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Kinetics of Molecular Encapsulation of 1-Methylcyclopropene into α-Cyclodextrin

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1-Methylcyclopropene (1-MCP), an ethylene inhibiting regulator, is commercially available in the form of an inclusion complex with α-cyclodextrin (α-CD). In this study, molecular encapsulation of gaseous 1-MCP into aqueous α-CD was investigated in a closed, agitated vessel with a flat gas–liquid interface. Molecular encapsulation of gaseous 1-MCP by α-CD is a simultaneous two-step reaction which involves the aqueous dissolution of gaseous 1-MCP and the encapsulation of the dissolved molecules by α-CD. The kinetics and mechanism of molecular encapsulation were analyzed based on the depletion rate of 1-MCP in the headspace of the vessel. The encapsulation rates could be explained quantitatively by the gas absorption theory with a pseudo-first-order reaction between 1-MCP and α-CD. The negative value of the calculated apparent activation energy of encapsulation (−24.4 kJ/mol) implied the significant effect of exothermic aqueous dissolution of 1-MCP. An encapsulation temperature of 15 °C was optimal; at this temperature, the highest 1-MCP yield and best inclusion ratio of inclusion complex were obtained. Changes in the X-ray diffraction pattern suggested that the crystal lattice structure of α-CD was altered upon inclusion of 1-MCP.

KEYWORDS: 1-Methylcyclopropene; α-cyclodextrin; gas encapsulation; inclusion complex; ethylene inhibitor

INTRODUCTION

1-Methylcyclopropene (1-MCP) is a four-carbon cyclic olefin that has a three-membered ring to which a methyl group is attached at the C1 position. 1-MCP is an ethylene inhibiting plant growth regulator that works at parts-per-billion levels (*1*). Its nontoxic mode of action and negligible residue make it an extremely favorable agrochemical in regards to humans, animals, and the environment (*2*). The mechanism of action of 1-MCP in inhibiting ethylene is presumed to be through tight binding to ethylene receptors of target plant materials, thereby blocking ethylene action (*3*). Under normal environmental conditions, 1-MCP is present in the gaseous state. It is chemically unstable and begins to self-react immediately. Furthermore, it also presents an explosive hazard when compressed. On account of the aforesaid difficulties, 1-MCP is never isolated in the manufacturing process for safety reasons. Instead, 1-MCP is produced as an inclusion complex with α-cyclodextrin (α-CD), by which its explosiveness is greatly diminished (*4*). Procedures for molecular encapsulation of 1-MCP with α-CD in both batch (*5*, *6*) and continuous manners (*7*) have been patented. Despite the fact that the study of the kinetics of the encapsulation reaction is essential for improvement of the encapsulation process, data on kinetic analysis of the encapsulation reaction are currently not available.

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Molecular encapsulation of 1-MCP by CD transforms the gaseous 1-MCP into powder form for easy and safe handling, storage, transportation, and application to target plant materials. Being encapsulated, 1-MCP does not exhibit a very high vapor pressure and is therefore protected from oxidation and other chemical degradation reactions (*5*). 1-MCP is released as a gas from a formulated CD powder through aqueous dissolution of the CD and release of the encapsulated gas (*1*).

Cyclodextrins (CDs) are natural starch derivatives resulted from enzymatic degradation by cyclodextrin glycosyltransferase (CGTase). They are a family of cyclic oligosaccharides with truncated molecular structure which are hydrophilic on the exterior and relatively hydrophobic in the cavity. The unique molecular structure of CDs imparts to them the ability to encapsulate either the whole or part of appropriately sized molecules which are less polar than water within their cavities. CDs are comprised of D-glucopyranose units linked through α-1,4 glycosidic bonds. Native CDs are named according to their numbers of D-glucopyranose units. Those made up of six, seven, and eight D-glucopyranose units are called α-, β-, and γ-CD, respectively. The cavity diameter of native CDs ascends with the number of D-glucopyranose units from 5.7 to 7.8 to 9.5 Å. The molecular encapsulation of carbon dioxide with α-CD has been utilized for the isolation of α-CD through the precipitation of a gas inclusion complex (*8*). Encapsulation and release of carbon dioxide into and from α-CD have also been reported previously (*9*).

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The physiological effects of 1-MCP have been widely studied on a wide range of fruits, vegetables, and ornamental crops including apple, banana, pear, broccoli, lettuce, pea, carnation, and rose. 1-MCP has also been employed as a new tool to elucidate the fundamental processes involved in various physiological changes in fruits and vegetables (*10*). Besides, a controlled release system of 1-MCP has been either patented (*11, 12*) or studied academically (*13*) to cater for different methods of application. To date, in spite of a considerable amount of research on this comparatively newly found organic compound, the kinetics of encapsulation of 1-MCP with α-CD still remains unexplored.

Our research objective was to characterize the 1-MCP/α-CD inclusion complex and the mechanism of molecular encapsulation of gaseous 1-MCP in aqueous α-CD, and to determine the parameters that influence the encapsulation reaction. The parameters studied include α-CD concentration, initial 1-MCP headspace concentration in the encapsulation system, encapsulation temperature, and agitation rate. This information is particularly essential in optimizing the encapsulation process. The encapsulation reaction was allowed to occur in a closed, agitated vessel with a flat gas–liquid interface.

