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https://doi.org/10.15017/1516862

出版情報：九州大学大学院システム情報科学紀要. 11 (2), pp.97-101, 2006-09-26. 九州大学大学院システム情報科学研究院
バージョン：
権利関係：

九州大学大学院
システム情報科学紀要
第11巻 第2号 平成18年9月

Research Reports on Information Science and
Electrical Engineering of Kyushu University
Vol.11, No.2, September 2006

Detecting Caffeine Using Lipid/polymer Membrane

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(Received June 16, 2006)

Abstract: The composition of lipid/polymer membrane to induce membrane potential change with an uncharged alkaloid, caffeine, has been studied using the taste sensor. The membrane of taste sensor is composed of a lipid and a plasticizer immobilized with a polymer. The difference in potential between the electrode with lipid/polymer membrane and the reference electrode was measured to detect potential change caused by caffeine. For the purpose of higher sensitivity to caffeine, the membrane forming materials such as dioctyl phosphate, trioctylmethylammonium chloride and tetradodecylammonium bromide were examined. We investigated concentration-dependency of caffeine for each lipid, and estimated the optimum composition to control sensitivity of caffeine.

Keywords: Alkaloid, Caffeine, Lipid, Taste sensor

1. Introduction

A taste sensor is based on the concept of modeling the mechanism of human taste recognition[1]. It is composed of a sensor unit and a personal computer; the sensor unit has a multi-channel electrode with artificial lipid membranes. Transducers of the multichannel taste sensor were composed of several types of lipid/polymer membranes immobilized with a polymer. The difference in potential between the multichannel electrode and an Ag/AgCl reference electrode was measured. The taste sensor transformed information of taste substances into electric signals. The electric signals obtained from the sensor was converted to a digital code by a digital voltmeter and transfered to a computer. The taste quality and intensity of the sample can be expressed graphically on a taste map in a two- or three-dimensional space. The taste sensor has been applied to many kinds of foodstuffs such as beer[2),3),4)], wine[5)], sake[6),7),8)], coffee[9)], milk[10),11)], rice[12)], soybean paste[13)].

On the other hand, the sensor has poor sensitivity for uncharged molecules comparing to charged ones because of the membrane potential measurement. Caffeine is a crystalline compound that is found especially in tea and coffee plants and induce a stimulant of the central nervous system. It is classified as a purine group of an alkaloid. An alkaloid is nitrogenous organic compounds of plant origin that have physiological actions on humans. A purine is a colorless crystalline compound with basic properties, forming uric acid on oxidation. Caffeine is consisted of a pyrimidine ring and an imidazole ring, and formed heterocyclic aromatic organic compound. Though an alkaloid is usually basic nitrogen compounds, caffeine does not have base property and it is an uncharged molecule.

In this study, we tried to detect caffeine by means of membrane potential measurement of the taste sensor. In the resent analytical approach, the binding and positioning in lipid of caffeine using NMR was reported[14)]. According to the study, binding-induced chemical interaction was observed between the membrane model composed of 1,2-dimyristelaidoyl-*sn*-glycero-3-phosphocholine (DMLPC) and caffeine. DMLPC is lipid that has a N-H group and a phosphoric group with alkyl chains. Interaction between the lipid and caffeine existed, for example, strongly at alkyl chains and weakly N-H group but not around phosphoric group. We therefore tried to form membranes with several types of lipids to induce membrane potential change by caffeine.

2. Materials and Methods

Figure 1 shows the schematic diagram of taste sensing system. The sensor unit has a multi-channel electrode with artificial lipid membranes. Lipids used for preparing membranes in this study are listed in **Table 1**. Each lipid was mixed with polyvinyl chloride and plasticizer (dioctyl-n-phenylphosphonate) dissolved in tetrahydrofuran. The mixture was then dried on a glass plate that was set on a hot plate of which temperature was controlled at about 30°C. The lipid/polymer membrane is a transparent film about

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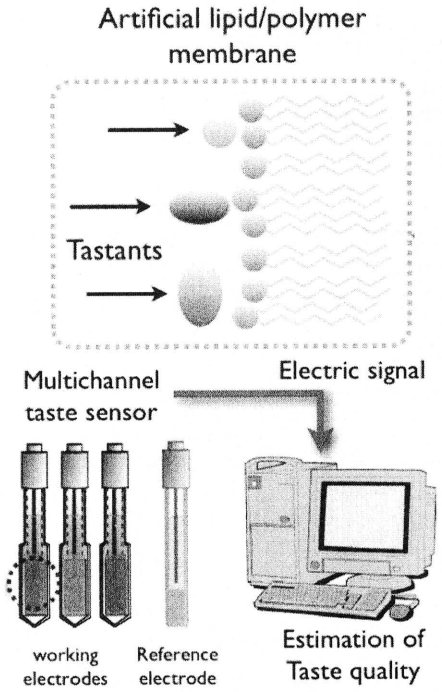

Fig. 1 Schematic diagram of taste sensor.

