OPTIMIZATION OF ENCAPSULATION METHODS AND CONDITIONS TO MAXIMIZE 1-MCP LOADING IN MODIFIED BETA-CYCLODEXTRINS

By

HAN ZHANG

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Kit L. Yam

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ABSTRACT OF THE THESIS

Optimization of encapsulation methods and conditions to

maximize 1-MCP loading in modified beta-cyclodextrin

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Thesis Director:

Kit L. Yam

1-methylcyclopropene (1-MCP) is a gas compound which can inhibit ethylene response in climacteric fruits. It is used to delay the ripening process and so to extend the shelf life of fresh produce. However, 1-MCP requires certain storage technology because it is highly unstable and flammable. Current storage technology is to use carrier systems such as α-cyclodextrin to encapsulate 1-MCP and thus protect and stabilize it. In the industry, high level of 1-MCP loading in carrier system is highly preferred to minimize the cost and maximize the efficiency. Therefore, encapsulation methods and conditions are critical for 1-MCP application as they determine the loading level of 1-MCP in the carrier system. The objective of this research is to identify optimum method and conditions to encapsulate 1-MCP into a newly-developed carrier system, modified β-cyclodextrin (MβCD), to maximize the loading of 1-MCP.

In this research, two encapsulation methods (solution method and solid method) and five condition factors (pH, MβCD concentration, temperature, 1-MCP concentration and encapsulation duration) were investigated to identify dominant factors for each method.

1-MCP concentration and encapsulation duration were found to be two dominant factors for both methods. Response Surface Methodology (RSM) was then used to optimize those two factors to identify optimum condition with maximum 1-MCP loading. Given by RSM, optimum condition of solution method was identified at ($1.33X10^5$ ppm, 21.2 hours) with maximum loading of 0.320% while optimum condition of solid method was at ($1.39x10^5$ ppm, 12.25 hours) with maximum loading of 0.529%. After testing the actual loading at optimum condition of the two methods, results showed 0.317% loading in solution method and 0.535% loading in solid method, which were -0.937% (solution method) and +1.13% (solid method) off to predicted values given by RSM respectively, confirming found optimum condition was valid.

In conclusion, solid encapsulation method gives maximum 1-MCP loading of 0.535% in MβCD at the condition of (12.25 hours, $1.39X10^5$ ppm). The result proves the effectiveness of the chemical modification of β-CD and provides further understanding of different encapsulation mechanisms followed by those two methods.

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I have always told myself: you are really a lucky guy. So far, you have a quite smooth life of 25 years. You never encounter any physical and mental issues about health. You enter your dream college and then graduate school. You have a good family always stands behind you and provide support. I would like to say thank you so much to all the people who care about me, give me concerns and bring me the luckiness.

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Thank you, my mom and dad! I know language is far less than enough to express that but I have to say here and prove it with my action.

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Special thanks to Dr. Liu from USDA. This elegant and lovely gentleman always has smile on his face that makes me feel relax. He treated me very nicely and provided me a lot of help when I worked in USDA. He agreed to be part of my committee without any hesitation when I asked him. I hope the smile will always be on your face and make you happy. Thank you so much!

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1. Introduction

1.1 Ethylene and ripening

Ethylene (figure 1.1) is a gaseous plant hormone which can trigger and accelerate ripening process in climacteric fruits and vegetables. Ethylene function requires ethylene molecules to bind ethylene receptors before functioning (Lacey & Binder, 2014).

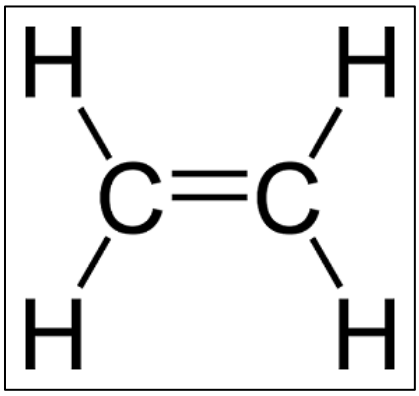

Figure 1.1 Molecule structure of ethylene

During ripening, fruits undergo numerous metabolic changes, including changes in color, texture and nutritional status (Meng et al., 2015). Usually, certain level of ripening is desired to develop preferred flavors, textures and nutrients content for human to consume (Mwithiga, Mukolwe, Shitanda, & Karanja, 2007). However, in postharvest stage, undesirable ripening will cause quality and shelf life problems in fruits by changing original appearance and texture as well as developing unpleasant odors and flavors. Therefore, as a key to the ripening process, ethylene activity needs to be inhibited in postharvest stage to improve quality and shelf life of such fresh produces.

Several strategies have been successfully developed to control ethylene activities (figure 1.2), where perception receptor inhibitors are proved to be one of the most effective approaches in chemical strategies. Groups of compounds have been discovered with ethylene inhibition activities, including silver thiosulfate (STS) (Veen, 1983), 2, 5-norbornadiene (2, 5-NBD)(Wang & Woodson, 1989), diazocyclopentadiene (DACP)(Margrethe Serek, Sisler, & Reid, 1994) and 1-methylcyclopropene (1-MCP)(M Serek, Sisler, & Reid, 1995).

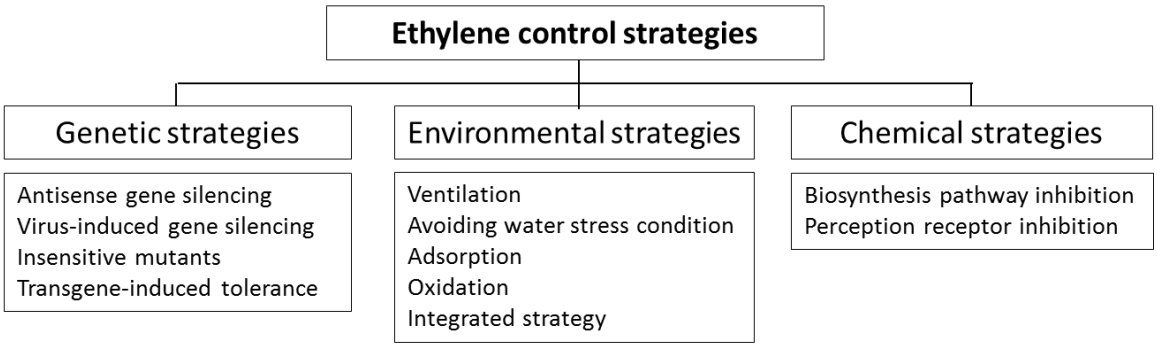

Figure 1.2 Schematic view of ethylene control strategies (Scariot, Paradiso, Rogers, & De Pascale, 2014)

1.2 1-MCP and its current product

1.2.1 1-MCP and its properties

1-MCP is an odorless gas compound (figure 1.3) under normal conditions. It was the first patented non-toxic ethylene action inhibitor and it has attracted a lot of interests in postharvest industry because of its effectiveness (Scariot et al., 2014). It has also been approved by the Environmental Protection Agency (EPA) in 1999 for use on ornamentals as well as edible horticultural products (Watkins, 2006).

1-MCP is able to affiliate ethylene receptors of plants and thus prevent ethylene molecules to bind to receptors (Sisler & Serek, 1997). By this way, ethylene response is inhibited and so that repining process is slowed. 1-MCP can function at very low concentration of ppb level with negligible residue (E.P.A, 2002).

However, as a flammable gas, 1-MCP is an explosive hazard when compressed. It can also easily go through degradation and dimerization process under certain conditions. All of those bring difficulties to store and transport 1-MCP through compressed gas tank as regular gases and so that limit its commercialization and application.

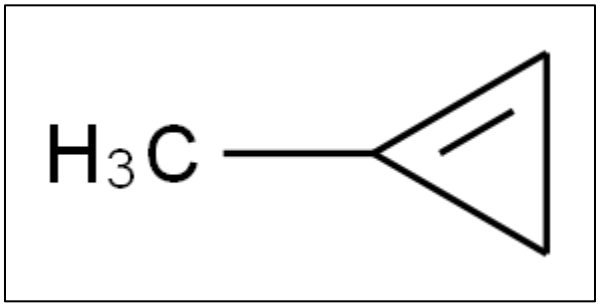

Figure 1.3 Molecule structure of 1-MCP

1.2.2 SmartFresh, current 1-MCP product in the market

For mass application of 1-MCP, non-volatile formulation is needed for safe storage and transportation. Several formulations have been reported using non-volatile 1-MCP chemical derivatives (Seglie, Sisler, Mibus, & Serek, 2010), micro-bubble technology (Pongprasert & Srilaong, 2014) and molecule encapsulation (Neoh, Koecher, Reineccius, Furuta, & Yoshii, 2010; Neoh, Yamauchi, Yoshii, & Furuta, 2007).