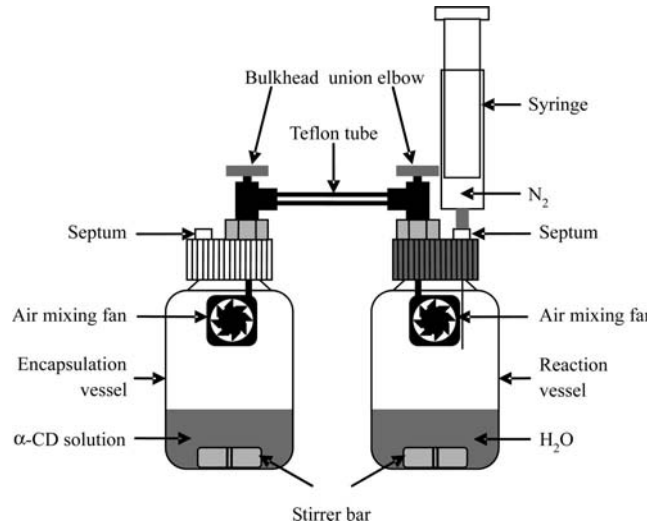

Figure 1. Experimental setup during transfer of 1-MCP from the reaction vessel to the encapsulation vessel. Nitrogen was injected into the reaction vessel to create atmospheric pressure in order to promote 1-MCP transfer.

MATERIALS AND METHODS

Materials. α-CD of 99% minimum purity was purchased from Ensuiko Sugar Refining Co., Ltd. (Tokyo, Japan). The α-CD powder was dried *in-vacuo* at 90 °C for 24 h before use. All the chemicals used were of reagent grade unless otherwise indicated. 3-Chloro-2-methylpropene (98%) and lithium diisopropylamide (30 wt % suspension in mineral oil) were purchased from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Potassium bromide (KBr) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nitrogen and isobutylene standard gas (100 μL/L) were purchased from Sumitomo Seika Chemicals Co., Ltd. (Osaka, Japan) in gas cylinders. Distilled water was used throughout the entire experiment.

1-MCP Synthesis. 1-MCP was synthesized according to the method reported by Sisler and Serek (*14*) with some modification. Approximately 2.4 mL of 3-chloro-2-methylpropene (98%) was withdrawn with a glass syringe and then injected into a screw-capped amber GC vial containing 21.42 g of lithium diisopropylamide (30 wt % suspension in mineral oil) through the butyl rubber stopper. Injection was carried out over 1 h while the chemicals were gently mixed with a magnetic stirrer. Mixing was continued after injection for another 30 min to ensure complete reaction. 1-MCP was formed as a lithium salt suspended in mineral oil. After reaction, vacuum was pulled to about 0.1 kPa on the suspension liquid to eliminate volatile impurities (mainly the remaining 3-chloro-2-methylpropene), and then the product was stored at −25 °C until use. 1-MCP gas could be produced by aqueous neutralization of the suspension liquid by adding it into distilled water.

Molecular Encapsulation of 1-MCP. Molecular encapsulation of 1-MCP into α-CD was carried out in a closed, agitated vessel with a flat gas–liquid interface. A 500-mL SCHOTT DURAN laboratory glass bottle (DURAN Produktions GmbH and Co. KG, Mainz, Germany) with a modified screw cap was used as the encapsulation vessel. α-CD solutions of 30, 50, and 87.3 mM at 20 °C were prepared in the encapsulation vessel, based on 100 g of distilled water by dissolving 3, 5, and 9 g of α-CD, respectively. Saturation of α-CD was reached at a concentration of 87.3 mM at 20 °C. The encapsulation vessel was first placed in an 80 °C water bath for about 15 min to fully dissolve the α-CD crystal. Subsequently, the vessel was immersed in a water bath at corresponding temperatures for temperature equilibration before the encapsulation process.

Encapsulation was carried out at three different initial 1-MCP head space concentrations of approximately 40,000, 80,000, and 100,000 μL/L. First, vacuum was pulled to about 0.27 kPa in a 500-mL SCHOTT DURAN laboratory glass bottle with a modified screw cap containing 100 g of distilled water using the PTFE diaphragm vacuum pump V-700 connected to the vacuum controller V-850 (BÜCHI Labotechnik AG, Flawil, Switzerland). This bottle was designated as the reaction vessel. Roughly 1, 2, or 3 g of previously thawed suspension liquid of lithium salt of 1-MCP was injected into the reaction vessel using a 20-mL syringe with a 18G needle through a rubber septum (Shimadzu Corp., Kyoto, Japan) fitted on the screw cap. Agitation was performed to promote complete aqueous neutralization for about 15 min. Upon its formation, the 1-MCP gas evaporated and accumulated in the head space of the reaction vessel.