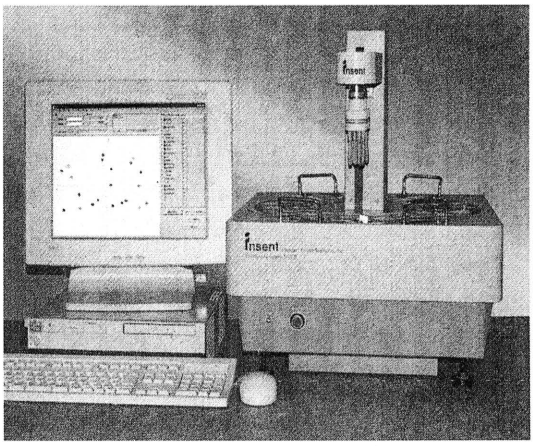

Fig. 2 Taste sensing system SA402B (Intelligent Sensor Technology, Inc.).

200μm thick.

Each electrode consisted of an Ag wire whose surface was plated with AgCl with an internal cavity filled with 3M KCl solution. The lipid/polymer membranes were adhered to the opening of the tube. The electrode potential difference between the multichannel electrode and the reference (Ag/AgCl with 3M KCl, saturated AgCl) was obtained by a high-input-impedance amplifier connected to a computer. The measurements were performed four times by a rotation procedure which involved one

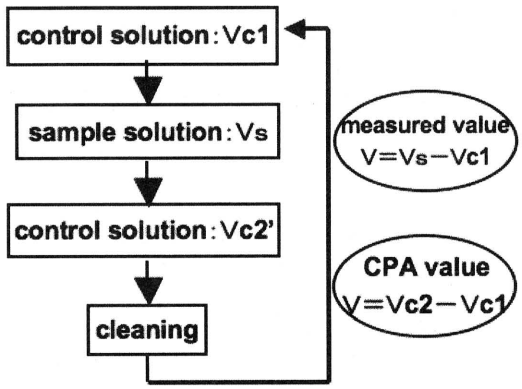

Fig. 3 Measurement procedure of taste sensor. Potential differences were obtained from Vs - Vc1 and Vc2 - Vc1.

round of measurements for all the samples. Taste discrimination was carried out by computer recognizing the signals obtained from these taste sensors in the form of patterns, so called "pattern recognition".

Figure 3 shows the measurement procedure of the taste sensor. First the control solution is measured (denoted as V_{c1}). The control solution has no taste and corresponds to the role of saliva in human. Then after the sample solution (V_s) is measured. After that the control solution is measured again. This value is called V_{c2}. V_{c2} is the measured value of the control solution after measurement in sample. The change in the measured values of the control solution before and after the sample is calculated (V_{c2} - V_{c1}). The difference is generally considered to be caused by changes in the charge density and structure of the membrane as a result of adsorption of the taste substance on the membrane. This value of V_{c2} - V_{c1} is called the "Change of membrane Potential caused by Adsorption", or CPA.

Tablel 1 shows membrane forming materials. **Figure 4** shows the chemical structure of 1,2-dimyristelaidoyl-*sn*-glycero-3-phosphocholine (DMLPC) and **Fig. 5** is structures of lipids and the plasticizer listed in **Tablel 1**.

Caffeine interacted with DMLPC with the region of alkyl chains, N-H group and boundary area between a phosphate group and alkyl chains[14]. We used simple structured lipids, which means the structure of DMLPC was divided into small pieces listed in **Fig. 5** to control sensitivity caffeine. Dioctyl phosphate (DOP) has alkyl chains and a phosphate group, which is negatively charged. Trioctylmethylammonium chloride (TOMA) and tetradodecylammonium

bromide (TDAB) were amine compounds with alkyl chains that interacted as hydrophobicity, and positively charged materials. The amount of di-n-octylphenylphosphonate (DOPP) and polyvinyl chloride (PVC) were fixed at 1.5 ml and 800 mg, respectively.

