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https://doi.org/10.15017/1498418

出版情報：九州大学大学院システム情報科学紀要. 4 (1), pp.37-41, 1999-03-26. 九州大学大学院システム情報科学研究科
バージョン：
権利関係：

九州大学大学院
システム情報科学研究科報告
第4巻 第1号 平成11年3月

Research Reports on Information Science and
Electrical Engineering of Kyushu University
Vol.4, No.1, March 1999

NIR Spectroscopic Study of the Effect of Taste Substances on Lipids

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(Received December 21, 1998)

Abstract: The effect of basic taste substances on lipids is investigated by mixing lipids, lecithin and DPPC (dipalmitoyl phosphatidylcholine), with five basic taste substance solutions at different concentrations and measuring their near infrared radiation (NIR) absorbance. On adding a taste solution to lecithin, second derivatives of the original curves revealed that certain peaks were slightly shifted. Out of the five taste substances (quinine, sucrose, NaCl, HCl and MSG) used, only sucrose maintained the transition temperature of hydrated DPPC.

Keywords: NIR, Taste substance, Lipid, Phase transition, Peak shift

1. Introduction

The food analysis began as long back as in the nineteenth century and was initially based on analytical techniques such as gravimetric and volumetric analysis. Because these traditional physical methods are not suitable for the food analysis of taste quality and the need for detailed quality specifications in food and beverage industries, other methods were introduced. One of the most successful methods used is the NIR (near infrared radiation) spectroscopy which has an unrivalled combination of speed, precision and experimental simplicity. Using NIR spectroscopy, samples are measured by transmittance or reflectance and mathematical processing of the spectral data enables absorption information to be extracted so that a wide range of constituents and quality attributes can be determined.

A multichannel taste sensor was developed to measure the five basic taste substances using the technique of electric potential change in the lipid/polymer membranes[1-5]. However, it is not adequate for measuring weak electrolytes and non-electrolytes as it is very sensitive to strong electrolytes. Therefore, another method of detecting taste substances using the technique of impedance change in the lipid/polymer membranes was introduced[6]. Membranes consisting of different lipid molecules showed different response characteristics of membrane resistance and capacitance to different taste substances. These two methods, using the potentiometric taste sensor and using membrane impedance change, depend on the characteristics of lipids. There is therefore need to research further on the characteristics of the interactions between lipids and taste substances in order to improve the performance of the taste sensor using lipid membranes. Having a thorough understanding of lipids can help us make suitable membranes of the sensors for different foodstuffs.

Generally, lipid bilayers are recognized as useful models of biological membranes. From this standpoint, the multichannel taste sensor using lipid membranes was developed. It is suggested that lipid molecules play an important role in receiving electrolytes, bitter substances and amino acids and that conformation of aggregated lipid molecules is very important in taste reception[1,2,5]. In certain ranges of temperature and concentration, mixture of lipids and water form homogeneous lamellae or lyotropic smectic liquid crystalline phases[7] consisting of lipid bilayers separated by layers of water.

Interaction of carbohydrates with dry lipids e.g. DPPC (dipalmitoyl phosphatidylcholine) was studied using differential scanning calorimetry (DSC)[8-10], infrared spectroscopy (IR)[9,11,12], nuclear magnetic resonance (NMR)[9,13]. It is believed that when a biological membrane is dehydrated, irreversible changes occur in its structural and functional integrity. However, recent researches showed that this integrity can be maintained under certain conditions. It was confirmed that dry organisms, e.g. fungal spores, yeast cells etc., can persist without water for decades[9,10,12,14] and the organisms rapidly resume active metabolism on becoming wet again. All these dry organisms, contain large quantities of sugar which is believed to play part in the

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survival of these organisms during dry states.

The multichannel taste sensor is also used in showing that phospholipid liposomes can suppress the bitterness of taste substances[15]. This is very important especially for pharmaceutical and food sciences. There is therefore need to find the relationship between phospholipids and other taste qualities.