Meanwhile, vacuum was also pulled to about 0.27 kPa in the encapsulation vessel containing temperature-equilibrated α-CD solution. The reaction and encapsulation vessel were connected to each other with a Teflon tube of 6 mm i.d. through the BVLM 20-0808 bulkhead union elbows (Pisco USA, Inc., Bensenville, IL) installed on the screw caps of each vessel (**Figure 1**). Circulation of the head space in both vessels was enhanced with a mixing fan (25 mm × 25 mm × 10 mm) (ICFAN, Shicoh Engineering Co., Ltd., Kanagawa, Japan). Transfer of 1-MCP from the reaction vessel to the encapsulation vessel, which was driven by a pressure gradient, was carried out by opening the valves of the elbows. The valves were then closed, and nitrogen was filled into the reaction vessel to create atmospheric pressure. The valves were reopened, and 1-MCP was transferred by the equalization of pressure in both vessels. This procedure was repeated until the pressure of both vessels reached the atmospheric value. Encapsulation was carried out at temperatures of 15, 20, 25, 27, and 30 °C. Encapsulation was promoted by agitation of the α-CD solution at agitation rates varied from 0, 50, 100, 200, to 300 rpm. The head space mixing fan was kept running throughout the experiment. During the encapsulation process, the depletion of 1-MCP in the head space of the encapsulation vessel was monitored over time by gas chromatography. Gas chromatography analysis of 1-MCP was performed as described in the later subsection. The depletion rate of 1-MCP during the initial stage of encapsulation (0–180 min) is postulated to reflect the encapsulation rate of 1-MCP into the α-CD cavity. The encapsulation time studied was 9 h. For each particular treatment, the encapsulation process was performed in duplicate. At the end of every encapsulation process, the α-CD solution containing the precipitate of the inclusion complex was centrifuged at 3000 rpm for 15 min. The supernatant was decanted, leaving the wet precipitate, which was then dried *in-vacuo* for 24 h before further analyses.

Quantitative Analysis of 1-MCP Included in α-CD. Gas chromatography was employed for quantification of the included amount of 1-MCP in α-CD as described in the following subsection. The inclusion ratio of 1-MCP is presented in a dimensionless term defined as the molar ratio of the included 1-MCP to α-CD. Procedurewise, approximately 10 mg of inclusion complex was weighed into a 60-mL

amber GC vial, which was then closed with a butyl rubber stopper and screw-capped. Roughly 3 g of distilled water was injected into the vial, and vortex mixing was performed for 5 min to ensure thorough dissolution of the inclusion complex. Subsequently, 0.1 mL of the head space was sampled with a gas tight syringe for gas chromatography. Calculation of the inclusion ratio was performed on a dry basis where the moisture contents of the inclusion complexes were determined by thermogravimetry (TG) as described in the later subsection.

1-MCP Analysis by Gas Chromatography. A 0.1 mL head space sample was separated with the GC-2010 gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with the ULBON HR-1 capillary column (30 mL × 0.53 mm i.d. × 5 μm film) (Shinwa Chemical Industries, Ltd., Kyoto, Japan) and a flame ionization detector. The injection port and detector temperatures were set at 110 and 210 °C, respectively. The initial column temperature was 40 °C with a hold time of 5 min, followed by heating to 100 at 20 °C/min, with a final hold of 12 min. Quantification of 1-MCP was accomplished using an external standard protocol (*15*). The isobutylene gas standard of 100 μL/L concentration was used. It was presumed to have a response factor similar to that for 1-MCP.

Powder X-ray Diffractometry (PXRD). Powder X-ray diffraction profiles of both uncomplexed α-CD and the 1-MCP/α-CD complex were recorded on the M03XHF X-ray diffractometer (MAC Science Co., Ltd., Tokyo, Japan) with X-ray Powder Research Software System (XPRESS). Measurements were performed using Cu Kα radiation (λ = 1.5406 Å) operating at 40 kV and 20 mA. Samples were scanned at a scanning speed of 4°/min over a diffraction angular range of $3° < 2\theta < 30°$. The divergence slit, receiving slit, and time constant were set at 1°, 0.15 mm, and 2 s, respectively.

Thermal Analysis of the Inclusion Complex. Thermogravimetric (TG) curves were recorded on the EXSTAR 6000 TG/DTA (TG/DTA 6200, SII Nano Technology Inc., Tokyo, Japan) equipped with the Muse Measurement software, version 3.7 (SII Nano Technology Inc.). Samples of 10 ± 0.5 mg were weighed into aluminum pans for analysis at a heating rate of 5 °C/min from 30 to 260 °C in a nitrogen atmosphere. The data were analyzed using the Muse Measurement software, version 3.7.

Correlation Analysis of 1-MCP Depletion Data. Data on 1-MCP depletion in the head space of the encapsulation vessel were empirically fitted to exponential decay-type curves using scientific graphing and analysis software (Origin7, OriginLab Corp., Northampton, MA). Correlation analysis was performed on 1-MCP retention *R*, with encapsulation time *t* for each treatment using the Avrami's equation:

$$R = \exp(-[kt]^n) \quad (1)$$

where *R* (unitless) is the 1-MCP retention, defined as the ratio of the 1-MCP head space concentration at time *t* (s) to its initial value, *k* (1/s) is the 1-MCP depletion rate constant, and *n* (unitless) is the depletion mechanism parameter. As the initial rate of 1-MCP depletion is postulated to reflect the encapsulation rate of 1-MCP, the *k* value is presumed to be equivalent to the apparent encapsulation rate constant and *n* is presumed to be equivalent to the encapsulation mechanism parameter. The apparent encapsulation rate constant *k* during the first 180 min of the encapsulation process was determined from eq 1 for comparison of the treatments. The encapsulation mechanism parameter, *n*, was fixed at 0.65 for all the treatments, as it allowed the most satisfactory fitting to the empirical data.

Statistical Analysis. All statistical analyses were carried out using Origin7. The apparent encapsulation rate constant determined by correlation of 1-MCP depletion data for each treatment during the first 180 min of the encapsulation process was subjected to analysis of variance (ANOVA) on a significant level of $P = 0.05$. Data were analyzed by parameters, namely α-CD concentration, initial 1-MCP head space concentration in the encapsulation system, encapsulation temperature, and agitation rate.

