Ryuji Takamatsu, Kiyoshi Toko, Hiroshi Takeguchi[1] and Akira Kawabata[2]

Department of Electronics, Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka 812–8581, Japan
[1]Center for Analytical Chemistry and Science, Inc. Yokkaichi, Mie 510–8530, Japan
[2]Center for Analytical Chemistry and Science, Inc. Ibaraki 300–0332, Japan

(Received December 15, 2000: accepted February 28, 2001)

Key words: HPLC, lipid membranes, bitter substances, separation and identification, multichannel taste sensor

A multichannel taste sensor is developed by imitating the mechanism in gustatory systems. Various foods have been measured using a taste sensor, and identification and quantification of a taste have become possible. However, there is no method for detecting and separating bitter substances in a solution. Development of such a method might contribute greatly to the understanding of foods, chemicals and medicines. The purpose of this study is to examine a method for detecting and separating bitter substances using a taste sensor and high-performance liquid chromatography (HPLC) and to develop the equipment.

1. Introduction

Humans have five senses: sight, hearing, touch, smell and taste. These senses are very important because humans act after receiving information from the outside world. Sensors have been produced to quantify the subjective and equivocal senses of humans by artificial methods and materials. In the field of taste, a multichannel taste sensor has recently been developed. This sensor imitates the mechanism in gustatory systems and produces as output the taste itself as information.[1–3] Various foods have been measured using the taste sensor, and identification and quantification of the taste have become possible.

There are five basic tastes: sweetness, saltiness, sourness, bitterness and umami taste. Bitterness among these tastes can be considered to be the signal of poison. Many animals mainly avoid bitter foods. Humans do not like bitterness either, aside from the bitterness of beverages like beer and coffee. When a sensory test is given to control the bitterness of foods or medicines, inspectors are under much stress.[4] Sometimes, it may also injure their health; the inspectors may be in danger when they are checking drugs.

In addition, the mechanism of the taste reception systems of the taste sensor resembles that of humans. The taste sensor imitates the mechanism in gustatory systems. Thus it becomes possible to detect bitterness quantitatively, by means other than a sensory test. However, it is difficult to separate bitterness and other taste qualities and to identify the bitter substance by the taste sensor. If such a detecting and separating method were developed, it might contribute largely to the food, chemical and medical industries. Thus we tried to examine a method for detecting and separating bitter substances using both the taste sensor and high-performance liquid chromatography (HPLC) and to develop the equipment.

2. Materials and Methods

2.1 *Multichannel taste sensor*

Generally, we used eight different membranes, each of which is an artificial lipid/polymer membrane that imitates a lipid bilayer, as the transducer in the taste sensor. Thus the taste sensor has a multichannel structure and eight outputs. There are two reasons we adopted these membranes as transducers. First, the biomembrane that receives taste substances is composed of proteins and a lipid bilayer. Second, these membranes imitate a lipid bilayer.[1-3]

The taste sensor is superior to that of humans in sensitivity, durability and reproducibility of measurements. Thus it is expected that the transducer will be able to be applied to food, medical and environment measurements.

The taste sensor shows large responses to bitter substances, since the bitter substances are bound to lipid membranes, as clarified by the change of membrane potential caused by adsorption (CPA) method.[5] Taste qualities such as sourness, saltiness, sweetness and umami taste are barely detected using the CPA method. That implies that lipid membranes have the potential to bind bitter substances selectively. This is reasonable because many bitter substances as well as the hydrocarbon chains of lipids are hydrophobic. This is the reason we adopted lipid membranes for the taste sensor in this study as the material to bind with bitter substances.

Table 1 shows the lipid membranes used in this study for each bitter substance. The response to these bitter substances is large and stable.[5,6] For example, the response of the taste sensor to caffeine is shown in Fig. 1.

2.2 *High-performance liquid chromatography*

Chromatography is a method used to separate a mixture into separate components. Two phases, the stationary phase and the mobile phase, maintain an equilibrium. A sample flows through a column. Each ingredient in the sample interacts with each phase and is

Table 1
Lipid membranes for each bitter substance.