The research on lecithins is very important because lecithin is used as the base material for the production, deoiling and purifications of phospholipids which are the building blocks of the body's cell membranes.

We used lecithin and DPPC for phospholipids in the present research. The word lecithin originated from a greek word *lekithos* which meant phosphorus containing lipids from egg yolk. However, nowadays, the industrial and commercial understanding of the term lecithin is used for the complex mixture of neutral lipids, polar lipids and carbohydrates. The physiological properties of lecithins mainly depend on the kind and portion of the various polar lipids. It was discovered[16] that the composition may vary considerably, depending on the origin of the used soybeans.

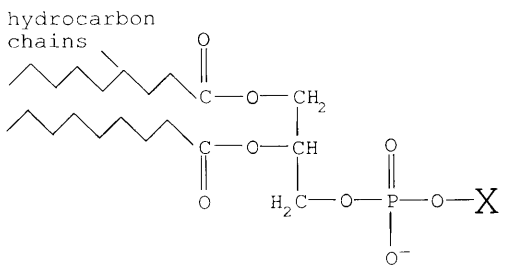

Fig.1 The lipid molecule.

Figure 1 shows the lipid molecule where X is choline for the case of phosphatidylcholine (PC), ethanolamine for the case of phosphatidylethanolamine (PE), inositol for the case of phosphatidylinositol (PI) and hydrogen for the case of phosphatidic acid (PA). The lecithin used is composed of these phospholipids.

The aim of the present paper is to find the NIR absorption characteristics of lipids, lecithin and DPPC with different taste substances at different concentrations. The results obtained here are helpful for improving the taste sensor and recognizing the biological interactions between lipids and taste substances.

2. Materials and Methods

The measurements were performed using the scanning spectrometer (UV-3100PC, Shimadzu). **Figure 2** shows a simplified diagram showing the setup for the measurement of absorbances of different samples. The wavelength range of NIR is 800 nm – 2500 nm (12500 cm^{-1} – 4000 cm^{-1}).

$$\text{absorbance} = \log[(I_o)/(I_t)] \quad (1)$$

Incident radiation, I_o, is applied to the 1 mm thick cell by the emitter and the transmitted radiation, I_t, is detected by the radiation detector. Equation 1 shows how the absorbance is calculated (Lambert-Beer's equation).

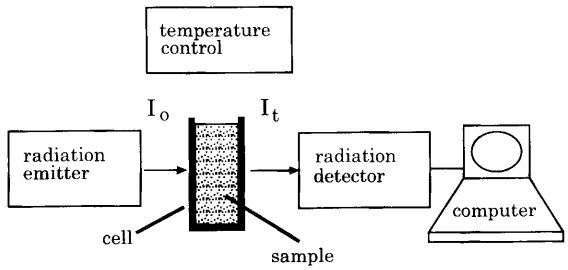

Fig.2 Setup for the measurement of the near infrared absorbances.

Solutions of sucrose (sweetness), trehalose (sweetness), NaCl (saltiness), quinine (bitterness), HCl (sourness) and monosodium glutamate (MSG, umami taste) with concentrations ranging from 0 M to 1 M were prepared. From these concentrations, 0.8 ml taste solution was mixed with 0.022 g DPPC. The mixture was stirred and warmed to a temperature of about 80°C until DPPC dissolved. The mixture was put in a 1 mm thick cell and near infrared absorbance was measured using the scanning spectrometer over the range 700 nm to 2500 nm. The temperature range was from 10°C to 60°C and measurements were taken for both increasing and decreasing temperatures. The scanning from 2500 nm to 700 nm took about 3 minutes. Each time after increasing or decreasing temperature, measurements were taken after waiting for about 3 minutes to allow the sample to attain the current set temperature.