RESULTS AND DISCUSSION

Effect of Initial 1-MCP Head Space Concentration. The 24-h stability of 1-MCP in the head space of an empty encapsulation vessel at a concentration of approximately 100,000 μL/L (data not shown) ruled out the possibility that its depletion might be attributed mainly to self-polymerization and decomposition. The 24-h stability of 10 μL/L 1-MCP has also been reported by Nanthachai et al. (*16*). Besides, a 5-h 1-MCP stability test has also been carried out in an encapsulation system comprising instead distilled water, revealing a negligible 1-MCP loss upon aqueous dissolution (data not shown). The finding is supported by the low Henry's law constant of 1-MCP (1.27×10^{-8} mol/L·Pa) (*4*), which further ruled out the possibility that 1-MCP depletion was attributable mostly to dissolution into the liquid phase. Both findings, therefore, strengthened the presumption that 1-MCP depletion in the encapsulation system containing the α-CD solution happened primarily due to its encapsulation by aqueous α-CD.

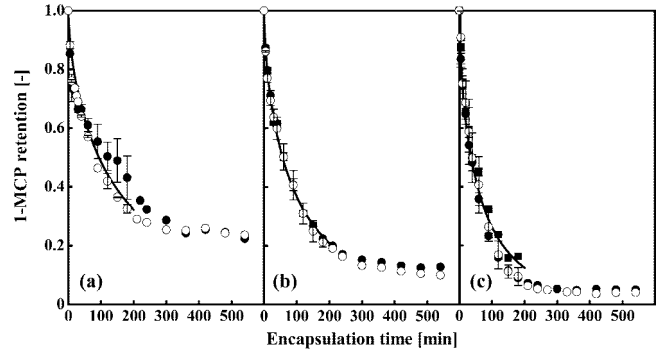

Figure 2. Depletion of 1-MCP in the head space of the encapsulation system containing α-CD solutions of concentrations at (a) 30 mM, (b) 50 mM, and (c) 87.3 mM. The solid lines represent data correlation using Avrami's equation. The initial 1-MCP head space concentration was tested at ≈80,000 μL/L (closed symbols) and ≈100,000 μL/L (open symbols). An initial 1-MCP head space concentration of 40,000 μL/L (■) was tested only for a 87.3 mM α-CD solution. Other parameters were set constant: encapsulation temperature, 20 °C; encapsulation time, 9 h; agitation rate, 200 rpm.

The effects of the initial 1-MCP head space concentration were studied by varying the initial concentration of 1-MCP at 40,000, 80,000, and 100,000 μL/L. α-CD solutions at concentrations of 30, 50, and 87.3 mM were tested, and the data were presented in parts **a**, **b**, and **c**, respectively, of **Figure 2**. The rest of the parameters, namely encapsulation temperature, encapsulation time, and agitation rate, were set at 20 °C, 9 h, and 200 rpm, respectively. As shown in **Figure 2**, after normalization of the 1-MCP head space concentration data against their initial values, in those treatments with identical α-CD concentration, the effect of initial 1-MCP head space concentration was negligible, where no significant difference was found between treatments that varied in initial 1-MCP head space concentration. The results implied that the dependency of encapsulation rate on the initial 1-MCP head space concentration could be described by a first-order relation. The formation of an inclusion complex between 1-MCP and α-CD was assumed to be on a 1:1 basis, and the first-order dependency on 1-MCP concentration could be described by the following reaction scheme:

$$\text{1-MCP} + \alpha\text{-CD} \rightleftharpoons \text{complex} \rightleftharpoons \text{precipitate} \quad (2)$$

At the early stage of encapsulation, the reaction could be approximated as an irreversible reaction where the rate of

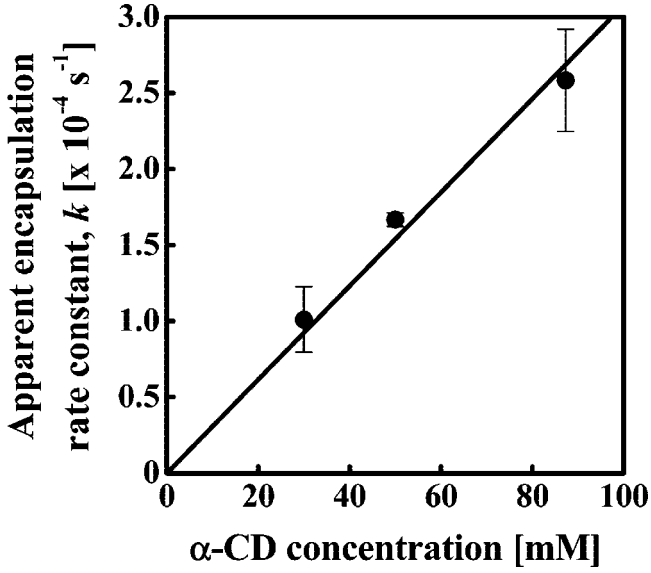

Figure 3. Linear correlation between the apparent encapsulation rate constant k and the α-CD concentration. The coefficient of determination R^2 was 0.99.