Bitter substance	Lipid membrane
Quinine hydrochloride	DOP (Dioctyl phosphate)
Caffeine	OA (Oleic acid)
Iso-α-acid	OAm (Oleyl amine)

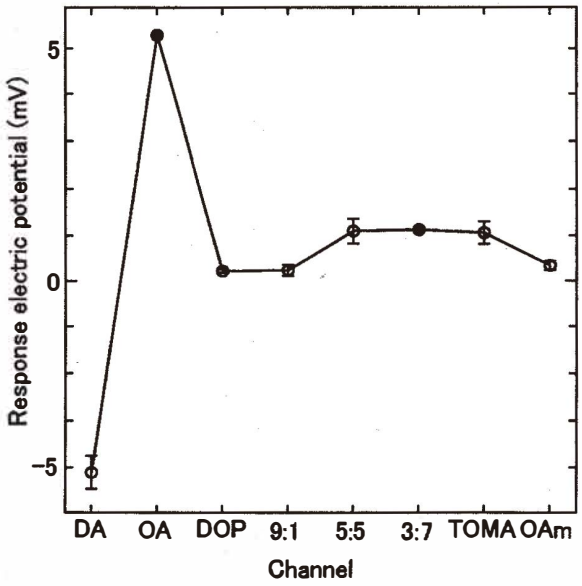

Fig. 1. Response of the taste sensor to caffeine. The horizontal axis is the channel of the taste sensor. Measurements were made three times. Each abbreviation identifies the lipid: DA, decyl alcohol; OA, oleic acid; DOP, dioctyl phosphate, TOMA, trioctyl methyl ammonium chloride; OAm, oleyl amine; and 9:1 is the molar ratio C:T=9:1; 5:5, C:T=5:5; 3:7, C:T=3:7.

separated. This separated component is measured qualitatively and quantitatively. In the case of using a liquid as the mobile phase, the chromatography is called liquid chromatography (LC). Specially, it is called high-performance liquid chromatography (HPLC) if the separation is carried out at high speed using a high-pressure pump and a high-performance filler.

The basic equipment for HPLC consists of five parts: a pump for propelling a liquid (mobile phase), an injector for introducing a sample, a column, a detector and data processing equipment. Figure 2 shows a schematic of typical HPLC equipment.

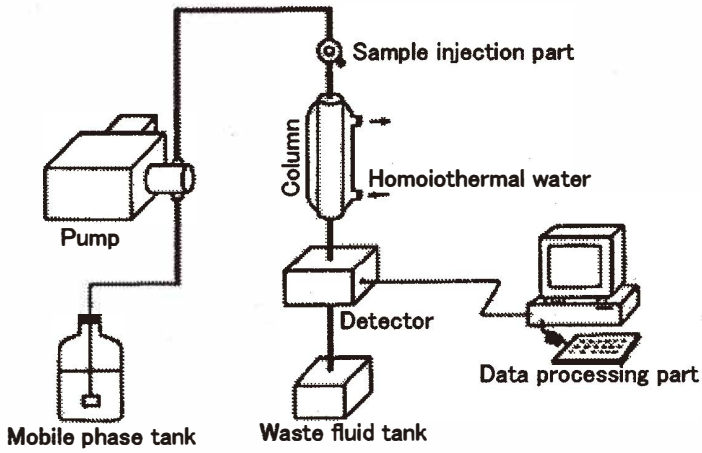

Fig. 2. Schematic of HPLC equipment. A solvent, the mobile phase, is propelled by the pump. The mobile phase is sent to the column by passing through plumbing shown by the bold line. A solid package, the stationary phase, fills the column. A small amount of sample is injected on the column from the sample injection port. The sample is separated in the column. The detection of each eluted ingredient is transformed into an electronic signal in proportion to the concentration and is recorded by the data processing equipment.

The HPLC instrument in this study was a Shimadzu LC-10AGVP equipped with a CTO-10ACVP column oven, a Shimadzu SPD-10AVP UV-VIS detector, a Shimadzu DGU-12A degasser, a Shimadzu CBM-10A communications bus module and a personal computer. The analytical column (0.46 × 15 cm) was a Shimpack ODS (Shimadzu Ltd.).

2.3 *Measurement method*

2.3.1 *Sample preparation and chromatography*

Figure 3 shows the method of preparing samples for HPLC measurement. Bitter substances adsorb to the lipid membrane in process ②, and the substances are extracted by ethanol in process ④. These steps successfully lead to the separation of bitter substances. We used 1 mM quinine hydrochloride as a typical positively-charged substance to show bitterness, 10 mM caffeine as a neutral substance and 20,000 ppm iso-α-acid as a negatively-charged substance. Some lipids can also be dissolved in ethanol in process ④. To take into consideration the effect of these lipids, we also prepared 1 mM KCl in place of bitter substances in the above method. The washing time in ③ was 5 s for iso-α-acid.