In another set of experiments, different taste solutions, quinine (10 mM, 1 mM, 0.1 mM and 0.001 mM), NaCl (100 mM, 10 mM and 1 mM), sucrose (1 M, 10 mM, and 10 mM), MSG (0.001 M, 0.01 M, 0.03 M and 0.1 M), and HCl (1 mM, 0.1 mM and

0.001 mM) were prepared and each of them was mixed with 1 g lecithin to give a lecithin-taste solution mixture with an approximately 80 water weight percentage. The set of concentrations was determined from human gustation. The lecithin (BMI-60, Kao) used had the following ingredients; 15-20% phosphatidic acid, 40% phosphatidylinositol, 10-15% phosphatidylethanolamine and 5% phosphatidylcholine. The so prepared samples were kept for about 4 hours before near infrared absorbances of these mixtures were measured using the same procedure as before. All the measurements were taken at 30°C.

The second derivative of the original absorbance spectrum curves of lecithin and taste substances were taken at a delta λ of 20 nm. The peak shifts and existence of new peaks can be hardly identified on the original absorbance curves but on second derivative curves and this explains why second order curves were considered. To find if there were any similarities on the peak shift patterns, correlations between the peak shift patterns with taste substances and concentrations were calculated.

3. Results

Figure 3 shows the absorbances of DPPC solution and DPPC-sucrose solution at 1300 nm and this wavelength was chosen arbitrarily. It can be seen that the level of absorbance for all samples change at about 42°C. This marks the phase transition from crystalline phase to liquid crystalline phase. The same result was obtained when trehalose was used instead of sucrose. **Figure 3** also shows that with increasing sucrose concentration, the absorbances decrease.

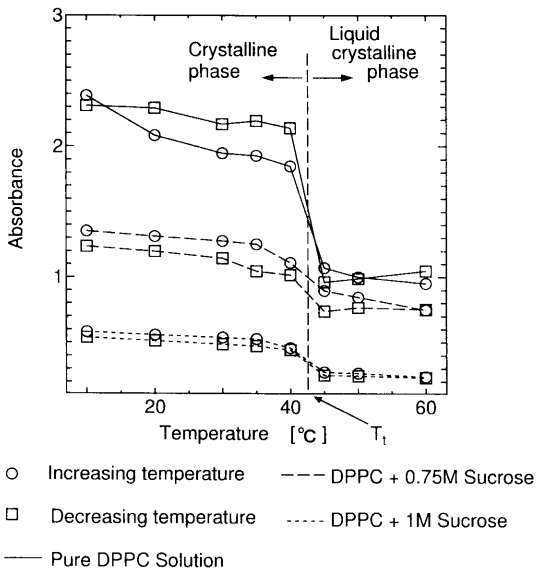

Fig.3 Absorbances of DPPC-sucrose solutions at 1300 nm showing the transition temperature, T_t, from crystalline phase to liquid crystalline phase.

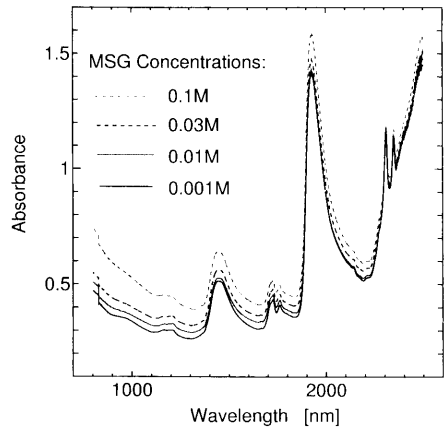

Fig.4 NIR absorbance curves of lecithin-MSG mixture.