The current 1-MCP product which has been approved to be used on edible horticultural products in the market is using encapsulation technology to stabilize and store 1-MCP. It

is under the brand name of SmartFresh, provided by AgroFresh Inc., a subsidiary of Rohm and Haas (Springhouse, PA) (Watkins, 2006). The concept of SmartFresh is to use α-cyclodextrin as carrier to hold and protect 1-MCP molecules so 1-MCP can be available in a powder or tablet form of product, making it much easier to store and transfer. When applied, the powder or the tablet will be placed in water (or other suitable solvents or buffer solutions) to dissolve and then release 1-MCP into the headspace, where fruits get exposed and treated.

1.2.3 α-cyclodextrin and molecular encapsulation

The successful development of SmartFresh relies on the molecular encapsulation of 1-MCP in α-cyclodextrin due to the special shape and size of α-cyclodextrin cavity. α-cyclodextrin is composed of a ring structure with 6 units of α-(1, 4)-glucopyranose (Fig 1.4 (a)). Topologically, it forms a cone shape (Fig 1.4 (b)) making it outer layer of the cone hydrophilic and inner layer hydrophobic. Therefore, 1-MCP, which is hydrophobic, can easily be entrapped in the cavity by hydrophobic interactions as well as physical entrapment (Ho, Howes, & Bhandari, 2014; Pinho, Grootveld, Soares, & Henriques, 2014). Some of the important properties of α-cyclodextrin can be found in table 1.1.

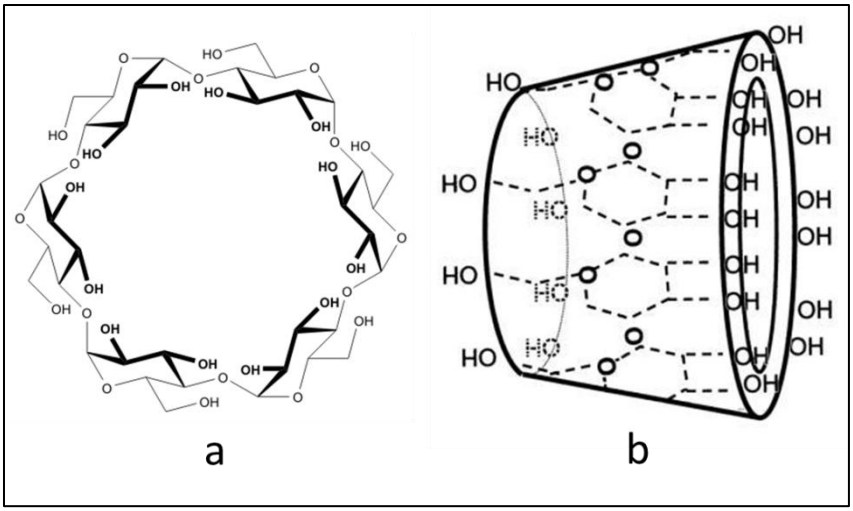

Figure 1.4 Molecule structure of α-cyclodextrin

The molecular encapsulation of 1-MCP in α-cyclodextrin usually occurs in aqueous solutions, where enthalpy-rich water molecules are displaced by hydrophobic guest molecules resulting in a more stable state with lower energy (Szejtli, 1998). This property of cyclodextrin has been widely recognized and used in industry to improve the stability, solubility and delivery of many products such as drugs (Fang, Comino, & Bhandari, 2013).

Vice versa, when cyclodextrin-MCP complex is dissolved in water, water molecule would get the chance to enter the inner cavity and push guest molecule which is 1-MCP in our case out of the cavity and trigger the release (Pande & Shangraw, 1995).

Table 1.1 Properties of α- and β-cyclodextrin(Del Valle, 2004)

Properties	α-cyclodextrin	β-cyclodextrin
Number of glucopyranose units	6	7
Molecular weight (g/mol)	972	1135
Solubility in water @25 °C (g/L)	145	18.5
Outer diameter (Å)	14.6	15.4
Cavity diameter (Å)	4.7-5.3	6.0-6.5
Height of torus (Å)	7.9	7.9
Cavity volume ($Å^3$)	174	262

1.2.4 Problems of current product

Even though, SmartFresh based on α-cyclodextrin is quite successful in the market, there still are some problems which may limit its applications and its further growth in business. First of all, the cost of α-cyclodextrin is quite high. Market price of α-cyclodextrin used to be as high as $ 100/kg making SmartFresh very expensive. Secondly, α-cyclodextrin is quite moisture sensitive as its solubility is 145g/L (Ho, Howes, & Bhandari, 2015; Kfoury, Auezova, Greige-Gerges, Ruellan, & Fourmentin, 2014; Wongmekiat, Tozuka, Oguchi, & Yamamoto, 2003). During storage, α-cyclodextrin will absorb moisture from the environment and then absorbed moisture will trigger the release of 1-MCP as discussed before. So the quality of the product will be compromised if stored improperly. Moreover, high solubility also makes the release of 1-MCP very fast resulting in a technical problem of designing a control release product, which may be directly applied in the field or the open environment.

1.3 A novel carrier system to overcome current limitations

To reduce the cost and improve the storage stability of 1-MCP encapsulated system, we were trying to synthesize a novel carrier system based on β-cyclodextrin, which has much lower market price and moisture sensitivity compared to α-cyclodextrin (Table 1.2).

Table 1.2 Price and solubility of α- and β-cyclodextrin(ChemFine, 2015)

	Price	Solubility
α-cyclodextrin	$ 60-100/kg	145g/L
β-cyclodextrin	$ 2-10/kg	18.5g/L

β-cyclodextrin has a similar structure as α-cyclodextrin but with a ring of 7 units of glucose. So it has a larger size of inner cavity compared to α-cyclodextrin with 6 units of glucoses (Fig 1.5). Some of the major properties of β-cyclodextrin are also showed in table 1.1.

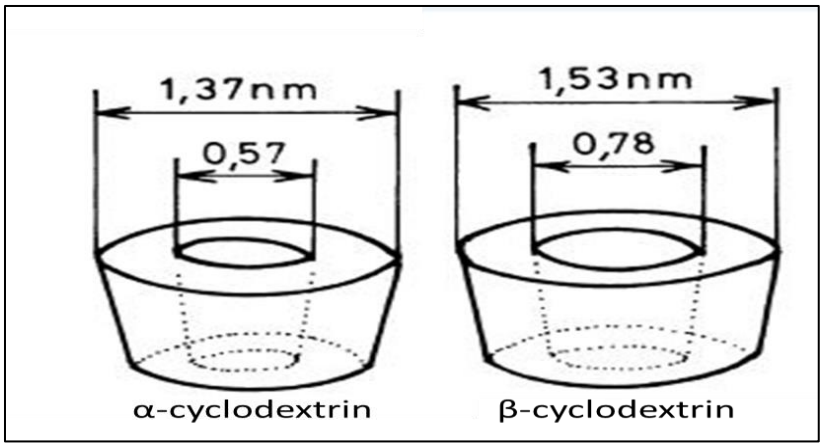

Figure 1.5 Difference of molecule size between α&β-cyclodextrin

Due to the increase of cavity size, 1-MCP cannot be tightly encapsulated only through physical entrapment and hydrophobic interactions in β-cyclodextrin anymore as it used

to be in α-cyclodextrin. As a result, the loading of 1-MCP in original β-cyclodextrin is less than 0.1% according to our results. Therefore, extra effort is needed to make the encapsulation of 1-MCP happen in β-cyclodextrin. The effort we put is to chemically modify the structure of original β-cyclodextrin to adjust the cavity size and properties according to other publications (Cavalli et al., 2010; Trotta & Cavalli, 2009; Trotta et al., 2011).

The idea of the new β-cyclodextrin based carrier system (modified β-cyclodextrin, MβCD) is to cross link β-cyclodextrin molecules with proper cross-linkers to build 3-dimensional sponge-like polymer structure. Fig 1.6 shows the modification reaction and the proposed structure formed by the reaction. After modification, on one hand, 1-MCP can be locked in the original cavity by polymer structure even the size of 1-MCP and original cavity in β-cyclodextrin is still not exactly compatible. On the other hand, extra encapsulation spots called inter-molecule cavities were created in the gap between β-cyclodextrin molecules and cross-linkers (Fig 1.7). Apparently, more encapsulation spots of 1-MCP were created through this reaction and thus loading capacity of the new carrier system is improved. This improvement will further reduce the cost of raw material for manufacturing at the same time and provide a solution of reducing the price of final product.

