MICROENCAPSULATION OF FLAVORS AND OIL BY CYCLODEXTRIN

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Abstract: Cyclodextrins are used to improve stability of flavor and/or oil via encapsulation of certain specific ingredients that exist by nature in food materials. The method is called often as “molecular encapsulation”, because the ingredients are encapsulated in the molecular cavity of cyclodextrins. Cyclodextrins form inclusion complexes with a variety of molecules including flavors, fats and colors. Most natural and artificial flavors are volatile oils or liquids and complexation with cyclodextrins provides a promising alternative to the conventional encapsulation technologies used for flavor protection.

Key Words: Cyclodextrin, Molecular inclusion, Flavor, Inclusion complex

1. INTRODUCTION

Cyclodextrins (CDs) are doughnut shaped cyclic oligosaccharides with an interior cavity and they form specific inclusion complexes with many organic compounds. CDs are made from starch using the CD transglycosylase enzyme to hydrolyze and cyclize the starch to form closed circular molecules or CDs. Typically, these CD molecules contain six, seven or eight glucose molecules and are called α-, β- or γ-CD. The CD molecule is composed of glucose units which are linked by α-1, 4 bonding found in starch. The hydrogen and glucosidic oxygen atoms face toward the inside of the CD and form an electron-dense or apolar lining for the cavity of the CD, which can interact with hydrophobic compounds that match the CD cavity to form an association or complex. On the other hand, the polar hydroxyl groups of the glucose monomers face toward the outside of the CD molecule and responsible for the aqueous-solubility properties of the CDs and their complex.

After a century of continuous research and development, CDs have gained certain recognition of its importance in various fields including foods, pharmaceuticals, agricultural, analytical, cosmetics, personal care, etc. Their applications are mainly intended for the entrapment of smaller molecules, stabilization of reactive intermediates, catalysis through encapsulation and as potential molecular transport and drug delivery device [1]. In food related applications, flavor compounds are being encapsulated into CDs for better retention and protection from various possible means of deterioration, as well as for controlled delivery. In pharmaceutical field, publications were made based on study on solubility enhancement of poor water soluble drugs.

CDs are also being utilized as carriers for controlled release of particular compounds entrapped within the cavities [2-5]. Particularly in pharmaceutical industry, controlled release systems are desirable to give optimized efficacy, safety and convenience because they can be designed to deliver a drug at a specified rate, for a specific period of time and even at a desired location [6]. Performance of CDs as carrier in controlled release systems can be evaluated by the release characteristics of inclusion complexes at various parameters of interest. Application of a specific inclusion complex is to a great extent dependent on its release characteristic.

2. FUNDAMENTALS OF INCLUSION

2.1. Minimum Water Content Needed for Inclusion

Recently, considerable attention has been focused on the water content required to encapsulate a guest with CDs, in order to study the possibility of the formation of inclusion complexes with low moisture content. The presence of water is essential for the formation of inclusion complexes between CDs and hydrophobic substances. However, no one has yet determined the number of water molecules needed for the inclusion of guest molecules in CDs. We determined the

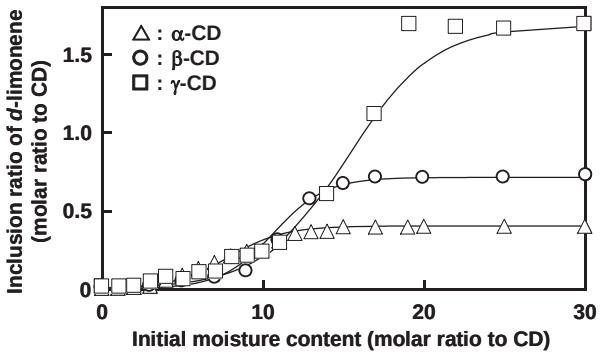

Fig. 1 The formation of inclusion complex between *d*-limonene and CDs with various water contents.

