

Apparent Diffusion Coefficient of Oxygen through Dehydrated Protein or Saccharide Films

Shuji ADACHI*, Koji MURAYA*, Tatsuji ISHIGURO*
and Ryuichi MATSUNO*

Thin dehydrated films of pullulan, maltodextrin, sodium caseinate, and gelatin were prepared without addition of any plasticizer. The apparent diffusion coefficient D_{app} of oxygen through them were measured under various conditions. Film formation at higher temperatures seemed to be unfavorable to reduce the D_{app} value. The D_{app} value for sodium caseinate film largely depended on the film thickness. There was no effect of relative humidity of air on the D_{app} values for all the films. The temperature dependence of D_{app} for sodium caseinate film obeyed the Arrhenius equation, but not for other films. Mixing of sodium caseinate with pullulan or maltodextrin resulted in an increase of D_{app} value. Pullulan films incorporated with emulsified methyl oleate were also prepared. The D_{app} values through them increased as the content of methyl oleate increased.

Keywords: Diffusion coefficient, Oxygen, Edible film, Permeability

INTRODUCTION

Encapsulation of liquid lipids, many of which originally possess physiological functions, into powdery matrixes of proteins and saccharides may provide some advantages or add new functions to the lipids¹⁾. One of them is a retardation of lipid oxidation²⁾. To estimate whether the retardation is due to only diffusional resistance of the dehydrated matrixes to oxygen or not, knowledges on both the oxidation kinetics and the diffusivity of oxygen

through the matrixes are required. Measurement of the diffusion coefficient of oxygen in small spherical matrixes of protein or saccharide, which is referred to as an entapping agent, may be difficult. Therefore, we prepared thin films of entrapping agents and measured the apparent diffusion coefficient of oxygen through the films.

Liquid lipids encapsulated disperse in the dehydrated droplets of proteins or saccharides. To estimate the mass transfer characteristics in the droplets, pullulan films including

* Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Sakyo-ku, Oiwake-cho, Kitashirakawa, Kyoto, 606-01 Japan

emulsified lipids were prepared and the apparent diffusion coefficient of oxygen through them were also measured. The effect of the content of lipid on the apparent diffusion coefficient was discussed.

MATERIALS AND METHODS

1. Materials

Maltodextrin (DE 2-5, Matsutani Chemical Industry), pullulan (PI-20, Hayashibara), sodium caseinate (Nacalai Tesque), and gelatin (CP-379, Osaka Kagaku-Gokin) were used as entrapping agents. Pullulan, gelatin, and three kind of polyethylene terephthalate (PET) films (normal film, film coated by silicone resin, and film treated by corona discharge) were kindly supplied from Osaka Kagaku-Gokin. Salts of analytical grades were purchased from Wako Pure Chemicals.

2. Preparation of protein or saccharide film

A dense solution of entrapping agent, the concentration of which is shown in Table 1, was degassed by aspiration to remove air bubbles. An adequate volume of the solution was poured on a PET film placed on a dick and flat glass, and then cast by an applicator (Yoshimitsu Seiki, YBA type). The clearance of the applicator could be adjusted to a given distance in a range of 0 to 250 μm . The cast solution was dehydrated by putting it in a temperature-controlled chamber to form its film. The film formed was peeled off and then used for measurement of diffusion coefficient of oxygen through it. Table

Table 1 Suitableness of polyethylene terephthalate films in preparation of entrapping agent films.

Entrapping agent	Concentration [% (w/v)]	PET ^a film	S ^b film	C ^c film
pullulan	25	○	×	○
maltodextrin	30	○	×	×
sodium caseinate	20	○	○	○
gelatin	20	○	○	○

a) PET: polyethylene terephthalate.

b) P: PET film of normal type.

c) S: PET film coated by silicone resin.

d) C: PET film treated by corona discharge.

The symbols ○ and × indicate that the PET film is adequate or preparation of entrapping agent film and not, respectively.

1 shows which type of PET film is usable for film preparation. The thickness of film was measured at some random positions (at least 5 points) by a dial thickness guage (Teclock Corp., SM-1201) and averaged.