Table 2 shows chromatographic conditions for three kinds of bitter substances. The solution in process ④ was measured by HPLC.

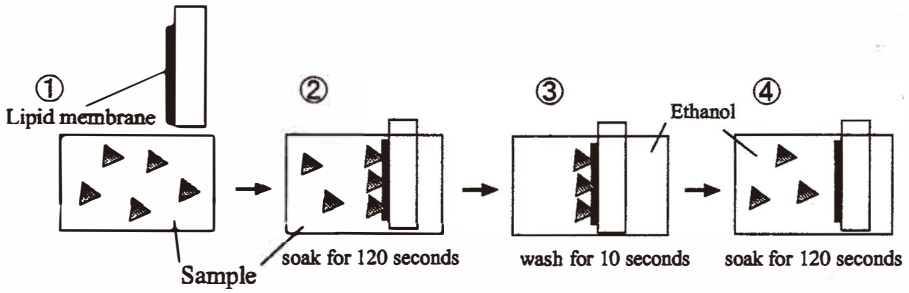

Fig. 3. Method for preparing samples for HPLC measurement.

Table 2
Chromatographic conditions for each bitter substance. Solvent A is a molar ratio of 10 mM phosphoric acid buffer: acetonitrile: methanol=2:1:2. Solvent B is a molar ratio of 10 mM phosphoric acid buffer: acetonitrile:methanol=9:1. Solvent C is methanol and water (72.5:27.5) containing 85% phosphoric acid (1.7 g/100 ml) and 10% tetraethylammonium hydroxide (2.95 g/100 ml).

	Quinine hydrochloride	Caffeine	Iso-α-acid
Mobile phase	Solvent A	Solvent B	Solvent C
Flow rate (ml/min)	0.1	1.0	1.5
Temperature of column oven (°C)	30	40	50
Detection wavelength (nm)	254	237	280

2.3.2 *Calibration and calculation*

The measurement of various concentrations of sample provided the calibration curves. The concentration of each bitter substance extracted using HPLC was calculated, using the calibration curves for quinine and caffeine in Fig. 4 and for iso-α-acid in Fig. 5.

Figure 5 shows three peaks: isocohumulon (co-), isohumulon (n-) and isoadhumulon (ad-). Five homologues exist in iso-α-acid because of differences in the acyl side chain, each homologue having two geometrical isomers: a cis form and a trans form. The ratio of the homologues depends on the variety of hop. The major homologues included in beer are co-, n- and ad-. Few differences in the bitter taste exist among the homologues.[7]

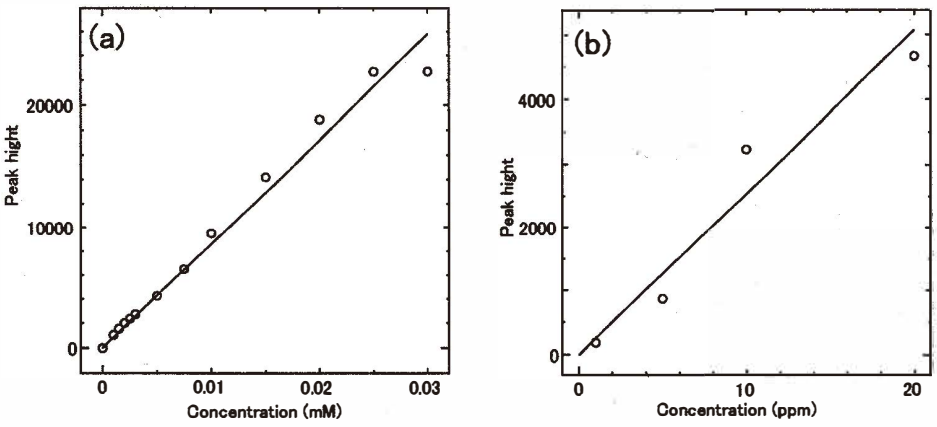

Fig. 4. Calibration curves of (a) quinine hydrochloride and (b) caffeine.

3. Results and Discussion

Because the peak of the lipid overlapped the peak of quinine hydrochloride, we deduced the chromatogram of the lipid from the chromatogram of quinine hydrochloride. The result is shown in Fig. 6(a). Figures 6(b) and 6(c) show the graphs of caffeine and iso-α-acid, respectively. These two chromatograms may be used as is because each analyte gives a single peak. There are three peaks corresponding to the three homologues, i.e., co-, n-, and ad-, in Fig. 6(c). Table 3 is obtained from Fig. 6 using the calibration curves of Figs. 4 and 5. It is clear from this result that the adsorption of these substances to the respective membranes brings about the response of the taste sensor to bitter substances, as is shown in the CPA measurement.[5]

We can thus obtain information about the adsorption of bitter substances to the lipid membranes by measuring various concentrations of bitter substances using this method. In addition, a response from the taste sensor implies a relationship between the strength of bitter tastes and adsorption.