minimum number of water molecules required to encapsulate *d*-limonene (the guest molecule) in α-, β-, and γ-CD by means of a micro-aqueous method, which is used to study enzymatic reactions in organic solvents [7]. First, a CD powder was mixed with *d*-limonene to make a suspension. A given amount of water was added to the resulting cloudy suspension of CD to obtain an inclusion complex. With this method it is possible to prevent the formation of large agglomerates of CD paste in which the water can not be distributed uniformly. Inclusion complexes between *d*-limonene and CDs were prepared with various proportions of water by the micro-aqueous method. The amount of an inclusion complex was significantly influenced by the water content in the liquid mixture, as shown in Fig. 1. Inclusion complexes were barely formed at zero water content with all the CDs. The amount of inclusion complex increased gradually in the low moisture content region. However, over specific initial moisture content for each CD, which is roughly 2, 4, and 10 for α-, β-, and γ-CD, respectively, the inclusion ratio increased exponentially and reached a maximal plateau. The maximum inclusion ratios of α-, β-, and γ-CD were 0.4, 0.7, and 1.68, respectively. These inclusion ratios suggested that the inclusion stoichiometries between CDs and *d*-limonene were roughly 2:1, 1:1, and 2:3 for α-, β-, and γ-CD, respectively.

2.2. Inclusion Complex in Organic Solvent

The most popular method for preparation of a

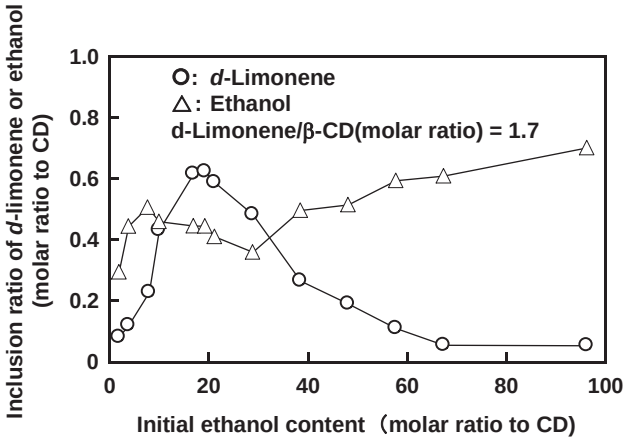

Fig. 2 The formation of inclusion complex of *d*-limonene and ethanol with various ethanol contents.

complex between CD and a drug is to form the inclusion complex in an aqueous solution of CD. However, since various pharmaceutical drugs have low solubility in water, it is very important to develop a new method for the effective formation of the inclusion complexes. Very poorly soluble guests cannot be complexed in any acceptable concentration without using a solvent. It has been shown that the addition of a selected third component such as alcohols or surfactants can affect the formation of CD complexes. However, there were no studies on the preparation of the inclusion complexes with pure organic solvents. This section presents the preparation of inclusion complexes between CDs and the guest in an organic solvent, ethanol. Figure 2 shows the inclusion ratios of *d*-limonene or ethanol in β-CD against the amount of ethanol added to the slurry [8]. With an increase in the amount of ethanol added to β-CD, the inclusion ratio of *d*-limonene increased up to a maximum value at the molar ratio of ethanol to β-CD of about 20, beyond which the inclusion ratio decreased. On the other hand, the inclusion ratio of ethanol to β-CD decreased to a minimum at about molar ratio of 30, and then increased with the increase in the ethanol content in the slurry. At higher ethanol content, *d*-limonene might be prevented by ethanol from the formation of inclusion complex with β-CD.