3. Preparation of pullulan film containing methyl oleate droplet

A mixture of methyl oleate (2 g) and 1% (w/v) Tween 20 solution (7.5 ml) was homogenized in a test tube for 1 min at power control 7 by Polytron PCU-2 (Kinematica Gmb) consisting of stator rotor with blade. The emulsions were used directly or after further emulsification, which was done as follows: the emulsions were diluted 5 times by 1% (w/v) Tween 20, and then passed repeatedly through hydrophilic cellulose acetate membrane with a pore size of 0.8 μm (Advantec Toyo, DISMIC-25cs). Hydrophobic membranes were inadequate for emulsification. In both cases, the emulsions were centrifuged for 30 min at 1000 rpm to form a cream layer. An adequate volume of the cream

layer was mixed with 25% (w/v) pullulan and degassed. Then, the pullulan film containing emulsified methyl oleate was prepared as mentioned above.

4. Apparatus for measurement of the apparent diffusion coefficient of oxygen

Fig. 1 (a) illustrates the apparatus used for measurement of the apparent diffusion coefficient of oxygen. Nitrogen gas or air was supplied through a saturated salt solution to the diffusion cell, the details of which is shown in Fig. 1(b) and which was kindly made by Kuri-

moto Iron Works. The diffusion cell consisted of two chambers isolated by a film, which was fastened after keeping it under atmosphere equilibrated with a given saturated salt solution for 2 days or longer. Air in both the chambers was first replaced by nitrogen gas. After the complete replacement, the valves of the upper chamber were closed and then air was begun to supply continuously to the lower chamber. The change of the partial pressure of oxygen in the upper chamber was continuously monitored by an oxygen electrode of Galvanic cell type (Oriental Denki, RA type) and recorded. It had been confirmed in preliminary experiments that the electrode made response proportional to the partial pressure of oxygen. The volume of the upper chamber V was 17.0 cm³ and the area of film available diffusion A was 19.6 cm².

5. Evaluation of the apparent diffusion coefficient of oxygen

In our experiments, the partial pressure of oxygen in the upper chamber began to increase after a short lag-time for each film. The lag-time was too short to evaluate precisely the solubility coefficient of oxygen to the film S. Therefore, we assumed a pseudosteady state and expressed the mass balance of oxygen in the upper chamber by Eq. (1) using the difference in concentration of oxygen between the lower and upper chambers as a driving force.

$$\frac{VdC}{dt} = k_{app} A(C_0 - C) \quad (1)$$

Fig. 1 Schematic diagram of the apparatus used for measurement of the apparent diffusion coefficient of oxygen through a film (a), and the details of the diffusion cell (b).

1; air compressor, 2; nitrogen gas cylinder, 3; three-way valve, 4; saturated salt solution, 5; flow meter, 6; stop valve, 7; diffusion cell, 8; film, 9; oxygen electrode, 10; oxygen meter, 11; recorder, and 12; thermostated water bath.

where C_0 and C are the concentrations of oxygen in the lower and upper chambers, respectively, and t is the time. The concentrations

can be easily related to the partial pressure of oxygen by the equation of state for ideal gas. k_{app} is the apparent mass transfer coefficient of oxygen through a film, and is related to the apparent diffusion coefficient of oxygen D_{app} by

$$k_{app} = D_{app} / L \quad (2)$$

Equation (1) can be solved under an initial Condition of $C=0$ at $t=0$, and gives

$$C/C_0 = 1 - \exp(-k_{app}At) \quad (3)$$

By plotting $\ln [1 - (C/C_0)]$ against t , the k_{app} value was determined and converted to the D_{app} value by multiplying the film thickness L . The measurement of D_{app} was made in duplicate or triplicate for a film, and the least value was adopted because the film preparation in that case might be the best.

The D_{app} value can be also determined by using the analytical solution of the diffusion equation^{3,4)}. Since both the methods gave a similar value of D_{app} , the method mentioned above was used because of its simplicity.

Although the permeability P is generally used to characterize the mass transfer properties of films, the D_{app} was evaluated in this study for purpose in use. The D_{app} value can be converted to the P value by $P = D_{app}/RT$, where R and T are the gas constant and the absolute temperature. The intrinsic diffusion coefficient within a film D is related to the permeability P by $P = SD$.