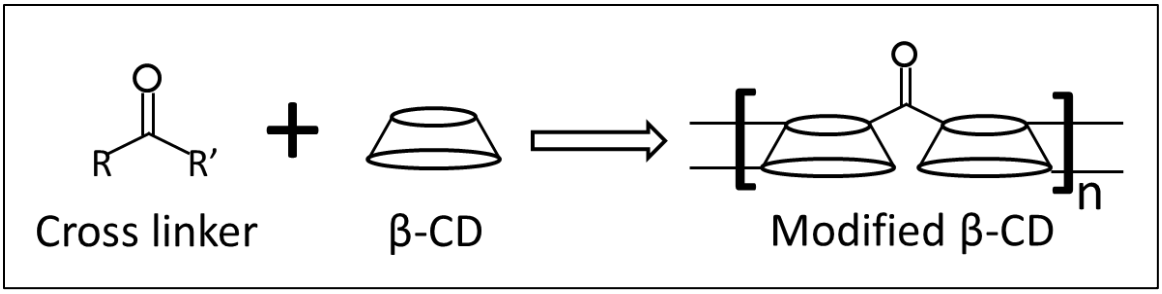

Figure 1.6 Process of β-cyclodextrin modification

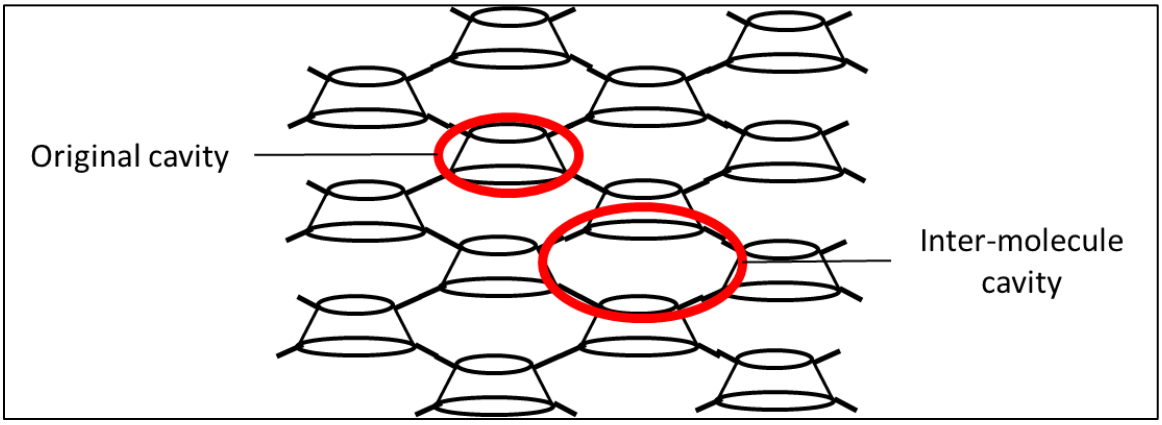

Figure 1.7 Proposed structure of modified β-cyclodextrin

Moreover, the new system (MβCD) is also good for the control release of 1-MCP because of its low sensitivity to moisture. While β-cyclodextrin already has a much lower water solubility compared to α-cyclodextrin due to the internal hydrogen binding formed between those hydroxyl groups within the molecule, cross linking reaction further consumes hydroxyl groups making the polymer poorly soluble in water(Chatjigakis, Doneze, Coleman, & Cardot, 1992). The solubility of the polymer is about 10g/L compared to 18.5g/L of β-cyclodextrin.

2. Rationale and objective

As introduced above, a new 1-MCP carrier system (MβCD) was developed to improve the loading capacity of 1-MCP as well as to reduce the cost and achieve the control release. However, the encapsulation process needs to be further investigated from the engineering point of view to identify the optimum method and conditions to encapsulate maximum amount of 1-MCP in MβCD.

The objective of this research is to compare 1) solid encapsulation method and solution encapsulation method and 2) optimize encapsulation conditions for each method to identify the optimum method and conditions with maximum 1-MCP loading in modified β-cyclodextrin.

3 sub-objectives are designed step by step to achieve the overall objective of this research.

1) Identify dominant factors for the encapsulation process of each method
2) Optimize found dominant factors to identify optimum conditions with maximum loading level
3) Confirm the validity of the optimum point and compare the final results of those two methods

Experiments were designed and accomplished based on the 3 sub-objectives and details would be discussed in section 4.

3. Materials, methods and set-up

All the chemicals were purchased from Sigma-Aldrich, Fisher Scientific and other certified suppliers of Rutgers. Further purification steps will be demonstrated if applied.

3.1 Synthesis of modified β-cyclodextrin

Modified β-cyclodextrin was synthesized based on the method provided in publication (Trotta & Cavalli, 2009). The reaction was showed in figure 1.6. Generally, CDI (carbonyldiimidazole, cross-linker) and β-cyclodextrin were dissolved in DMF (Dimethylformamide). The solution was allowed to react for 3 hours at 100°C in water bath. Then water was added into reaction solution to precipitate MβCD polymer. Precipitated MβCD was then filtered out and washed with 100% ethanol for several times before dried under vacuum. The set-up is showed in fig 3.1 and Fig 3.2 shows the appearance of the MβCD after drying.

The cavity size and structure of MβCD could be manipulated by controlling the molecule ratio of CDI: CD in the reaction (such as 2:1, 4:1 and 8:1). The structure and morphology of MβCD could be further modified using ultra-sonication.

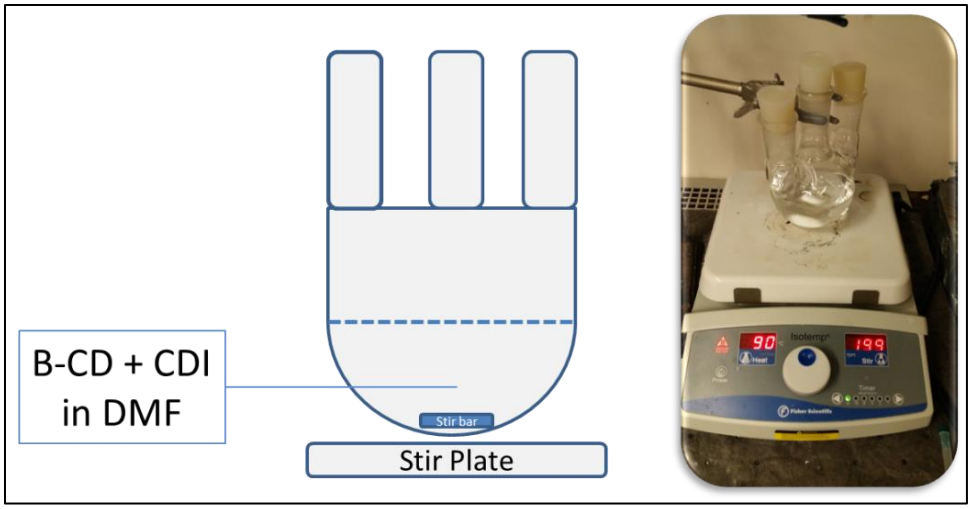

Figure 3.1 Set up for MβCD synthesis

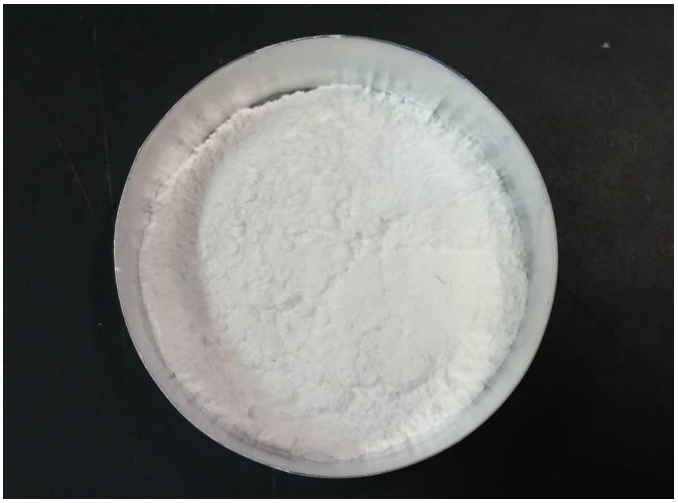

Figure 3.2 Appearance of MβCD after drying

3.2 Characterization of modified β-cyclodextrin

3.2.1 FT-IR analysis

FTIR analyses (Nexus 870 FT-IR, Nicolet Instrument Corporation, WI) were conducted to confirm the formation of cross-linking. Four samples (β-cyclodextrin, modified β-cyclodextrin, and two CDI samples from two different suppliers) were tested. A DTGS KBr detector and KBr beamsplitter were used to prepare samples for the test. The

spectra were collected at room temperature within the wave number range from 400 cm^{-1} to 4000 cm^{-1}.

Figure 3.3 FT-IR instrument

3.2.2 H-NMR analysis

H-NMR instrument (fig 3.4) (Varian VNMRS 400MHz) at Rutgers Chemistry department was utilized to identify the location of cross-linking between hydroxyl groups. H-NMR samples was prepared by dissolving MβCD in DMSO-d6 and then centrifuged. The clear supernatant was collected and placed in the NMR tube to run the test.