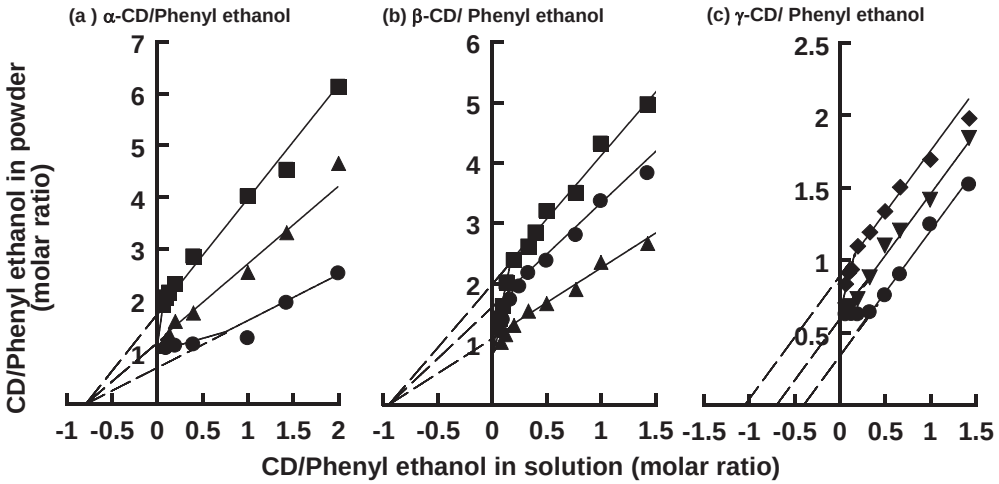

Fig. 3. Correlation of the inclusion ratio of phenyl ethanol in CDs by the inhibited enzyme kinetics of the Michaelis-Menten type. ●, *d*-limonene/CD (molar ratio)=0; ▲, 2; ■. 5; ▼, 10; ◆, 20.

2.3. Competitive Inclusion of Flavors in Cyclodextrin

In the previous section, we have demonstrated that there were some competitions of inclusion between two flavor guest compounds. For the inclusion of binary and ternary systems, Furuta *et al.* [9] demonstrated that the addition of linear alcohol enhanced the inclusion of *d*-limonene, particularly alcohols of high polarity and short alkyl chain length. In this section, the competitive inclusion of a target flavor molecule into CDs for binary systems is described. The preparation of inclusion complex between the guest compounds and the CDs is basically by the same method described in the previous section. Phenyl ethanol/*d*-limonene and methyl *n*-hexanoate/*d*-limonene were used as model binary systems, using phenyl ethanol and methyl *n*-hexanoate as target flavors, and *d*-limonene as an inhibitor. The inclusion ratio of phenyl ethanol or *d*-limonene of the inclusion complex was investigated against the molar ratio of phenyl ethanol added to CD. For α-CD, the inclusion ratio of phenyl ethanol increased rapidly with an increase of the initial amount of phenyl ethanol added, followed by a plateau value which depended on the amount of *d*-limonene. On the other hand, *d*-limonene included in α-CD decreased with the increase in the initial phenyl ethanol content. This implies that phenyl ethanol and *d*-limonene might be included competitively in the molecular cavity of CD. Double reciprocal plots for phenyl ethanol at three different *d*-limonene concentrations are depicted in Figs. 3(a), (b), and (c) for α-, β- and γ-CD, respectively [10]. The competitive inclusion between phenyl ethanol and *d*-limonene for each CD could be successfully represented by the double reciprocal plot for the enzyme inhibition kinetics of Michaelis-Menten type. At the low concentration of phenyl ethanol, the reciprocals of the inclusion fractions of phenyl ethanol for α- and β-CD were correlated well by lines of different slopes, depending on the mixing ratio of *d*-limonene to phenyl ethanol and intersecting the x-axis at a definite point. This means that noncompetitive inhibited inclusion could be applied between phenyl ethanol and *d*-limonene for α- and β-CD. For γ-CD, on the other hand, the uncompetitive type of inhibition was seemed to take place, since the correlation lines had the same slope. The difference of the inhibition type may be attributed to the cavity diameter of the given CD. A larger cavity of γ-CD could include both phenyl ethanol and *d*-limonene together, indicating the uncompetitive inhibited inclusion. At higher concentration of phenyl ethanol, the correlation lines converged at a point on the y-axis, independent of the concentration of *d*-limonene and the type of CD. This implies that in the concentration region studied the competitive inhibited inclusion was true between phenyl ethanol and *d*-limonene.

3. INCLUSION AND OXIDATION OF PUFA INCLUDED IN CYCLODEXTRIN

3.1. Inclusion Complex of PUFA by Cyclodextrin

The ω-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have important physiological functions. These PUFAs are chemically quite reactive, requiring