The measurement of the diffusion coefficient of oxygen in methyl oleate was also made using the diffusion cell shown in Fig. 1. Polyfuron film (Advantec Toyo, PF050) with opening of 70% and with the thickness of 400 μm was im-

mersed in methyl oleate and kept under reduced pressure. After wiping away excess oil on the film, the film was fastened in the diffusion cell. The diffusion coefficient was obtained by multiplying the k value, determined by the above method, by the thickness of film L and then by dividing by the opening.

6. Differential scanning colorimetry of films and powders

Differential scanning calorimetric (DSC) measurements of entrapping agents (powders) and their films were made at the temperature-raising rate of 5 or 7 K/min using a differential scanning calorimeter (Seiko Denshi Kogyo, DSC-100). The films were cut to small pieces and 21 mg of them was used in DSC measurement. The same weight of Al_2O_3 was put in a reference pan.

RESULTS AND DISCUSSION

1. Effect of drying temperature on D_{app}

Fig. 2 shows the effect of drying temperature, at which films were prepared, on the D_{app} values at 25°C. There seems to be a tendency that dehydration at higher temperatures causes higher diffusivities. This might be due to that rapid dehydration at higher temperatures is liable to result in film defects.⁵⁾ All the following experiments were made by using the films prepared at 50°C.

2. Effect of film thickness on D_{app}

Films with various thickness were prepared and the D_{app} values, which were the small-

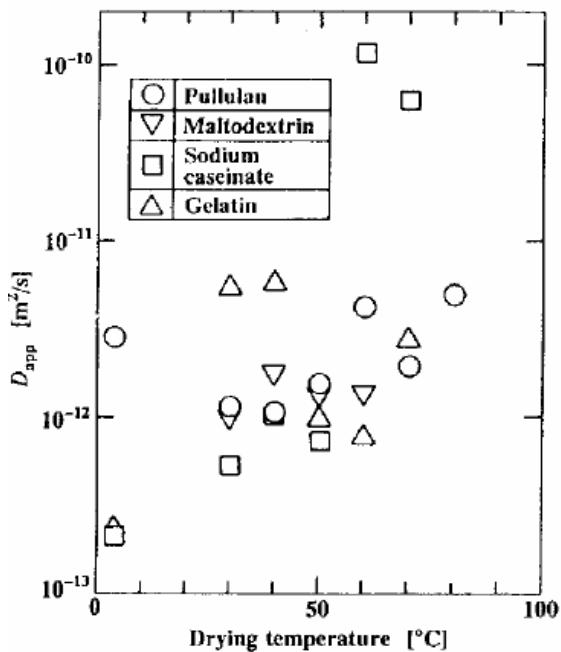


Fig. 2 Effect of the drying temperature in preparation of films on the apparent diffusion coefficient D_{app} of oxygen through films at 25°C.

est at 5 μm intervals, were plotted against film thickness in Fig. 3 for each entrapping agent. The D_{app} value through sodium caseinate film remarkably depended on the thickness. Similar dependency has been reported in terms of water vapor permeability through sodium caseinate film⁶. If the film consisted of dense and rough layers and the thickness of the dense layer was constant, such a tendency could be qualitatively explained. We observed the section of the film by a scanning electron microscope. However, such two-layer structure could not be clearly observed. For other films, the D_{app} values seemed to depend slightly on the thickness. The DSC measurements were also made for sodium caseinate and pullulan films with different thickness to evaluate their enthalpy changes ΔH . However, we could not get conclusive results due to a problem in registration of ΔH by the DSC apparatus used.

3. Effect of relative humidity on D_{app}

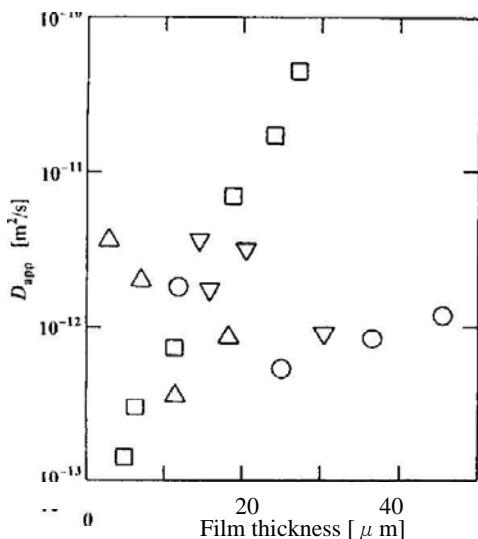


Fig. 3 Effect of the film thickness on the apparent Diffusion coefficient D_{app} of oxygen through films at 25°C.