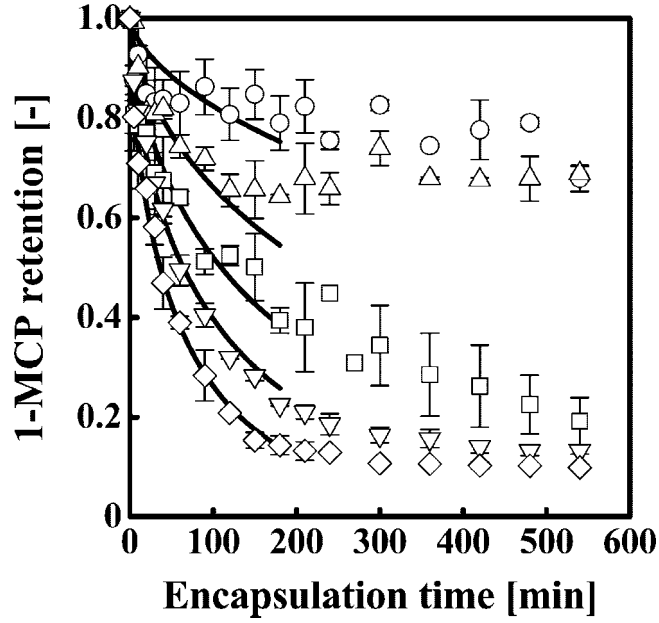

Figure 4. Depletion of 1-MCP in the head space of the encapsulation system under various agitation rates of 0 rpm (○), 50 rpm (△), 100 rpm (□), 200 rpm (▽), and 300 rpm (◇). Other parameters were set constant: initial 1-MCP head space concentration, ≈80,000 μL/L; α-CD concentration, 50 mM; encapsulation temperature, 20 °C; encapsulation time, 9 h.

the encapsulation reaction could be described as follows:

$$r_{1\text{-MCP}} = r_{\alpha\text{-CD}} = k_2 C_{\alpha\text{-CD}} C_{1\text{-MCP}} \quad (3)$$

where $r_{1\text{-MCP}}$ is the encapsulation reaction rate with respect to 1-MCP, $r_{\alpha\text{-CD}}$ is the encapsulation reaction rate with respect to α-CD, k_2 is the second-order encapsulation reaction rate constant, and $C_{\alpha\text{-CD}}$ is the concentration of the α-CD solution. $C_{1\text{-MCP}}$ is the concentration of 1-MCP dissolved in the α-CD solution, which could be described by $HP_{1\text{-MCP}}$, where H is the Henry's Law constant and $P_{1\text{-MCP}}$ is the partial pressure of 1-MCP in the head space of the encapsulation system.

At the early stage of the encapsulation process, since the concentrations of the α-CD solutions, $C_{\alpha\text{-CD}}$, were incomparably greater than the concentration of dissolved 1-MCP, $C_{1\text{-MCP}}$, $C_{\alpha\text{-CD}}$ could be regarded as constant. Thus, the complexation reaction could be approximated to be a pseudo-first-order reaction with respect to the 1-MCP concentration.

Effect of α-CD Concentration. Three different concentrations of α-CD solutions, namely 30, 50, and 87.3 mM, were studied to examine the effect of α-CD concentration on the encapsulation rate of 1-MCP (**Figure 2a**, **b**, and **c**). The solid lines were the fitting results performed using eq 1, which also reflected the encapsulation of 1-MCP. The fitting was satisfactory, with a fixed n value of 0.65. The 1-MCP head space concentration dropped at a rather high rate at the beginning of the encapsulation process, and the depletion rate decreased gradually until the 1-MCP head space concentrations eventually leveled off to some constant values which depended on the α-CD concentration. Correlation analysis was performed until the first 180 min of encapsulation. The apparent encapsulation rate constant k was found to be significantly different among treatments of different α-CD concentrations. The k values increased with the increase of α-CD concentration. When plotted as a function of α-CD concentration, the k values were well described by a linear equation which has an intercept at the origin, with a coefficient of determination of 0.99 (**Figure 3**). The strong linear correlation substantiated that the apparent encapsulation rate constant k varied proportionally with $C_{\alpha\text{-CD}}$.

Based on film theory, for the encapsulation reaction of gaseous 1-MCP in aqueous α-CD, which approximates the absorption with an irreversible pseudo-first-order reaction, the 1-MCP absorption rate, $N_{1\text{-MCP}}$, could be described by the following equation (*17*):

$$N_{1\text{-MCP}} = C_{1\text{-MCP}} \sqrt{k_2 C_{\alpha\text{-CD}} D_{1\text{-MCP}}} \quad (4)$$

where $D_{1\text{-MCP}}$ is the diffusion coefficient of 1-MCP in the aqueous phase.

In view of the fact that the correlation analysis on 1-MCP depletion was satisfactory using a fixed n value of 0.65, the apparent encapsulation rate could also be deduced as varying proportionally with $C_{\alpha\text{-CD}}$. This result was in rather good agreement with the film theory which states that the 1-MCP absorption rate, $N_{1\text{-MCP}}$, is proportionally dependent on $C_{\alpha\text{-CD}}$.

From the above findings it can be inferred that the rate-limiting step of 1-MCP gas encapsulation into aqueous α-CD is gas absorption with a pseudo-first-order reaction between 1-MCP gas and aqueous α-CD. The apparent encapsulation rate is approximately proportional to the square root of the α-CD concentration.

Effect of Agitation. Agitation of the α-CD solution during encapsulation significantly affected the encapsulation rate. The effects of agitation were studied by varying the agitation rate from 0, 50, 100, 200, to 300 rpm. All other parameters were set constant: initial 1-MCP head space concentration, ≈80,000 μL/L; α-CD concentration, 50 mM; encapsulation temperature, 20 °C; encapsulation time, 9 h.