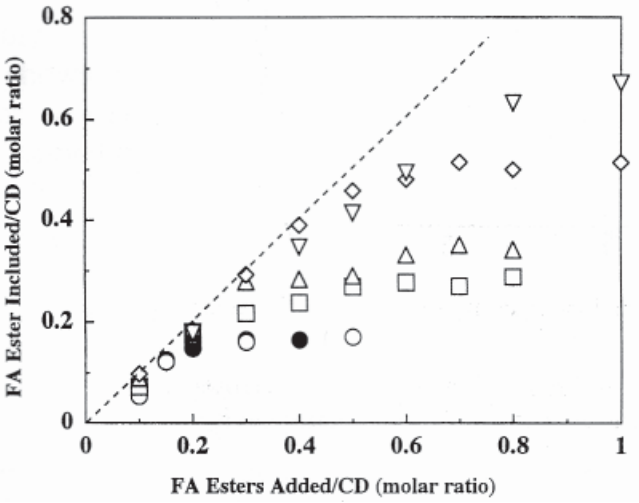

Fig. 4. Effect of the addition molar ratio of various FAME to α-CD on the inclusion fraction of α-CD complex of fatty acid methyl/ethyl ester. ▽ CAPME; ◇ CPRME; △ MYRME; □ LINME; ○ EPAEE; ● DHAEE

proper encapsulation in a powder form to protect against autoxidation. A molecular inclusion by cyclodextrin was applied for encapsulation of PUFA into powder form. In this section, inclusion of fatty acid methyl/ethyl esters (FAME) with cyclodextrin, and the oxidation process of the powdery ethyl eicosapentaenoate included in α-, β- and γ-CD are discussed. The inclusion complex powders of FAME with cyclodextrin were prepared by a self-cleaning twin-screw kneader [11] in a nitrogen atmosphere. The dried CD was mixed with FAME of in a nitrogen atmosphere, followed by adding distilled water to an initial moisture content of 30% on dry basis. The powdered mixture was supplied to the twin-screw kneader and kneaded. Nitrogen gas was passed through the kneader to prevent the oxidation during kneading. The kneaded wet slurry was dried *in vacuo*, followed by grinding and stored in a refrigerator until use. Fig. 4 shows a plot of the amount of FAME included in α-CD against the initial amount of FAME added to α-CD [12]. The dotted line in Fig. 4 indicates the theoretical value of the included FAME if all of it was completely included into α-CD. The included FAME in α-CD increased linearly with the amount of added FAME and reached a plateau, indicating the maximum inclusion value. This maximum value decreases with the chain length of the FAME. From the plateau value, we estimated that the average number of α-CD molecules required for one mole of EPAEE or DHAEE is about 6.

3.2. Isothermal Oxidation of EPA Included in Cyclodextrin

Isothermal autoxidation of EPA inclusion complex powders was investigated with a gas flow type equipment. Since all EPA mixed and kneaded with CDs could not be included in CDs, the dried powders were washed with diethyl ether to remove the adsorbed EPA on the surface of the CD powders. About 0.1 g of the complex powder was placed in a glass bottle, which was stored in an air bath at 50 ºC. The gas mixture of oxygen and nitrogen of an equal volume ratio was passed through the glass bottles. After a timed period, the glass bottles were taken out, and the residual amount of EPA was measured with gas chromatograph. The time courses of oxidation of

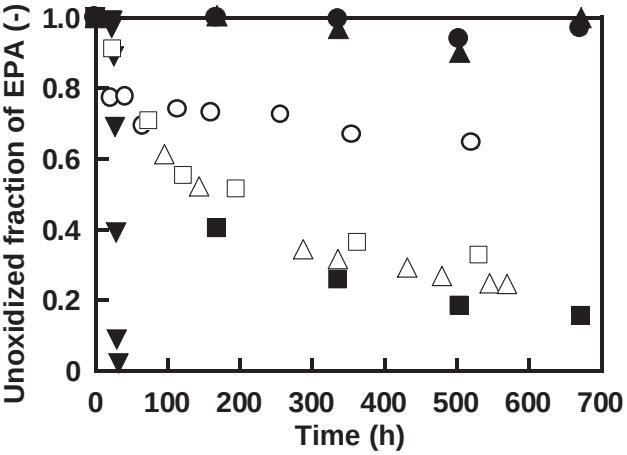

Fig. 5 Retardation of the autoxidation of powdery EPA. ▼ Liquid EPA. ●○ in α-CD, ▲△ in β-CD, ■□ in γ-CD. Open symbol are unwashed powders, and closed symbols are washed powders. 50 ºC and 0% relative humidity.