The original absorbance spectra of lecithin-MSG solutions are shown in **Fig. 4**. The absorbances increase with MSG concentration. This observation was opposite for the case of DPPC-sucrose solutions because on increasing the sugar solution, the absorbances decreased, as shown in **Fig. 3**. The reason is that di-saccharides form hydrogen bonds with the polar head groups of lipid molecules and thereby strengthens the already existing hydrogen bonds between OH groups from water and the polar head groups[8–10]. Although the hydrogen bonds were strengthened, the phase transition temperature was not affected by the addition of sugar because the lipid-water and lipid-sugar solution interactions are similar. On the other hand, the interactions between the lipid and other taste substance solutions are different from lipid-water interactions. As a result, the transition temperature was not maintained at about 42°C and absorbances increase on increasing the concentration of these taste substances. In other words, sugar decrease the density of the lipid molecules in the mixture through hydrogen bond bridging between polar groups of lipid molecules while other taste substances increase the lipid density.

The second derivative curves of lecithin-MSG solutions are as shown in **Fig. 5**. Peak shifts can be observed easily in these second derivative curves because the peaks were magnified by the derivative operation. The peak at 2175 nm shifted with changing concentration of MSG.

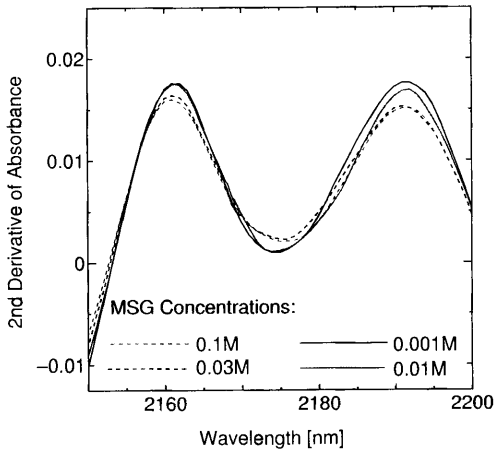

Fig.5 Second order spectrum curves of lecithin-MSG mixtures.

The peak shifts for other taste substances at this wavelength are summarized on **Fig. 6**. It shows that when the concentrations of HCl, MSG and

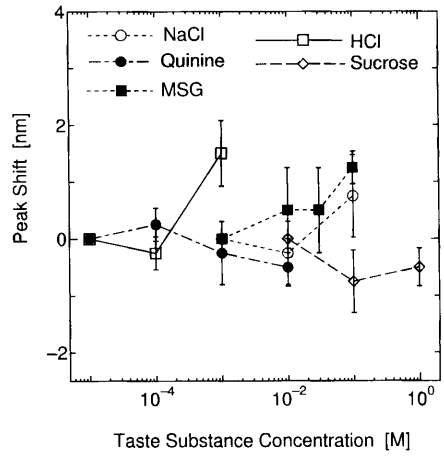

Fig.6 Peak shift of the second order curves of lecithin-taste substance solution at 2175 nm.

NaCl are increased, the peak of the second order curve at 2175 nm is shifted to higher wavelengths while in the case of quinine and sucrose, the peak is actually shifted to lower wavelengths. Peak shifts at other five wavelengths (at 2123 nm, 2192 nm, 2162 nm, 2233 nm and 2269 nm) were investigated and summarized as was done on the peak at 2175 nm. The patterns of the peak shifts were different from those at 2175 nm shown in **Fig. 6**. This implies that the patterns differ from one peak to the other.

Figure 7 shows patterns constructed from six wavelengths for different taste substances at typical concentrations.

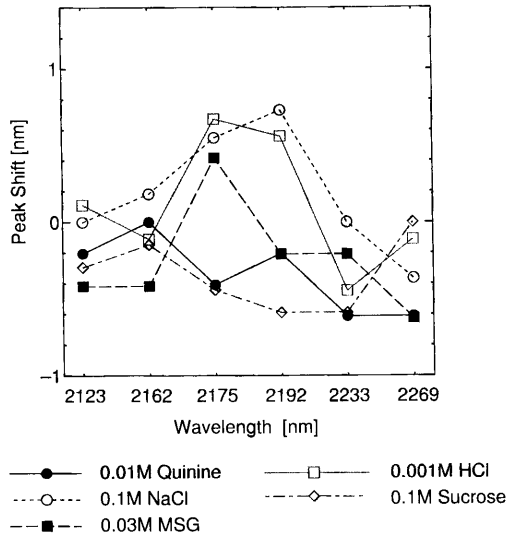

Fig.7 Pattern constructed from six wavelengths for different taste substances with typical concentrations.