Quinine hydrochloride is positively charged in aqueous solution, while caffeine is neutral and the iso-α-acids are negatively charged. This result indicates the possibility of detecting any bitter substance using this method, if the chromatographic conditions are prepared for each bitter substance. However, the weak point in this method is its low sensitivity. In general, substances whose adsorption power is weak seem to be difficult to detect. For example, 0.1 mM quinine hydrochloride could not be detected. The sensitivity can be increased by improving the extraction conditions.

This method requires the chromatographic conditions to be changed for each substance. When an unknown solution is measured, several chromatographic conditions must be prepared. We suggest the following process to achieve the separation and identification of an unknown solution. First, the property of a bitter substance is understood using the taste sensor; second, the chromatographic conditions are determined, and finally, samples are

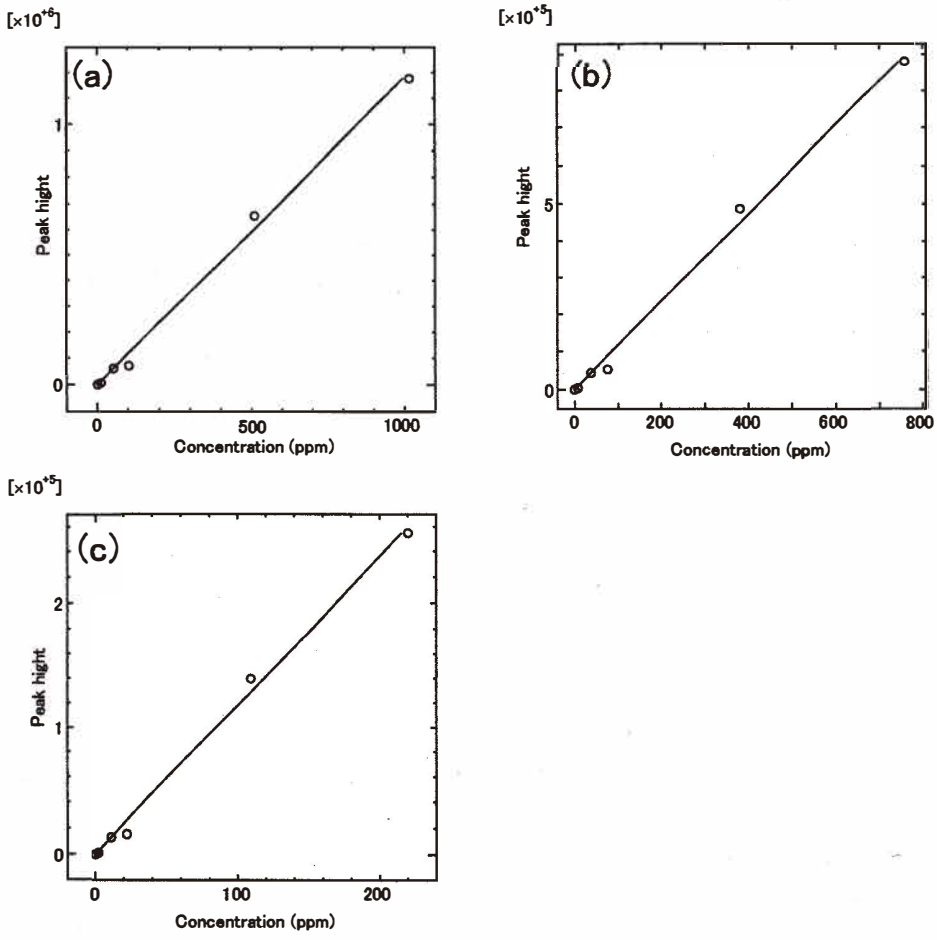

Fig. 5. Calibration curve of (a) iso-α-acid(co-), (b) iso-α-acid(n-) and (c) iso-α-acid(ad-).

measured. These improvements enable the practical use of this technology for separating and identifying bitter substances.