EPA included in CDs at 50 ºC in a dry gas are illustrated in Fig. 5 [13]. The open symbols are the

results for the unwashed powders, and closed symbols represent for washed ones. The oxidation of liquid EPA was illustrated as a control sample in Fig. 5. EPA included in all CDs is quite stable compared to liquid EPA, irrespective of the washing operation. However, the oxidation stabilities of EPA included in α- and β-CD are markedly dependent on the washing operation. The washed powdery EPA in α- or β-CD was quite stable against oxidation, and most EPA remained unoxidized for 670 h. The resistances against autoxidation of the unwashed powders are also improved in comparison with liquid EPA, but are inferior to the washed powder. For unwashed complexed α-CD powder, about 25% of EPA was oxidized abruptly in the initial period, which was nearly corresponding to the EPA by the first washing operation. The unwashed EPA powder of β-CD could be oxidized markedly in the initial period, and then gradually with time. For γ-CD, on the other hand, there were no clear differences of the oxidation time course of EPA between the washed and unwashed powder. From these results, it could be concluded that EPA truly included in α-CD may be quite resistant to oxidation.

4. INCLUSION OF FOOD FLAVORS IN CYCLODEXTRIN

4.1. Formation of Inclusion Complex of Flavors in Natural Cyclodextrin and the Release

Molecular inclusion of flavors has been used as a method of converting liquid flavors into a dry form. For food applications of the inclusion complex powder of flavors, a controlled release property has been needed for many food products such as microwave entrees, snacks and desserts, etc. The pharmaceutical industry has utilized the technique as a drug delivery system. However, the release kinetics of the guest compounds from the inclusion complex powder has not yet been fully understood. In this section, the release rate of allyl isothiocyanate (AITC) was investigated at various temperatures and relative humidities for different cyclodextrins. The release of AITC was investigated at various temperatures and relative humidities for different cyclodextrins. The release time courses of AITC from the inclusion complex powders were measured with

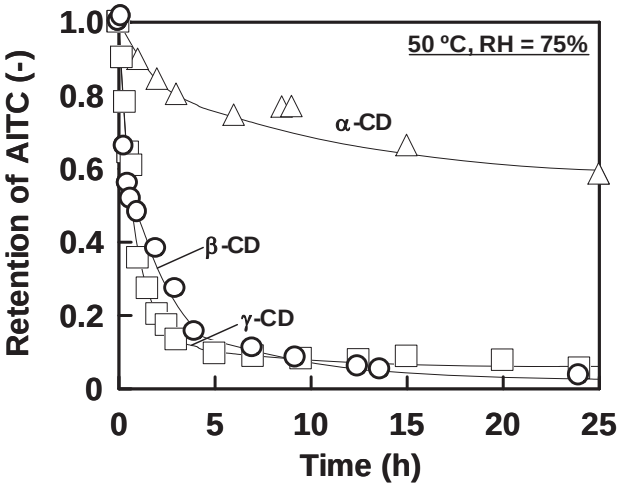

Fig. 6. Release time course of AITC included in the different cyclodextrins.

the same equipment as reported previously [14]. The inclusion powder (ca. 0.1 g) was weighed in glass bottles, and stored in an air bath at a constant temperature. Humid air was blown into the glass bottle to sweep out the AITC released from the powder. At prescribed intervals, the glass bottle was taken out, and the residual AITC was measured by gas chromatography. The retention of AITC was defined as the ratio of the residual amount of AITC to the initial one. Fig. 6 shows the comparison of the retention time-courses of AITC included in α-, β-, and γ-CD against the release time at 70 ºC and 60% of relative humidity. The AITC included in α-CD exhibited an extended release. After 25 hours, 60% of the initial amount of AITC still remained in the powder at such a high temperature and humidity. On the other hand, the AITC included both in β- and γ-CD was considerably released during the initial period of release, showing quantitatively a similar release behavior in both cases. This implied that AITC included in α-CD had good controlled released properties. The qualitatively similar results were observed for the different temperatures and humidities used. The effect of relative humidity on the time-course of the AITC release is shown in Fig. 7 for the inclusion powder with β-CD [15]. The effect of the relative humidity on the release of AITC was pronounced. As the relative humidity was increased, the release of AITC was greatly accelerated, particularly between 50 to 60% of relative humidity.