The key are the same as in Fig. 2.

The humidity of air introduced to the diffusion cell was controlled by passing it through saturated salt solution (potassium acetate (0.23), magnesium chloride (0.33), potassium carbonate (0.44), magnesium nitrate (0.55), sodium nitrate (0.65), sodium chloride (0.75), and lithium sulfate (0.85), where the figures in the parentheses indicate the water activity of saturated aqueous solution). The films were equilibrated at the same humidity for 2 days or more before use. We had not confirmed whether the period was enough for equilibration or not. In Fig. 4, the D_{app} values are plotted

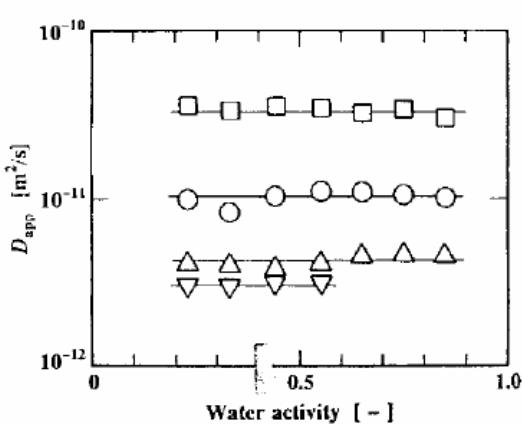


Fig. 4 Dependence of the apparent diffusion coefficient D_{app} of oxygen through films at 25°C on the relative humidity of air.
The keys are the same as in Fig. 2.

against the relative humidity of air introduced to the diffusion cell. In our experiments, the relative humidity did not affect the diffusivity of oxygen through the films, although it was reported that the permeability of oxygen through pullulan films depended on the relative humidity⁷⁾. The reason for this discrepancy remains unclear.

4. Effect of temperature on D_{app}

Fig. 5 shows the Arrhenius plot of D_{app} observed at the relative humidity *ca.* 0.75 of air introduced. The D_{app} value for gelatin film was almost independent of the temperature. That for sodium caseinate film could be expressed by the Arrhenius equation, the apparent activation energy being 45 kJ/mol. The activation energies for oxygen permeation process through corn zein, wheat gluten, and wheat gluten/soy protein isolate films have been reported to be *ca.* 46 kJ/mol⁸⁾, which is close to that mentioned above. The D_{app} for pullulan and gelatin films

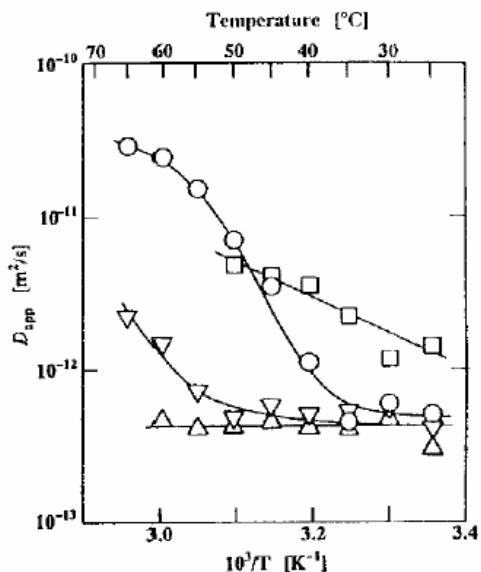


Fig. 5 The Arrhenius plots of the apparent diffusion coefficient D of oxygen through films
The keys are the same as in Fig. 2.

showed anomalous dependences on the temperature. As a reason for this, the phase transition of the films might be considerable. The DSC measurements of the films and the powders of the film-forming materials were made (Fig. 6). Although it was showed that all the films changed their structures within a range of temperature where the D_{app} values were measured, it was difficult to find the correlation between the D_{app} values and the DSC measurements. As another reason for the anomalous dependence of the D_{app} on the temperature, existence of pinholes in the films used for the D_{app} measurement may be considered⁹⁾. However, we have not examined in this point.