Figure 3.4 Rutgers NMR instrument

3.2.3 Thermo-gravimetric analysis

Thermo-gravimetric analysis was conducted to test the thermo-stability of modified β-cyclodextrin. TGA results were also used to optimize the chemical modification process of β-cyclodextrin. Additional information such as the existence of residual chemicals or impurities of small molecules with low boiling point can also be obtained. Four samples were tested: α-CD, β-CD, modified β-CD washed by ethanol and modified β-CD washed by acetone.

3.2.4 SEM and TEM pictures

Surface and internal morphology was characterized using SEM at USDA Eastern Regional Research Center and TEM at Rutgers Chemistry Department.

3.2.5 Porosity characterization

The porous structure and pore size distribution of MβCD was characterized using automated micro-pore gas analyzer Autosorb-1 MP (Quantachrome Instruments) with high pressure N_2 adsorption and desorption. Data of pore size, void volume and pore surface area was collected and analyzed to provide better understanding of the structure and potential 1-MCP encapsulation capacity.

3.3 1-MCP generation

1-MCP was generated through the reaction reported by publications (Fisher & Applequist, 1965; Magid, Clarke, & Duncan, 1971). Generally, LDA (lithium Diisopropylamide) was placed in THF (tetrahydrofuran) to form a solution. Then 3-chloro-2-methylpropene was added drop by drop to react with LDA for certain period of time. After reaction was complete, the solution was mixed with mineral oil and then placed under vacuum to remove all the impurities (fig 3.6) and volatile solvent. Final product was an orange suspension with MCP-Li complex suspended in mineral oil (fig 3.7). Fig 3.5 shows the set-up for 1-MCP preparation.

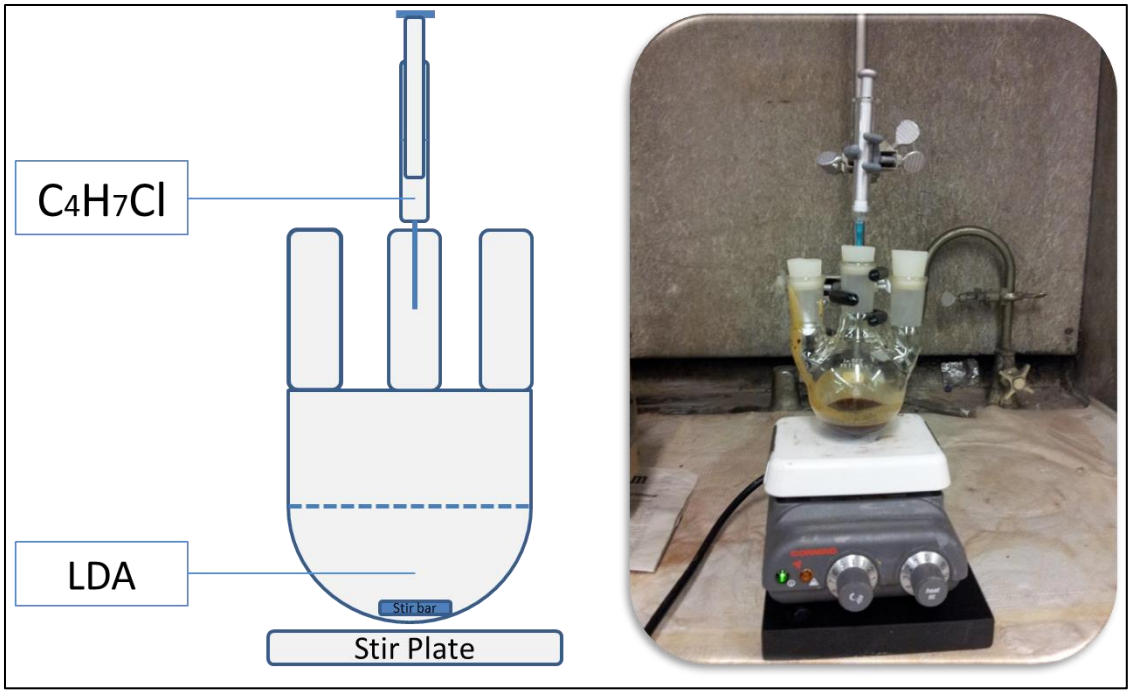

Figure 3.5 Set-up for 1-MCP synthesis

Figure 3.6 Vacuum applied to remove impurities

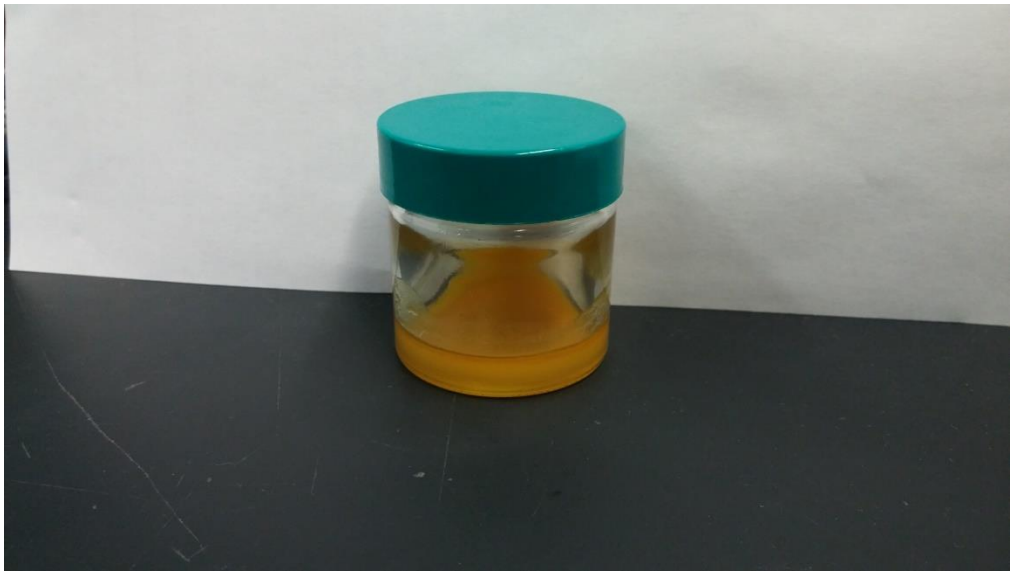

Figure 3.7 1-MCP-Li complex suspended in mineral oil

The suspension can be stably stored in freezer for several months. When used, the suspension is mixed with suitable amount of water. MCP-Li complex will react with water and release gaseous 1-MCP.

3.4 Encapsulation of 1-MCP into MβCD

Two most commonly used encapsulation methods were applied to load 1-MCP into MβCD system and for further comparison.

1) Solution method

   Solution encapsulation method is showed in fig 3.8. Generally, pre-prepared MCP-Li complex was put in the left vessel to react with water to generate gaseous 1-MCP. Generated 1-MCP was induced to the right vessel and accumulated to desired concentration in the headspace. MβCD was dissolved or suspended in water and interacted with 1-MCP in the headspace through

agitation. When the encapsulation process was finished, the precipitated MβCD particles were filtered and dried for further test.

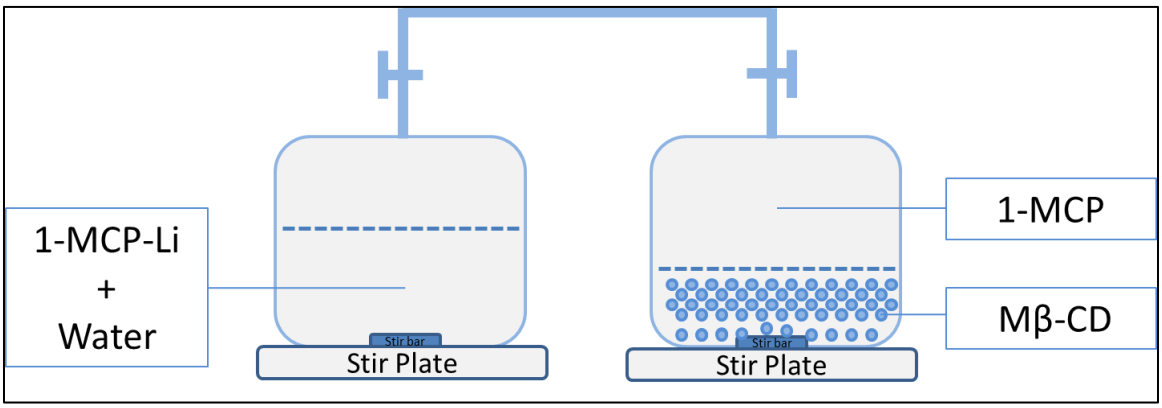

Figure 3.8 Set up for solution encapsulation

2) Solid method

Solid encapsulation method is showed in fig 3.9. Similar set-up as solution method was applied. However in right vessel, MβCD powder was directly in contact with 1-MCP in the headspace without any moisture. After encapsulation, powder was taken out for further test.