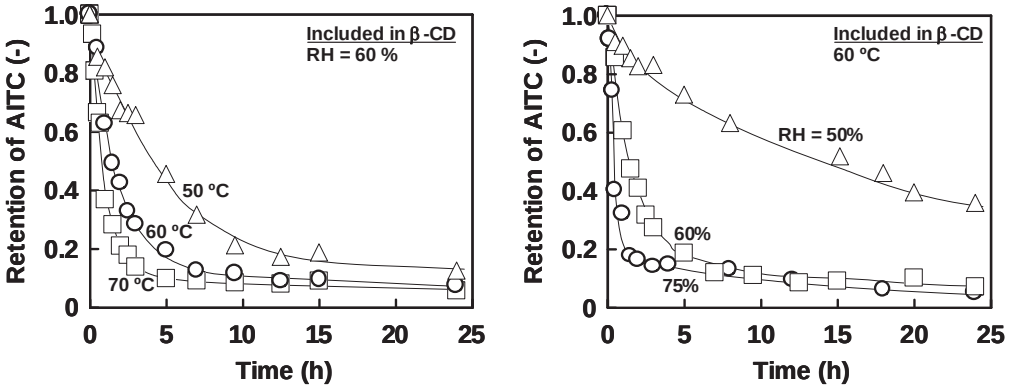

Fig. 7 Effects of temperature and relative humidity on the release of AITC from the inclusion complexes

Table 1. The maximum inclusion ratios of various flavors in modified

Flavor	HP-β-CD	RM-β-CD	TA-β-CD
d-Limonene	0.4	0.66	5
AITC	0.37	0.85	0.4
l-Menthol	0.73	0.68	1.1
Ethyl Butyrate	0.48	0.70	0.46
Ethyl propionate	0.45	0.83	0.2-

This suggested that the release of AITC was closely related to the presence and the concentration of water molecules surrounding the powder.

4.2. Inclusion of Flavors in Modified Cyclodextrins and the Release

Recently, some modified CDs were developed and used for inclusion of many chemical and pharmaceutical compounds, since they remarkably enhance the solubility. In this section, three kinds of modified CDs, namely 2-hydroxypropyl-β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD), and triacetyl-β-CD (TA-β-CD), were used to include various flavors such as *d*-limonene, allyl isothiocyanate (AITC), and *l*-menthol [16]. Various flavors were included into modified CDs by solution method and spray drying. In the case of TA-β-CD, owing to its insolubility in water, acetone was used as the medium for encapsulation. Table 1 shows the inclusion ratio (molar ratio of flavor compound to CD in the inclusion complex) of various flavors for the modified CDs. Inclusion ratio depended on the combination of the flavor and CD. The inclusion ratios of complexes from HP-β-CD and RM-β-CD were less than 1. In the case of TA-β-CD, a continuous increase of inclusion of inclusion ratio of *d*-limonene with the increase of the initial *d*-limonene/CD molar ratio was observed. Flavor/RM-β-CD complexes had higher inclusion ratio than that from HP-β-CD except for Menthol/RM-β-CD complex. Flavor/TA-β-CD complexes showed different inclusion behavior from MT-β-CD and HP-β-CD complexes. Among the three modified CDs, HP-β-CD had the lowest ability of inclusion of all the flavors used. Inclusion complexes were subjected to release experiments under constant relativity humidity (75% and 31%) at constant temperature (50 °C). The release rate constants of flavors were estimated by the following Avrami equation on the basis of the release time-courses of the included flavors.

$$R = \exp[-(k \cdot t)^n] \quad (1)$$

where R (-) is the retention of flavor in the complex powder at time t (s), k (s^{-1}) the release rate constant and n the release mechanism parameter.