5. D_{app} through film prepared by mixing protein and saccharide

Proteins were mixed with saccharides at var-

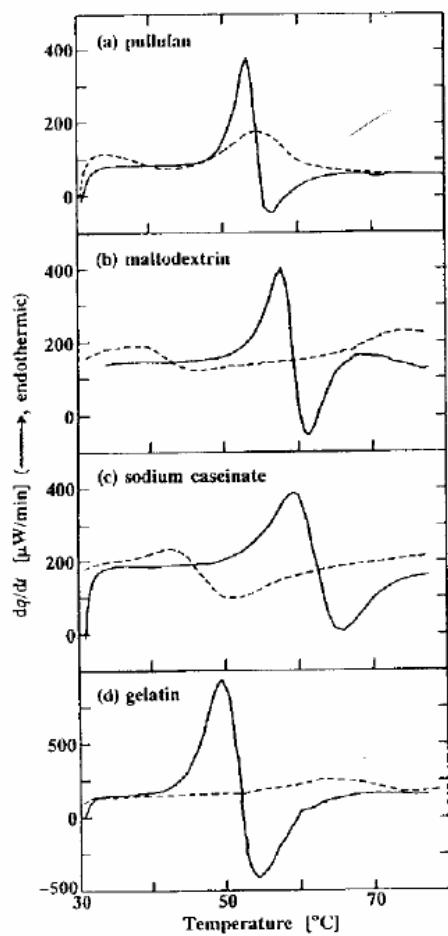


Fig. 6 Differential scanning calorimetric measurements of dehydrated films and their constituent powders. The solid and broken curves indicate the films and powders, respectively. The temperature-raising rate was 7°C/min except for pullulan (5°C/min).

ious ratios and the films of the mixtures were prepared. The D_{app} values through them were observed at 25°C (Fig. 7). Mixing of sodium caseinate with saccharides resulted in an increase of D_{app} , while the mixing of gelatin showed no significant effect on D_{app} . This may be due to the molecular shape of protein mixed.

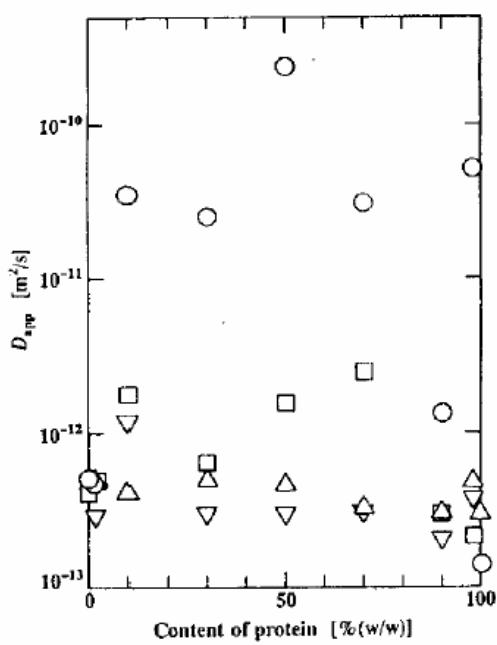


Fig. 7 Effect of mixing of protein and saccharide on the apparent diffusion coefficient D_{app} of oxygen through films at 25°C.
Symbols: ○, pullulan + sodium caseinate; △, pullulan + gelatin; □, maltodextrin + sodium caseinate; ▽, maltodextrin + gelatin.