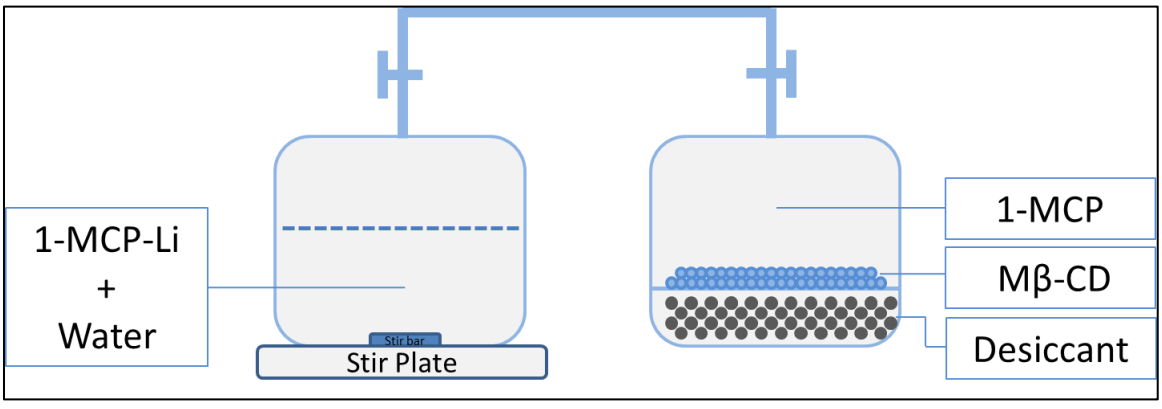

Figure 3.9 Set up for solid encapsulation

Fig 3.10 shows a real set-up of solid encapsulation as an example in the lab.

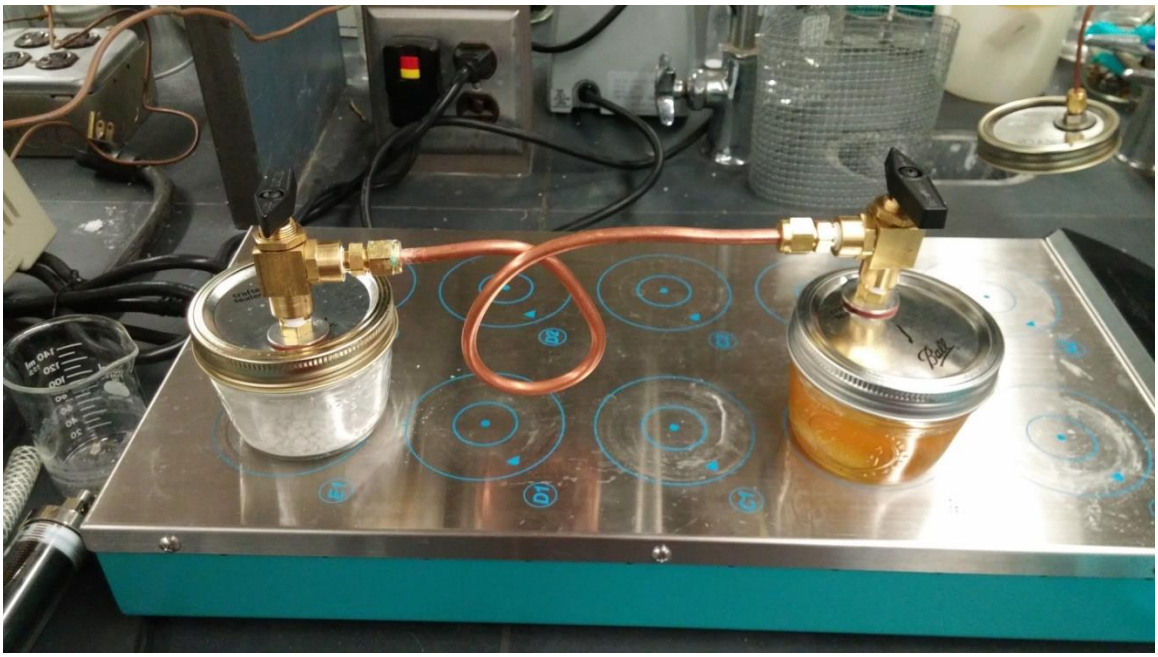

Figure 3.10 Solid encapsulation in the lab

3.5 Identification and quantification of 1-MCP

Commercial available 1-MCP product was used to generate 1-MCP and the generated 1-MCP was tested in GC-FID to find out retention time as well as to produce the standard curve.

Gas Chromatography (Agilent 6890 series, Fig 3.11) with Flame Ionized Detector was utilized to test 1-MCP in the headspace.

When testing our sample, 1-MCP encapsulated MβCD powder was placed in sealed GC bottle with water in it and then shaken for 30 minutes to completely release 1-MCP to the headspace.

Figure 3.11 GC system used for 1-MCP identification and quantification

4. Design of Experiment

To address the overall objective of this research, which is to find out the optimum method and conditions to maximize 1-MCP loading in MβCD, 3 steps are designed and discussed in this section. Each step is corresponding to one of the three sub-objectives.

4.1 Single factor experiments

The purpose of step 1, single factor experiments, is to identify two to three dominant factors from all the potential factors that affect 1-MCP encapsulation process and find out boundaries of those factors for further optimization as well.

According to the literature and previous work of our lab, we found that temperature, pH of encapsulation solution (solution method only), MβCD concentration in encapsulation

solution (solution method only), 1-MCP concentration in head space and encapsulation duration are 5 factors having potential impact on the encapsulation process and may affect the final loading of 1-MCP in MβCD. So in this section, encapsulation was done in the practical range of each factors using both solid and solution method (pH and MβCD concentration only applied in solution method). Table 4.1 shows different levels of each condition that have been investigated.

Table 4.1 design of single factor experiments

		Range of interest	Middle Point
Temperature	Solid	0°C – 20 °C	10°C
	Solution	0°C – 20 °C	10°C
pH	Solid	---	---
	Solution	4 – 10	7
MβCD concentration	Solid	---	---
	Solution	5g/L – 15g/L	10g/L
1-MCP pressure	Solid	0.05 atm. – 0.15 atm.	0.08 atm.
	Solution	0.05 atm. – 0.15 atm.	0.08 atm.
Encapsulation time	Solid	3h – 15h	9h
	Solution	5h – 30h	18h

When one factor was being tested, other factors remained fixed at their middle point as showed in the table. For instance, when temperature was tested from 0°C to 20°C, pH was set at 7, MβCD concentration at 10g/L, 1-MCP pressure at 0.08atm and encapsulation duration at 18h for solution methods and 9h for solid method.

Dominant factors as well as their boundaries were identified according to the results and will be discussed in section 5.

4.2 Optimization with Response Surface Methodology (RSM)

Once dominant factors and target boundaries were identified, Response Surface Methodology (RSM) would be applied to deal with the optimization and so find out the optimum point with maximum loading of 1-MCP in MβCD for each method.

RSM is an efficient analytical tool to deal with optimization problems. Briefly, it applies suitable mathematic model (usually polynomial model) to fit in preliminary data and finds out the relationships between independent variables (factors) and dependent variables (responses). The model developed by RSM is used to identify optimum points according to the target of optimization. It can also be well applied when a response or a set of responses of interest are influenced by several variables (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Tarley et al., 2009).

In this research, there is only one response which is the loading of 1-MCP in MβCD while factors are one or more of the following: temperature, pH, 1-MCP concentration, MβCD concentration and encapsulation duration depending on the results of step 1.

4.3 Optimum point confirmation

Once optimum point for each method was found, which means optimum conditions were predicted by the model, a test would be followed under the optimum conditions to check the real loading is close to the predicted value or not.

If the difference of real loading and predicted loading was in the acceptable range, then we would trust the optimum conditions given by RSM were reliable. However, if the

difference was huge and out of the acceptable range, then either a more effective design needed to be applied or some key factors with great impact were ignored.

5. Results and discussion

5.1 Method development

5.1.1 Chemicals used to generate 1-MCP

The mechanism of generating 1-MCP-metal complex is to use a strong base to trigger the α-elimination of 3-chloro-2-methylpropene. According to the literature, several groups of metal-organic compounds can be utilized in this reaction. In this research, we compared the efficiency of sodium amide ($NaNH_2$), lithium diisopropylamide (LDA) and Phenyllithium (Ph-Li) in terms of 3-chloro-2-methylpropene transfer rate (fig 5.1), in order to select most efficient chemicals to generate 1-MCP.