Release characteristics of complexed flavors prepared by freeze drying and spray drying

Release characteristics distinctively depended on the type of CD and guest flavor compound. Figure 8 illustrates the release time-courses of the included flavors in RM-β-CD by both freeze drying and spray drying. Generally aromatic flavors such as *d*-limonene and *l*-menthol were hard to release. However AITC was found to have the most unstable host-guest interaction. The solid lines in the figure are the correlation curves by equation (1), showing good agreement with the empirical data. Effect of relative humidity of air on release characteristics

Figure 9 shows the release time-courses of *d*-limonene and AITC for freeze dried inclusion complex powder of HP- and RM-β-CD under 31% and 75% RH at 50 °C. The release of *d*-limonene complexed in RM-β-CD is markedly dependent on the relative humidity, while for HP-β-CD the release was almost independent of the relative humidity. On the other hand, the release of AITC is dependent on the relative humidity for both CDs. As the storage humidity rises higher, the inclusion complex becomes less stable and the release rate becomes higher.

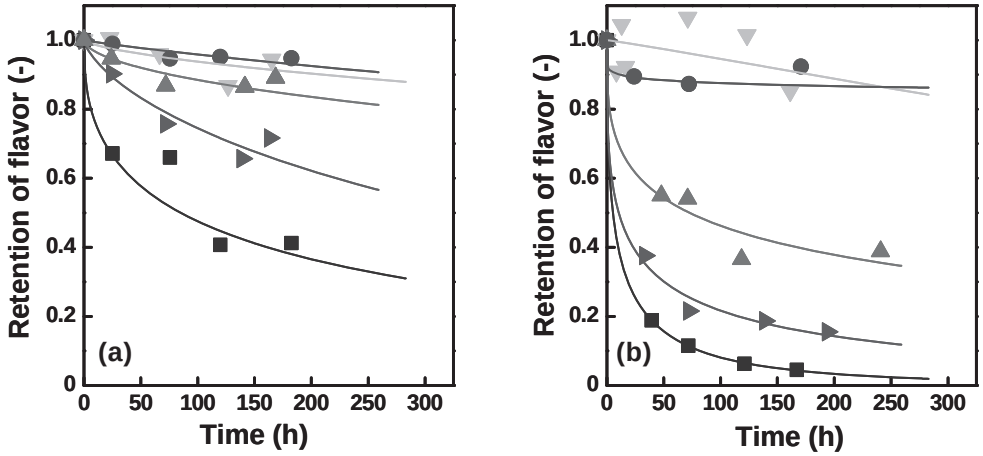

Fig. 8. Release characteristics of complexed flavors prepared by (a) freeze drying and (b) spray drying. ●*d*-Limonene, ▼ *l*-Menthol, ■ AITC, ▲ Ethyl *n*-butyrate, ▶ Ethyl *n*-propionate.

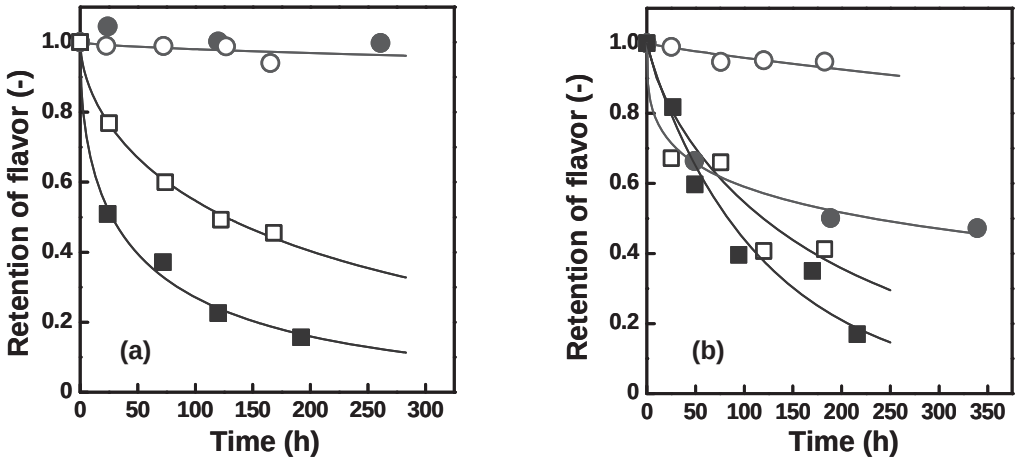

Fig. 9. Effect of relative humidity of air on release characteristics of *d*-limonene and AITC included in (a) HP-β-CD and (b) RM-β-CD. *d*-Limonene:● 75% RH,○ 31% RH. AITC: ■ 75% RH, □ 31% RH.

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