The 1-MCP head space concentration depleted at decreasing rates until the first 3 h and almost stopped diminishing thereafter (**Figure 4**). Encapsulation was accelerated by faster agitation. In the treatment without agitation (0 rpm), the 1-MCP head space concentration diminished only around 20% at the end of the experiments, whereas, at 300 rpm agitation, the 1-MCP headspace concentration stabilized only after depletion of nearly 85%.

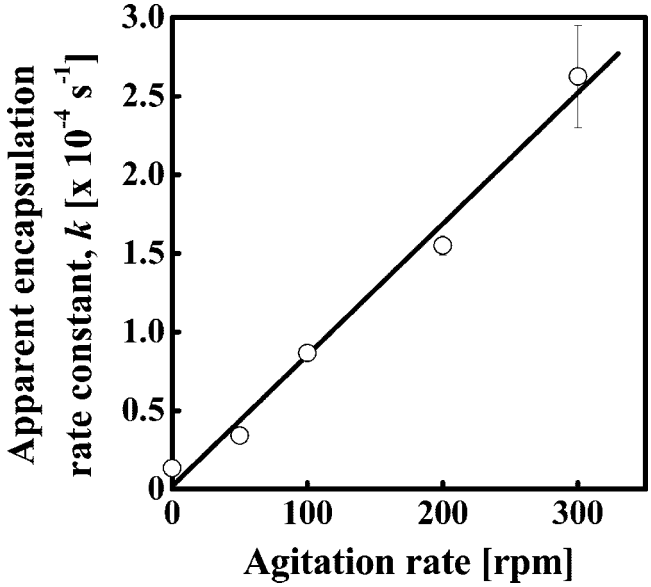

Figure 5. Linear correlation between the apparent encapsulation rate constant k and the agitation rate during the encapsulation reaction. The coefficient of determination R^2 was 0.99.

The apparent encapsulation rate constant k was plotted against the agitation rate as shown in **Figure 5**. The data was well fitted by a linear equation that intercepts the origin, with a coefficient of determination of 0.99. The strong correlation between the encapsulation rate constant and the agitation rate is persuasive in postulating the effect of agitation on encapsulation of 1-MCP in aqueous α-CD.

We presume that encapsulation occurs predominantly at the gas–liquid interface. Owing to the low aqueous solubility of the 1-MCP/α-CD inclusion complex, the inclusion complex molecules crystallize on the gas–liquid interface, apparently upon formation. As inclusion complex formation and crystallization continues, a thin film made up by crystallized inclusion complex is formed, covering the gas–liquid interface. This thin layer of crystals acts as a barrier which obstructs the diffusion of 1-MCP into the α-CD solution. At low agitation rates (0 and 50 rpm), the agitation force was too weak to destroy and sink the layer of inclusion complex crystal, thus hindering the encapsulation reaction from progressing further. A thin layer of the above-described crystallized inclusion complex was vividly observed at the end of the encapsulation processes carried out at low agitation rates. The mechanism of action of encapsulation hindrance, however, is still not fully understood.

Effect of Encapsulation Temperature. The effect of encapsulation temperature was studied within the range 15−30 °C. All other parameters, namely initial 1-MCP head space concentration, α-CD concentration, encapsulation time, and agitation rate, were set at ≈80,000 μL/L, 50 mM, 9 h, and 200 rpm, respectively. **Figure 6** shows the inclusion ratio of inclusion complexes and the 1-MCP yield at different encapsulation temperatures. The 1-MCP yield is defined as the ratio of the total amount of 1-MCP recovered in the inclusion complex to the total amount of 1-MCP depleted in the head space of the encapsulation vessel, while the inclusion ratio is defined as the molar ratio of 1-MCP to α-CD in the collected inclusion complex. The influence of encapsulation temperature on the inclusion ratios of inclusion complexes prepared at temperatures of 15−27 °C was not significant, probably due to the fact that only the 1-MCP/α-CD inclusion complex precipitated during the encapsulation process. At 30 °C, however, an acute drop of the inclusion ratio to almost zero

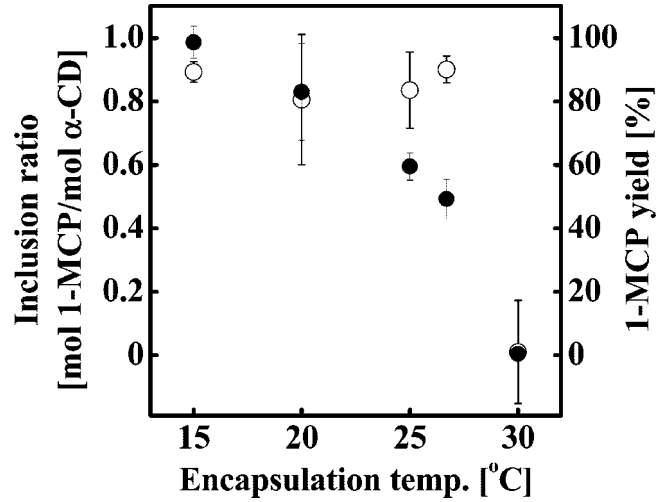

Figure 6. Inclusion ratio of inclusion complexes (open symbols) and 1-MCP yield (closed symbols) obtained from the encapsulation reaction performed at encapsulation temperatures between 15 and 30 °C.