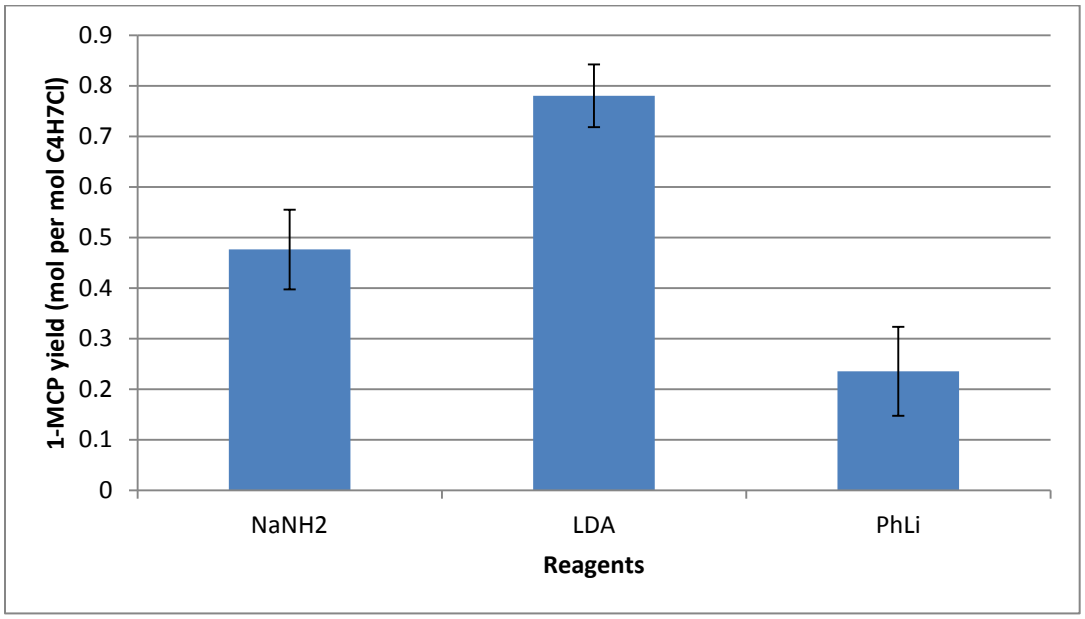

Figure 5.1 Comparison of efficiency of 1-MCP generating chemicals

From fig 5.1, the transfer rate of 3-chloro-2-methylpropene is 48% when reacted with $NaNH_2$, 78% with LDA and 24% with PhLi. LDA shows about 1.5 times more efficient than $NaNH_2$ and 3 times more efficient than PhLi. Moreover, LDA is also the most cost-effective and convenient agent for us to use. Pre-packed LDA-THF solutions are commercially available while $NaNH_2$ is only sold in powder form, which needs to be put into solutions for reaction. 100 ml LDA solution is $55.40 which is only 63% of PhLi price, which costs $87.50 for 100 ml.

So LDA is selected for the generation of 1-MCP.

5.1.2 Standard curve for 1-MCP quantification

To quantify the 1-MCP loading in MβCD using GC-FID, calibration curve is needed. Calibration curved is produced based on current commercially available 1-MCP product, with 1-MCP loading of 0.63% per tablet. The equation 5.1 below is used to calculate the 1-MCP concentration in the headspace.

$$\mathrm{C}\ (1-\mathrm{MCP}) = \frac{M \times 0.63\% \times 22.4}{54.09 \times V} \times 100$$

Equation 5.1 Calibration equation of 1-MCP

C (1-MCP) = concentration of 1-MCP in the headspace, %

M = weight of commercial product, g

0.63% = 1-MCP content in commercial product

22.4 = volume of 1 mol gas under standard conditions, L/mol

54.09 = molecular weight of 1-MCP, g/mol

V = volume of the headspace, L

100 = coefficient to transfer value to percentage

In GC chromatograph, retention time of 1-MCP is 4.7 min, calibrated by commercial product sample. Based on the principle of GC, we know peak area is proportional to the concentration and the concentration is proportional to the total amount of 1-MCP in the headspace according to the equation above. So a linear equation can be found to describe the relationship between the peak area (PA) and the amount of 1-MCP in the headspace through calibration.

Table 5.1 GC calibration data

tablet powder (g)	PA	1-MCP (mg)	Concentration (mg/mL)	ppm
0	0	0	0	0
0.0023	89.55316	0.01472	0.000064512	29.32643
0.0056	222.36548	0.03584	0.000159289	71.403483
0.0103	403.38495	0.06592	0.000292978	131.33141
0.0208	739.34235	0.13312	0.000591644	265.21294
0.0355	1296.7811	0.2272	0.001009778	452.64708
0.0388	1430.85	0.24832	0.001103644	494.72413

As showed in table 5.1, 2.3, 5.6, 10.3, 20.8, 35.5 and 38.8 mg tablet were weighted and then 1-MCP content as well as concentration in the headspace was calculated based on the weight of tablet samples. After injection of the headspace, peak area results were recorded and PA vs. 1-MCP weight was plotted (fig 5.2).

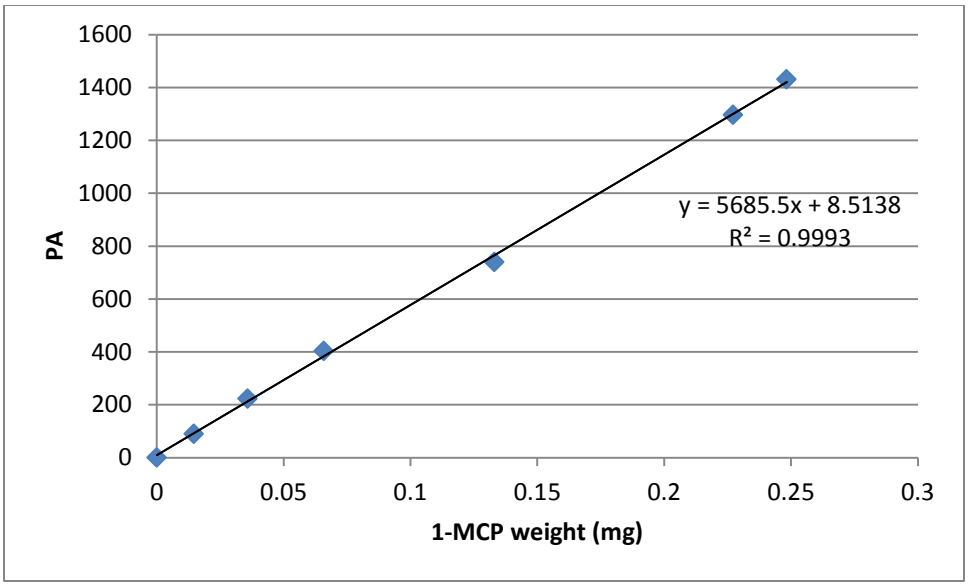

Figure 5.2 Linear relationship between 1-MCP content and peak area

As showed in fig 5.2, the relationship between 1-MCP content and peak area is linear described by the equation below with R^2=0.999.

$$\mathrm{PA} = 5685.5 \times \mathrm{M}(1 - \mathrm{MCP}) + 8.514$$

The curve was calibrated every week by adding data points on it and was reproduced every month through the same procedure to ensure the accuracy.

5.2 MβCD characterization

5.2.1 FT-IR analysis

FT-IR was used to confirm the formation of the ester bond between cyclodextrin molecules.