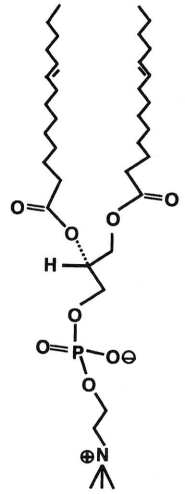

Fig. 4 1,2-dimyristelaidoyl-*sn*-glycero-3-phosphocholine (DMLPC).

Table 1 Materials used for the membrane forming process.

Material	Abbr.
Dioctyl phosphate	DOP
Trioctylmethylammonium chloride	TOMA
Tetradodecylammonium bromide	TDAB
Di-n-octylphenylphosphonate	DOPP
Polyvinyl chloride	PVC

3. Results and Discussion

First, we measured caffeine responses for membranes containing DOP, TOMA and TDAB, respectively. Caffeine dissolved in the control solution composed of 30 mM KCl and 0.3 mM tartaric acid. In the following figures, the vertical axis shows the potential difference between the caffeine sample and the control solution, and the lateral axis is the caffeine concentration.

In **Fig. 6**, electric responses were obtained compared to the membrane without adding DOP. However, the responses to caffeine did not show the concentration-dependency as DOP concentration was increased, implying that caffeine had weak or no interaction with the region of phosphate group and thus did not cause potential changes.

Figure 7 is the result of membranes contained

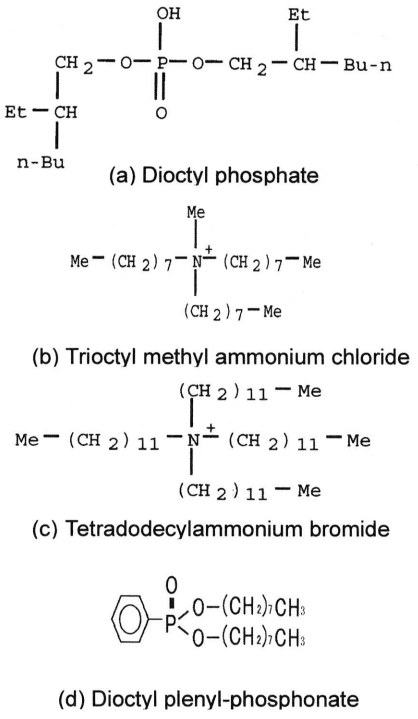

Fig. 5 (a) Dioctyl phosphate (DOP), (b) Tetradodecylammonium bromide (TDAB), (c) Trioctylmethyl ammonium chloride (TOMA) and (d) Di-n-octylphenylphosphonate (DOPP).

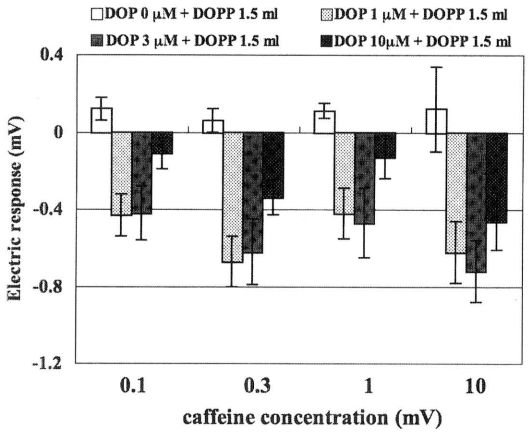

Fig. 6 Electric responses for caffeine as DOP concentration was increased.

TOMA. In this case, the membrane composed of 3μM TOMA shows concentration-dependency for caffeine, implying that caffeine had interaction with the region of N-H group on some level and therefore caused potential differences. The same results were obtained with the membrane consisted of TDAB. The membrane of 3μM TDAB also has sensitivity and concentration dependency to caffeine samples as

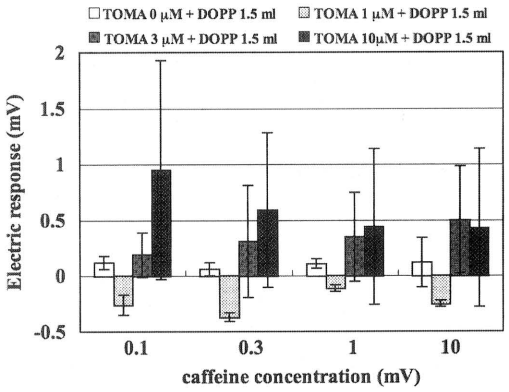

Fig. 7 Electric responses for caffeine as TOMA concentration was increased.