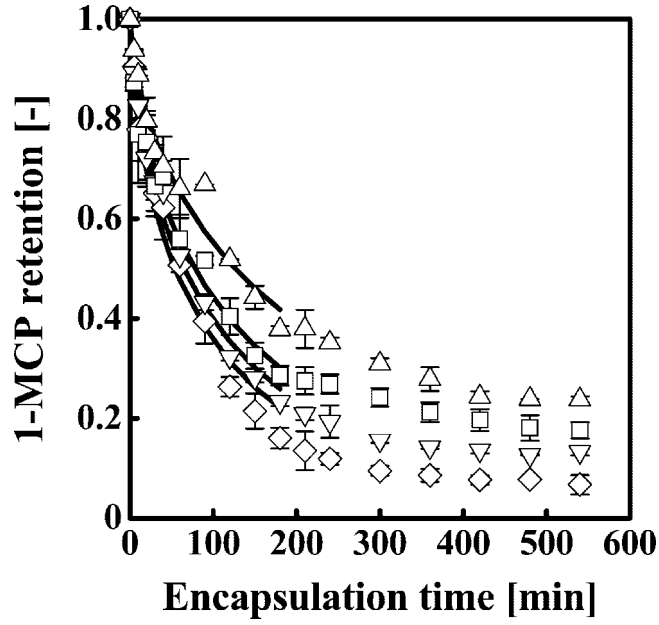

Figure 7. Depletion of 1-MCP in the head space of the encapsulation system studied at encapsulation temperatures of 15 °C (◇), 20 °C (▽), 25 °C (□), and 27 °C (△). Other parameters were set constant: initial 1-MCP head space concentration, ≈80,000 μL/L; α-CD concentration, 50 mM; encapsulation time, 9 h; agitation rate, 200 rpm.

was observed. Meanwhile, the 1-MCP yield decreased exponentially from 100% at 15 °C to almost 0% at 30 °C. It is conceivable that the phenomenon resulted from the increase of solubility of the inclusion complex and also the increase of the tendency to self-polymerize between the dissolved 1-MCP. The optimal encapsulation temperature was thus determined at 15 °C. The highest inclusion ratio achieved of approximately 0.95 mol 1-MCP/mol α-CD suggested a 1:1 ratio of 1-MCP/α-CD complexation.

The increase in encapsulation temperature decreased the encapsulation rate and thus the apparent encapsulation rate constant, k (**Figure 7**). Nonetheless, the effect of encapsulation temperature on the encapsulation reaction was insignificant. Referring to the results of the 1-MCP yield and the inclusion ratio, the 1-MCP depletion at 30 °C might be perceived as resulting from a number of reactions that contributed to 1-MCP loss. The 1-MCP depletion at 30 °C is thus not presented in **Figure 7**.

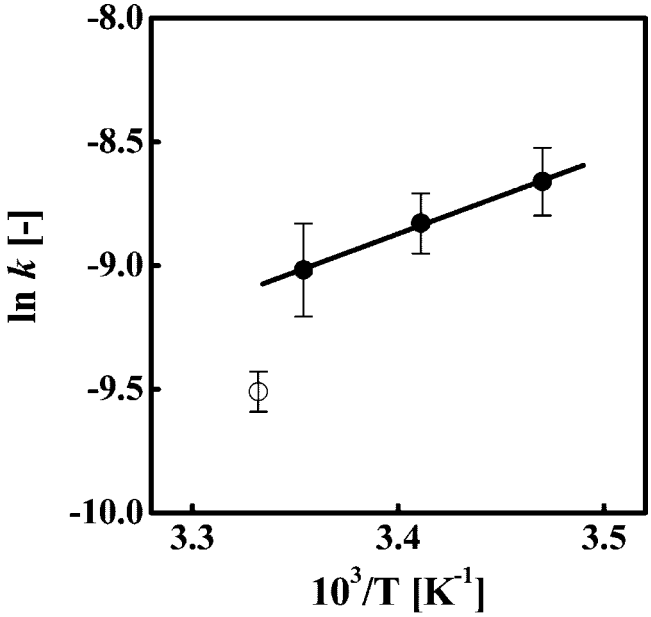

Figure 8. Arrhenius plot of the apparent encapsulation rate constant. The open symbol represents the logarithmized *k* value obtained at the encapsulation temperature 27 °C. Closed symbols represent values obtained within the temperature range 15−25 °C.

Figure 8 is an Arrhenius plot where the logarithmized *k* values are plotted as a function of the reciprocal temperature. A linear function could be well fitted to the logarithmized *k* values between 15 and 25 °C. When the encapsulation temperature went just slightly up, to 27 °C, the *k* value decreased drastically, where the particular linear function could no longer fit the data. These results imply a different mechanism of encapsulation at 27 °C from that at temperatures between 15 and 25 °C. The slope of the straight line fitted between 15 and 25 °C leads to the apparent activation energy of encapsulation E of −24.4 kJ/mol with a pre-exponential factor of 6.85×10^{-9} s^{-1}.

The encapsulation reaction was conceived to be comprised of a series of reactions that occurred almost simultaneously, namely the dissolution of gaseous 1-MCP into the aqueous phase, the diffusion of dissolved 1-MCP in the aqueous phase before being encapsulated, and the inclusion of the dissolved 1-MCP into α-CD.