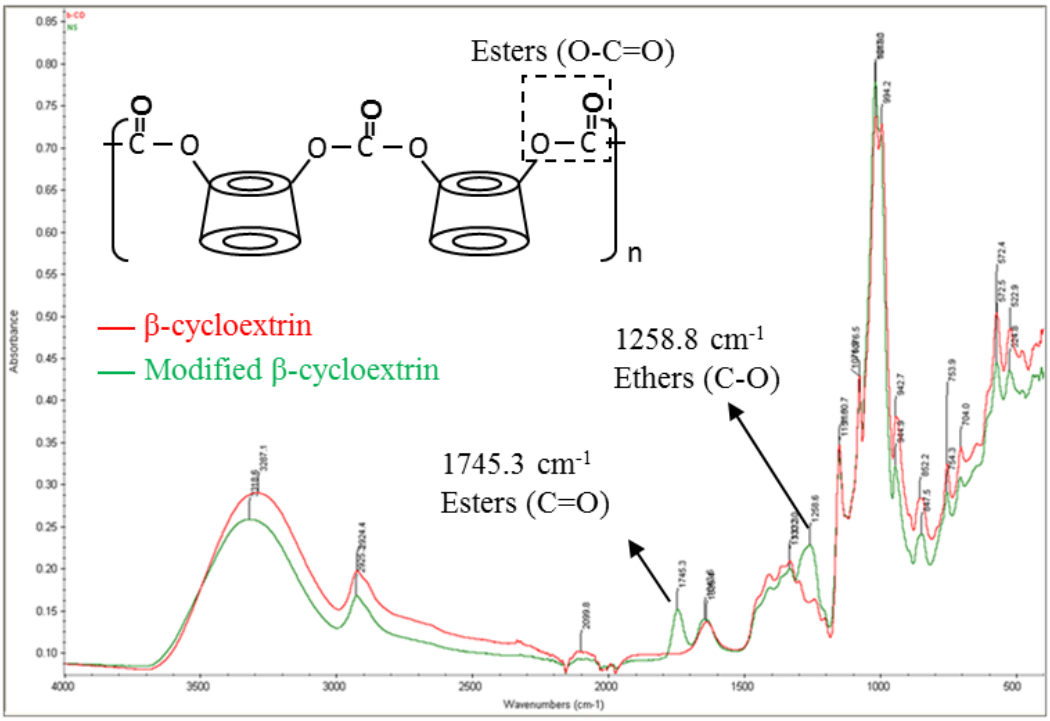

Figure 5.3 FT-IR spectrum of MβCD

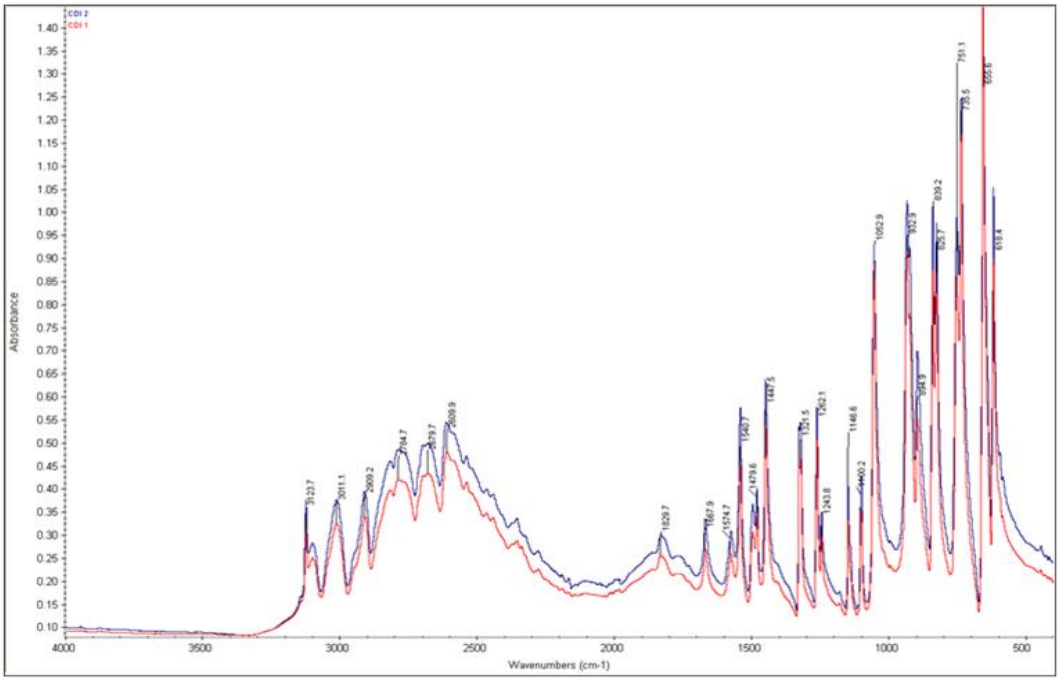

Figure 5.4 FT-IR spectrum of CDI (cross-linker)

In the spectra above, the green spectrum is for modified β-cyclodextrin, the red spectrum is for pure β-cyclodextrin (reference). As shown, there are two distinct peaks at 1258.8 cm^{-1} and 1745.3 cm^{-1} appears after the modification process, which are also the only difference between the two spectra. The peak at 1745.3 cm^{-1} represents the C=O bond of the ester bond and the peak at 1258.8 cm^{-1} represents the C-O bond of the ester bond. In the spectrum of CDI, no distinguishing peaks at 1745.3 cm^{-1} and 1258.8 cm^{-1} are observed, proving that new peaks are not caused by the residue of CDI in MβCD. So those 2 new peaks confirm the formation of the ester bond, which indicate the successful cross-linking of β-cyclodextrin.

5.2.2 H-NMR analysis

The NMR analysis is used to detect where the ester bonds are formed between hydroxyl groups of β-cyclodextrin rings. As showed in fig 5.5A below, the area of OH-2 and OH-3, 0.13 while the area of H-1 is 0.07 and area of OH-6 is 0.07. So in terms of ratio of area, (OH-2, OH-3): (H1) equals to 2 and (OH-6): (H-1) equals to 1. The results match the number of H atoms in those groups. After modification reaction (fig 5.5B), (OH-2, OH-3): (H1) changes to 1.66 and (OH-6): (H-1) changes to 0.89. We know H-1 will not get involved in the reaction so the area should be constant. The only explanation of the ratio change is cross-linking occurs at OH-2, OH-3 and OH-6, which cleaves H atoms in those hydroxyl groups and causes ratio change.

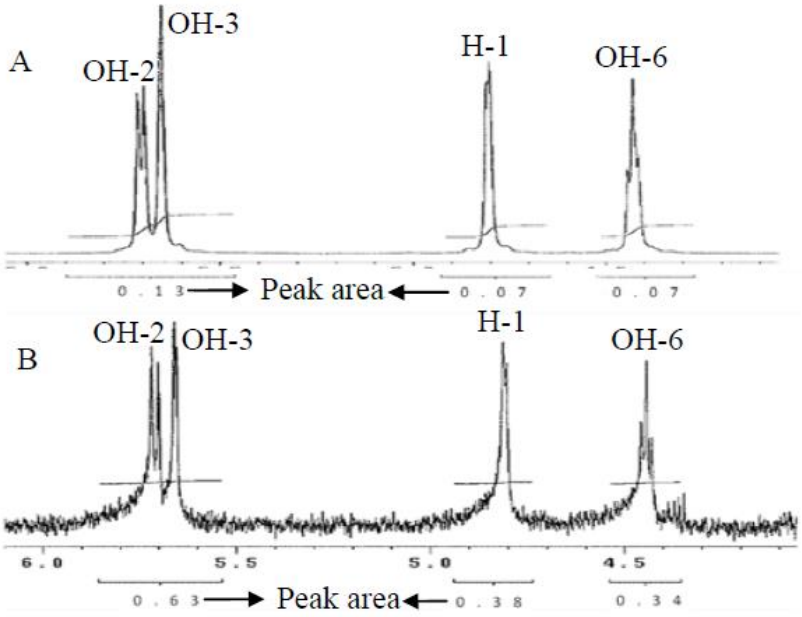

Figure 5.5 H-NMR spectrum of MβCD (A: βCD, B: MβCD)

5.2.3 Thermo-gravimetric analysis

Thermo-gravimetric analysis (TGA) is used to characterize the thermo-stability of MβCD as shown in fig 5.6. Original α- and β-CD are listed as reference for comparison. In fig 5.6, A represents α-CD (black), B represents β-CD (red), C represents MβCD washed by ethanol and D represents MβCD washed by acetone.

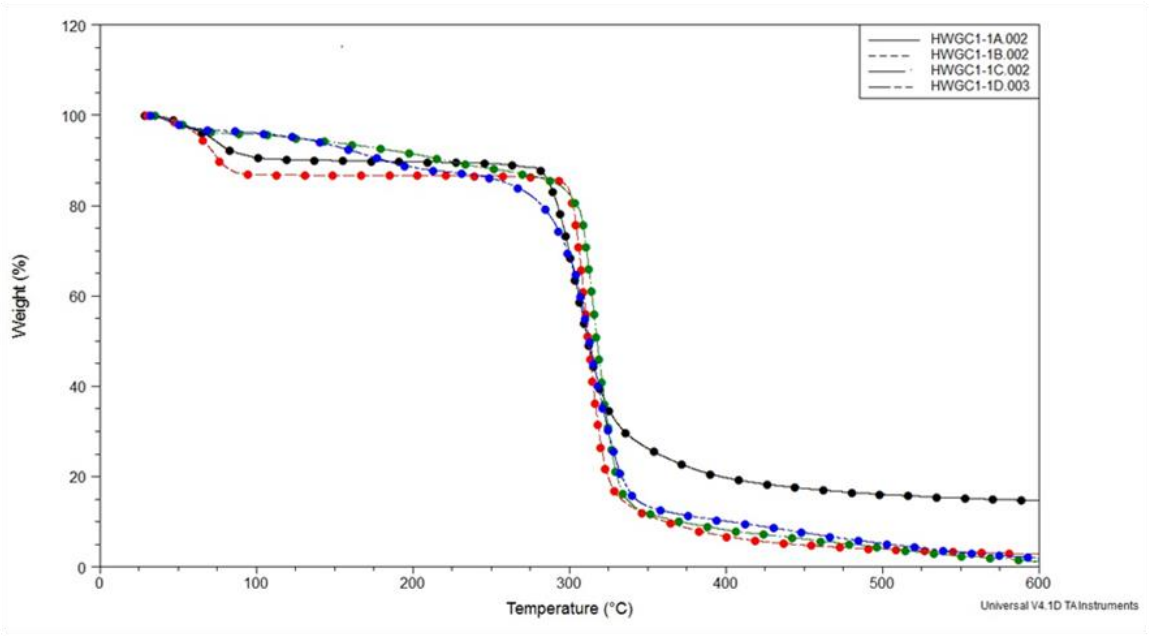

Figure 5.6 TGA results

(A: α-CD, B: β-CD, C: MβCD washed by ethanol, D: MβCD washed by acetone)

In fig 5.6, the first drop occurs around 100°C is the evaporation of water. The continuous weight decrease of C and D from 100°C to 200°C is caused by residual chemicals and solvents within MβCD matrix, which have been addressed by our most recent washing and drying process. At 300°C, cyclodextrin starts its thermal decomposition to smaller molecules and releasing carbon dioxide and this is the huge drop of weight occurs at around 300°C.