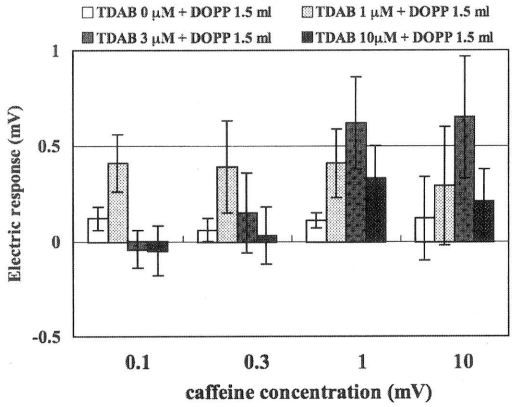

Fig. 8 Electric responses for caffeine as TDAB concentration was increased.

shown in **Fig. 8**.

From the above results, it was indicated that amine compounds such as TOMA and TDAB had possibility to increase sensitivity to caffeine, though the responses were still quite small compared to electrolyte components. We next investigated the effect of plasticizer, DOPP. TOMA and TDAB are positively charged materials, while DOPP is negatively charged one. In our early study, DOPP was negatively charged because of derivatives such as phenylphosphonic acid monooctyl ester, though DOPP itself was an uncharged compound[16]. Therefore, it would resist changes in potential and thus decrease the sensitivity to caffeine. We substituted DOPP with 2-nitrophenyl octyl ether (NPOE). **Figure 9** shows chemical structures of 2-nitrophenyl octyl ether (NPOE).

Figure 10 is the electric responses of membranes composed of TDAB, PVC and NPOE instead of DOPP. As shown in the figure, the membrane consisted of NPOE and PVC, without TDAB, has small electric

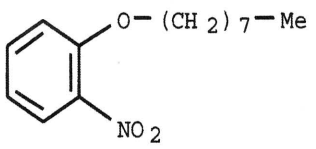

Fig. 9 2-nitrophenyl octyl ether (NPOE:plasticizer).

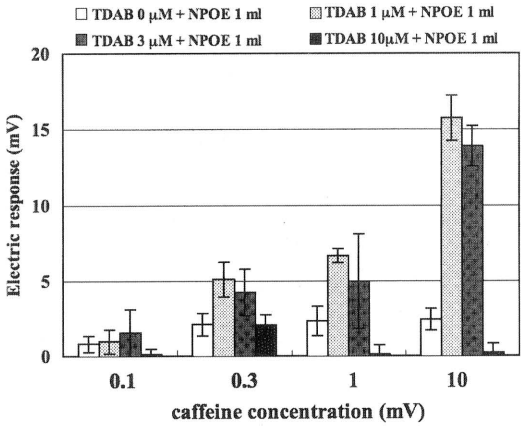

Fig. 10 Electric responses for caffeine obtained from membranes composed of TDAB and NPOE.

responses. However, the sensitivity to caffeine was dramatically improved by addition of TDAB, implying that strong interaction between TDAB and caffeine molecules existed.

As shown in figures, there is the highest sensitivity for caffeine samples with the membranes contained 1 to 3μM TDAB. From our pervious study, the electric response for an adsorptive molecule such as an alkaloid do not inrease linearity but has maximum potential, increasing the amount of lipid in the membrane[15]. It is because of hydrophobic interaction between the membrane surface and the molecule. Caffeine is also an adsorptive molecule, suggesting that the obtained peak potential around at 1 to 3μM TDAB would have some interaction related to hydrophobicity.

4. Summary

Caffeine is found in the leaves and beans of the coffee or in tea. It is defined as alkaloid reagent that is nitrogenous organic compounds, while it has no basic property and electric charge. A taste sensor with lipid/polymer membranes was required to be sensitive enough to such an nonelectrolytes. In this study, we used three kinds of lipids, DOP, TOMA and TDAB as the agent for caffeine. The potential changes was obtained from amine compounds

TOMA and TDAB, but not from a phospholipid DOP. It is known that alkaloids interact with biological materials because of hydrophobic property. As the resent practical approach using NMR showed[14], there exist cross-interaction between lipids and caffeine. Our results also indicated that the interaction played an important role in the sensitivity to caffeine, though the detailed mechanism of which induces potential change need to be worked out through further experiments.

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