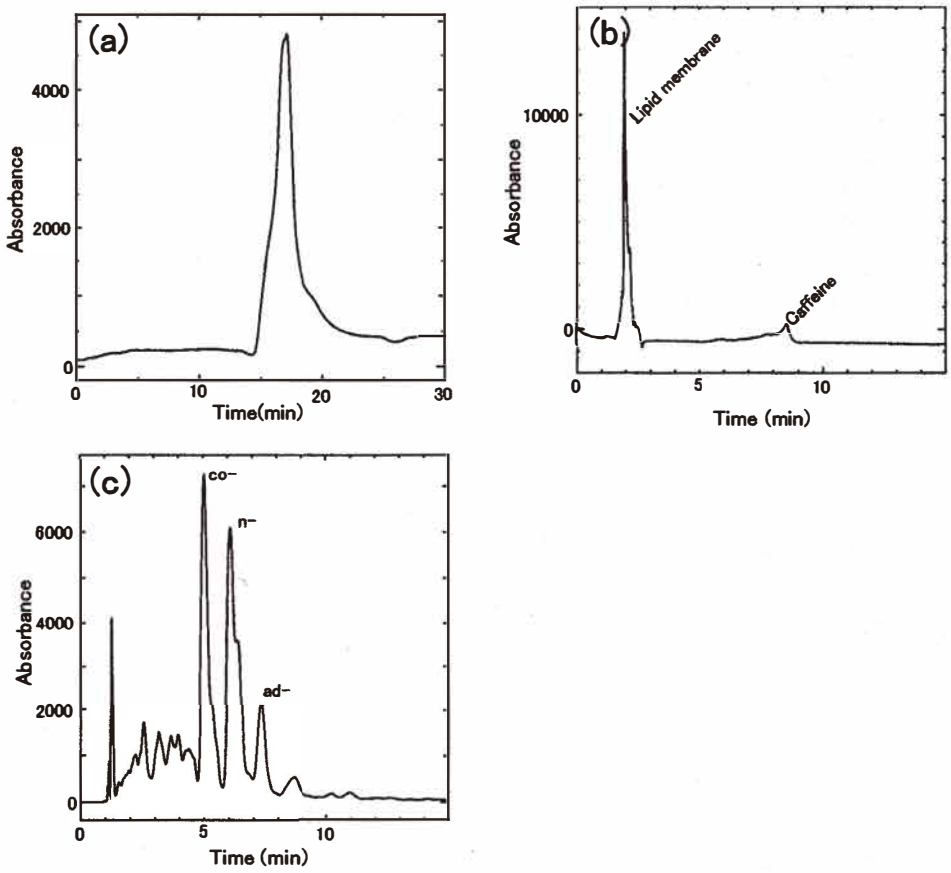

Fig. 6. The result of (a) 1 mM quinine hydrochloride, (b) 10 mM caffeine and (c) 20,000 ppm iso-α-acid.

Table 3
Concentration of each chemical listed in Table 1 extracted using HPLC from the lipid membranes to which each is bound.

1 mM quinine hydrochloride	1.08 μM
10 mM caffeine	0.438 μM
20,000 ppm iso-α-acid (co-)	8.99 ppm
20,000 ppm iso-α-acid (n-)	7.52 ppm
20,000 ppm iso-α-acid (ad-)	2.60 ppm

Acknowledgement

We sincerely thank Dr. H. Ikezaki of Anritsu Co., Ltd. for his support of this work.

References

1 K. Toko: Biomimetic Sensor Technology (Cambridge University Press, Cambridge, 2000) Chap. 6.
2 K. Toko: Sensors and Actuators B **64** (2000) 205.
3 K. Toko: Meas. Sci. Technol. **9** (1998) 1919.
4 M. Sato and N. Ogawa: Saishin Mikaku no Kagaku (Science of Taste, Asakura, Tokyo, 1997), Chap. 2. [in Japanese]
5 H. Ikezaki, A. Taniguchi and K. Toko: IEE Japan **118**-E (1998) 506. [in Japanese]
6 S. Takagi, K. Toko, K. Wada, H. Yamada and K. Toyoshima: J. Pharm. Sci. **87** (1998) 552.
7 N. Hashimoto: Biru no Hanashi Part 2 -Oisisa no Kagaku (Topics of Beer, Gihodo Publishing, Tokyo, 1998) Chap. 9. [in Japanese]
8 Y. Izumi, M. Ogawa, S. Kato, J. Shiokawa and T. Shiba: Kikibunseki no tebiki **2** (Kagakudojin, Kyoto, 1996) Chap. 2. [in Japanese]