6. D_{app} through pullulan films with emulsified methyl oleate

The D_{app} values are plotted against the volumetric content of methyl oleate in Fig. 8. The D_{app} values did not depend on the method for emulsification, mechanical and mechanical + membrane emulsifications. The size distributions of emulsions, which were measured by a centrifugal particle size analyzer (Shimadzu, SA-CP3L) after dissolving the pullulan films by 0.1% (w/v) sodium dodecyl sulfate, is shown in Fig. 9. The solid curves I and II in Fig. 8 were the D_{app} values calculated by the series and parallel models, respectively. They are expressed by

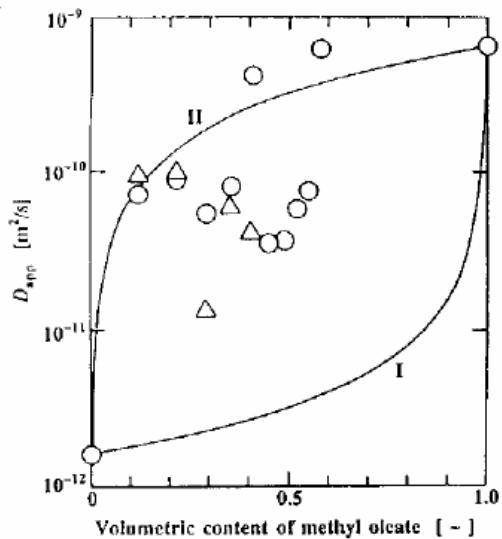


Fig.8 Dependence of the apparent diffusion coefficient D_{app} of oxygen through pullulan films containing emulsified methyl oleate at 25°C on the volumetric content of methyl oleate. The curves I and II are those calculated by the series and parallel models, respectively. The symbols ○ and △ indicate that the emulsions were prepared by mechanical and mechanical + membrane methods.

$$D_{app} = \frac{(D_{xL}HRT/\phi)(D_{xG}/(1-\phi))}{D_{xG}/(1-\phi) + D_{xL}HRT/\phi} \quad (4)$$

$$D_{app} = \phi D_{xL}HRT + (1-\phi)D_{xG} \quad (5)$$

where D_{xO} and D_{xL} are the diffusion coefficients of oxygen in dehydrated entrapping agent film and methyl oleate, respectively. H is the solubility coefficient of oxygen to methyl oleate. Because we could not find the H value for methyl oleate, the H value for soybean oil¹⁰, which is rich in oleate, was used in the calculation. ϕ indicates the volumetric content of methyl oleate in the films. The observed D_{app} values are between the curves I and II, and if anything, are close to the curve II. This may be due

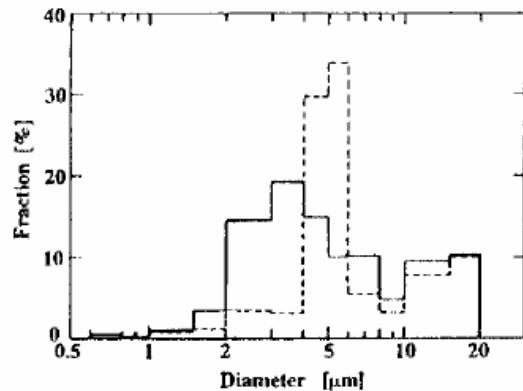


Fig.9 Size distribution of methyl oleate droplets entrapped in pullulan film. The solid and broken lines are for emulsions prepared by mechanical and mechanical + membrane methods, respectively.

to that there were large droplets in the films, which were in the same order as the film thickness.

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タンパク質および糖の乾燥薄膜における酸素の見掛けの拡散係数

安達修二* 村谷浩二* 石黒達治* 松野隆一*

プルラン、マルトデキストリン、カゼインナトリウムおよびゼラチンの乾燥薄膜を調整し、種々の条件下で酸素の見掛けの拡散係数 D_{app} を測定した。製膜時の乾燥温度を高くすると、 D_{app} が大きくなる傾向にあり好ましくなかった。カゼインナトリウム膜での D_{app} は膜厚に大きく依存した。いずれの膜についても、相対湿度は測定範囲内(0.23~0.85)で D_{app} に影響を及ぼさなかった。カゼインナトリウム膜では D_{app} の温度依存性はアレニウスの式で整理できたが、他の膜では適用できなかった。カゼインナトリウムをプルランやマルトデキストリンに混合して調整した膜では D_{app} が大きくなり好ましくなかった。エマルジョン化したおれいん酸メチルを含むプルラン膜では、脂質含有率の増加に伴い D_{app} が大きくなかった。

キーワード：拡散係数、酸素、可食性フィルム、透過性