4. Discussion

The change in the level of absorbance shown in **Fig. 3** signifies the transition temperature where DPPC solution and DPPC-sugar solution changes from gel phase to liquid crystalline phase. Although the absorbances show that the phases are reversible, the absorbances are not exactly the same and this might imply that the phases need longer time for them to return to their original state on increasing and decreasing temperature. According to some work covered before, besides this main phase transition, there are also two sub-transitions at 17°C and 34°C for hydrated DPPC[17]. At 17°C, the state changes from the crystalline phase to the gel phase whereas at 34°C, it changes from the gel phase to a different gel phase. However, NIR absorbances did not show these sub-transitions. This shows that conventional near infrared, so as infrared[18], have limited success in a detailed study on DPPC solution and DPPC-sugar solution phases because of intense water absorption in this region. This is the reason why most researches are done using other methods e.g. DSC[8–10], freeze drying[17], NMR[9,13]. However, the transition temperature for the samples with DPPC solution only and DPPC-sucrose solution are exactly the same, it therefore implies that adding water to DPPC is similar to adding either trehalose solution or sucrose solution. The phase transition was not maintained when DPPC was mixed with NaCl, HCl, quinine and MSG separately. This therefore agrees with the work covered

before[9, 12] that if a di-saccharide sugar e.g. trehalose or sucrose, is added to dry DPPC, hydrogen bonds (as in the case of water) are formed between the OH groups of the sugar and the polar head groups of DPPC. This also explains why the transition temperature of dry DPPC is 70°C while that of hydrated DPPC is 41°C. The hydrogen bonds alter the spacing of the polar head groups, causing an increase in the surface area per phospholipid[9, 10] and this may thereby decrease the van der Waals interactions in the hydrocarbon chains of DPPC. This is the same way sugar biologically stabilizes dry vesicles and it is also the same reason why anhydrobiotic organisms contain large quantities of sugar in their bodies. Since **Fig. 3** showed that with increasing the concentration of the sucrose solution, the absorbance decreases. This suggests that if sucrose is added to hydrated DPPC, stronger hydrogen bonds occur and as a result, the absorbances decrease. In the case of other taste substances, e.g., MSG, the increasing absorbances with MSG implies that MSG disturb the hydrogen bond formation.

The pattern of peak shifts on adding taste substances to lecithin differs from one peak to the other. **Figure 6** shows an example of the patterns at 2175 nm. The shift of peaks can be used for grouping taste substances according to their taste qualities. The difference among peak shift patterns from one peak to the other is attributable to the fact that peaks represent chemical bonds. Effects of the interaction of lecithin and taste substances is different from peak to peak. The taste quality of chemical substances mainly depend on the interaction characteristics between lipid membrane and taste substances. Therefore, the peak shift patterns shown in **Fig. 7** may have information about taste quality of the used substances. On the other hand, the present results imply that five basic taste substances influence the lipid molecule in five different ways.

Concentrations of taste substances used for making samples were different from one taste substance to the other since they were chosen according to human taste sensation. This was so because basic taste stimuli is different from one taste quality to the other.

5. Conclusion

The main phase transition of hydrated DPPC was not affected by the addition of trehalose and sucrose. It was also found that within the 10°C - 60°C temperature range, the phases are reversible although they need some time to reanneal to their original state. The OH groups from both water and sugars form hydrogen bonds with the polar head groups and this affects the phase transitions. The peaks of the absorbance curves of hydrated lecithin changed their positions after adding different taste substances at different concentrations and this can be used for grouping foodstuffs according to their taste qualities.

Acknowledgement

The authors give sincere thanks to Hayashibara Biochemical Laboratories, Inc. for providing trehalose samples.

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