The temperature dependence of the second-order encapsulation reaction rate constant, k_2, the diffusion coefficient of 1-MCP in the aqueous phase, $D_{1\text{-MCP}}$, and the Henry's law constant of 1-MCP, H, in eq 4 could be described respectively by the Arrhenius equation, and the whole equation could be rewritten as follows:

$$N_{1\text{-MCP}} = H_0 \exp\left(-\frac{\Delta H_S}{RT}\right) \times P_{1\text{-MCP}} \sqrt{k_0 \exp\left(-\frac{E_E}{RT}\right) C_{\alpha\text{-CD}} D_0 \exp\left(-\frac{E_D}{RT}\right)} \quad (5)$$

where H_0 (M/Pa), k_0 (1/s), and D_0 (m^2/s) are the pre-exponential factors, ΔH_S (kJ/mol) is the dissolution heat of 1-MCP, E_E (kJ/mol) is the activation energy of encapsulation, E_D (kJ/mol) is the activation energy of 1-MCP diffusion in aqueous phase, R is the universal gas constant (8.314 J/K·mol), and T (K) is absolute temperature. Rearranging eq 5 gives the following:

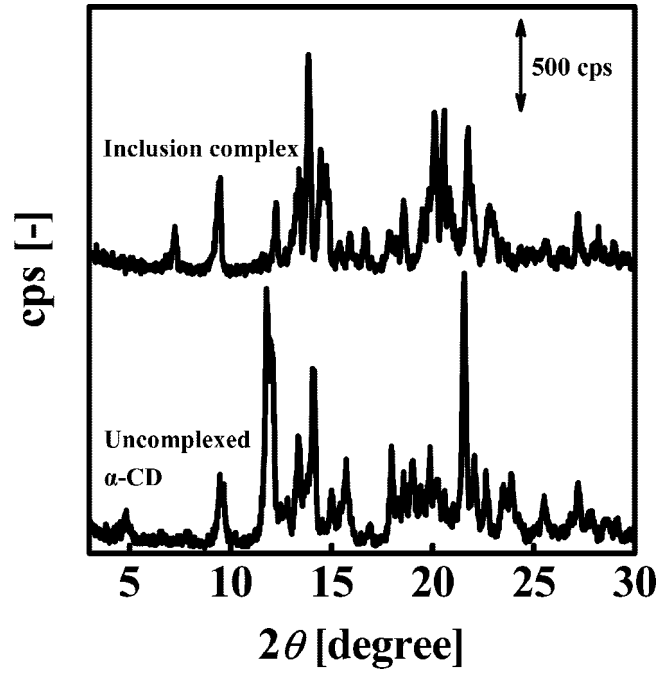

Figure 9. Powder X-ray diffraction patterns of uncomplexed α-CD and the 1-MCP/α-CD inclusion complex.

$$N_{1\text{-MCP}} = H_0 P_{1\text{-MCP}} \sqrt{k_0 C_{\alpha\text{-CD}} D_0} \exp\left(-\frac{\Delta H_S + \left(\frac{E_E + E_D}{2}\right)}{RT}\right) \quad (6)$$

The term of $\Delta H_S + \left(E_E + E_D / 2\right)$ would be equivalent to the apparent activation energy of encapsulation E. As E is the sum of ΔH_S and the average value of E_E and E_D, ΔH_S is shown to be the dominating component in E.

The dissolution of short-chain hydrocarbons is often an exothermic reaction. For example, the heat of aqueous dissolution of ethylene was reported at about −19 kJ/mol (*18*), whereas, by calculation from the data reported by Huq and Wood (*19*), the activation energy of ethylene diffusion in water is about 17 kJ/mol. By the assumption that 1-MCP also has ΔH_S and E_D values of similar magnitudes as those for ethylene, E_E would then be predicted as −28 kJ/mol. The predicted exothermic value shows that 1-MCP encapsulation by α-CD happens rather readily in the aqueous phase. This could be explained by the ability of α-CD cavities to provide hydrophobic microenvironments that are capable of stabilizing hydrophobic substances in the aqueous phase. The readiness of CDs to encapsulate organic compounds such as Triflumizole has also been reported with exothermic ΔG values (*20*).

Powder X-ray Diffractometry (PXRD). Figure 9 presents the PXRD patterns of uncomplexed α-CD and the 1-MCP/α-CD inclusion complex. The inclusion complex was prepared from a 30 mM α-CD solution at 20 °C. The initial 1-MCP head space concentration was 100,000 μL/L, the agitation rate was 200 rpm, and the encapsulation time was 9 h. Sharp peaks in the X-ray diffractograms suggested crystallinity of both samples.

The characteristic peaks of uncomplexed α-CD at $2\theta = 4.8°$, 11.8°, 14.1°, and 21.6° decreased in intensity or even dissipated with encapsulation, while the characteristic peaks of the inclusion complex were found at $2\theta = 7.2°$, 12.3°, 13.9°, 14.5°, 14.7°, 20.1°, 20.6°, and 21.8°. The four peaks at $2\theta = 9.5°$, 18.6°, 25.5°, and 27.2° were just slightly shifted or stayed unchanged after encapsulation of 1-MCP. Comparison of the

PXRD patterns of the uncomplexed α-CD and the inclusion complex substantiated that the changes resulted from encapsulation. Based on these results, it could be deduced that inclusion of 1-MCP in the α-CD cavity changed the crystal lattice structure of α-CD.

In conclusion, the probable ratio of complexation between 1-MCP and α-CD is 1:1. The rate-limiting step of encapsulation is the gas absorption, with a pseudo-first-order reaction between the host and guest molecules. The apparent activation energy of encapsulation was calculated as −24.4 kJ/mol, suggesting a strong domination by the activation energy of 1-MCP dissolution over the activation energies of both encapsulation and diffusion. Under the tested conditions, the optimal encapsulation temperature was determined at 15 °C.

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