5.2.4 SEM and TEM pictures

TEM shows the image of MβCD clusters in water (fig 5.7A). The round dark zone is the cluster, the lighter zone is background. As shown in the magnified image (fig 5.7B), there are dark and light spots in a cluster in which dark spots are MβCD polymers and light spots are the pores and inherent cyclodextrin cavities.

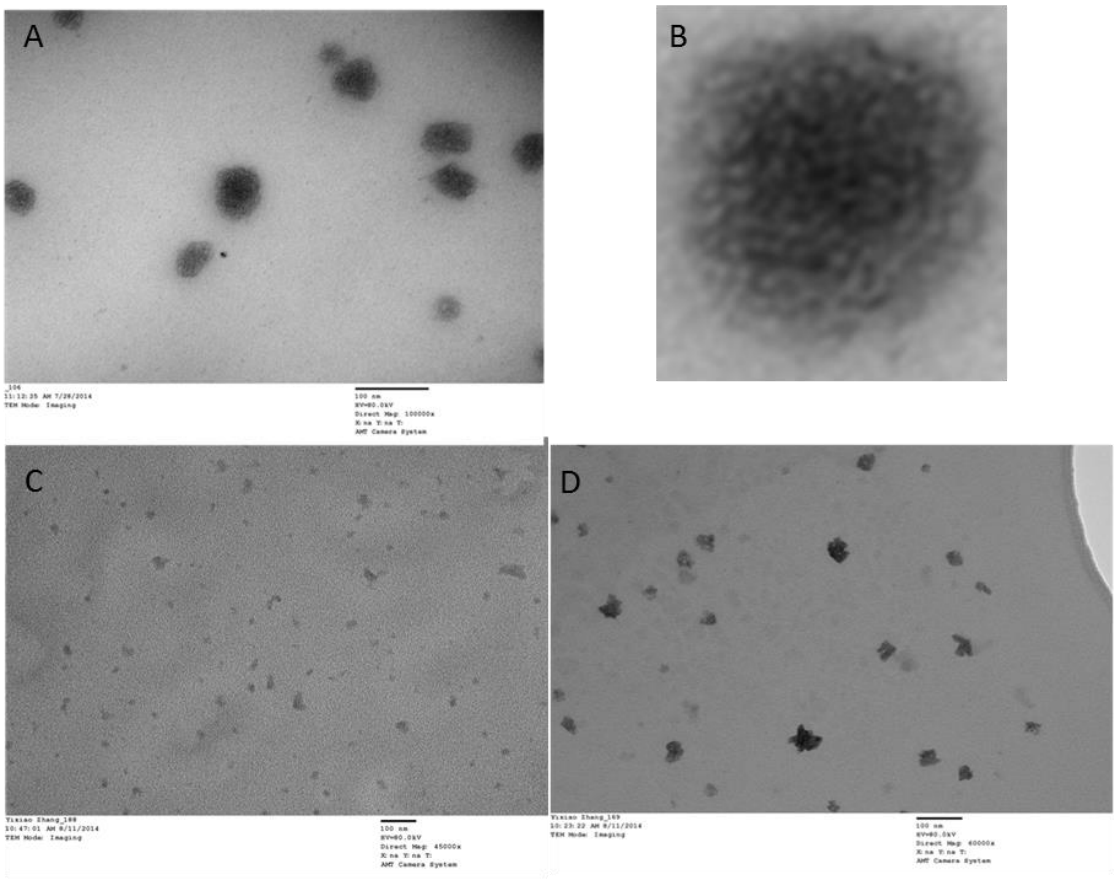

Figure 5.7 TEM picture

(A: MβCD, B: magnification of single cluster in A, C: α-CD, D: β-CD)

The average diameter of the clusters is around 53 nm. The average diameter of the pores is around 1.5 nm or 15 Å (measured using Nano Measurer 1.2 software). The porous structure is clearly showed in the magnified image indicating the encapsulation sites or spots are successfully constructed.

Compared with original α-cyclodextrin (fig 5.7C) and β-cyclodextrin (fig 5.7D), cluster size of MβCD is much larger, which indicates polymer structure formed with multiple β-cyclodextrin molecules.

SEM pictures are showed in fig 5.8. A and B represents original α-cyclodextrin and β-cyclodextrin respectively while C represents MβCD. Fig 5.8 clearly shows the difference between MβCD and original cyclodextrins with a porous, sponge-like polymer structure while original cyclodextrins are more like blocks in pictures.

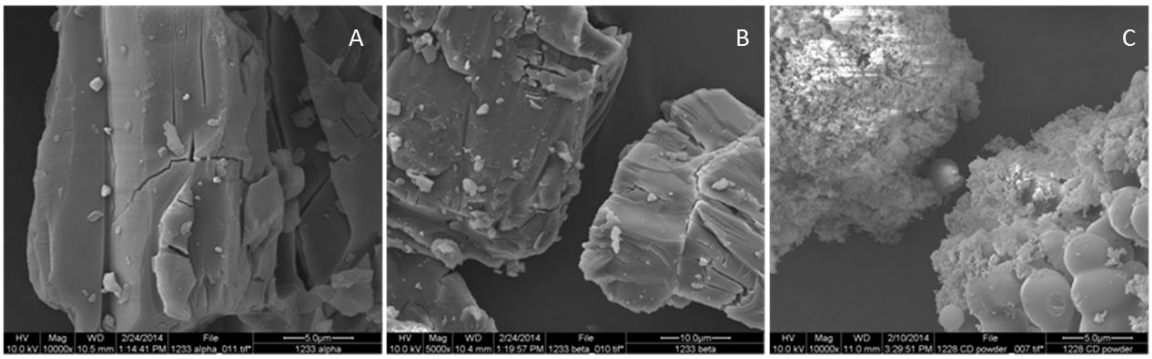

Figure 5.8 SEM pictures

(A: α-cyclodextrin, B: β-cyclodextrin, C: MβCD)

5.2.5 Porosity characterization

Table 5.2 below shows the porosity information of α-cyclodextrin, β-cyclodextrin and MβCD including BET surface area, pore volume and average pore size. As we know, the pore size of α-cyclodextrin, which is 7 Å, is suitable for 1-MCP encapsulation, pore size of MβCD has been reduced significantly from 32 to 14 after modification. Even it is still larger than α-cyclodextrin, the loading capacity of 1-MCP has already been improved a number of times. The pore size results are constant with pore volume. However, decrease of surface area of MβCD after modification is observed compared to original β-cyclodextrin. The explanation may be the dense network structure formed through cross-linking makes those sites in the core unavailable for BET test.

Table 5.2 Porosity information of α-cyclodextrin, β-cyclodextrin and MβCD

Sample name	α-CD	β-CD	Modified β-CD
BET surface area (m2/g)	3.8	7.2	6.7
Pore volume (cc/g)	0.0051	0.025	0.014
NLDFT Average pore size (Å)	7	32	14

5.3 Optimization

5.3.1 Single factor experiments

5.3.1.1 Influence of temperature

Temperature theoretically could have influence on encapsulation process because it can change the dynamics of 1-MCP molecules entering or leaving cavities of MβCD. Experiments were conducted to investigate 1-MCP loading at 3 different temperature levels (0°C, 10°C and 20°C). The temperature range of 0 - 20°C was selected because storage temperatures of most fresh produce fall into this range. For example, optimum storage temperature of apple is 0 - 4°C, ripe banana is 13 - 16°C and ripe avocado is 3 - 7°C (Facundo, Gurak, Mercadante, Lajolo, & Cordenunsi, 2015; Kweon et al., 2013; Zauberman & Jobin-Decor, 1995).

However, according to our results, temperature is not a dominant factor of our process. As showed in fig 5.9, no significant difference of loading at 3 different temperatures was observed.

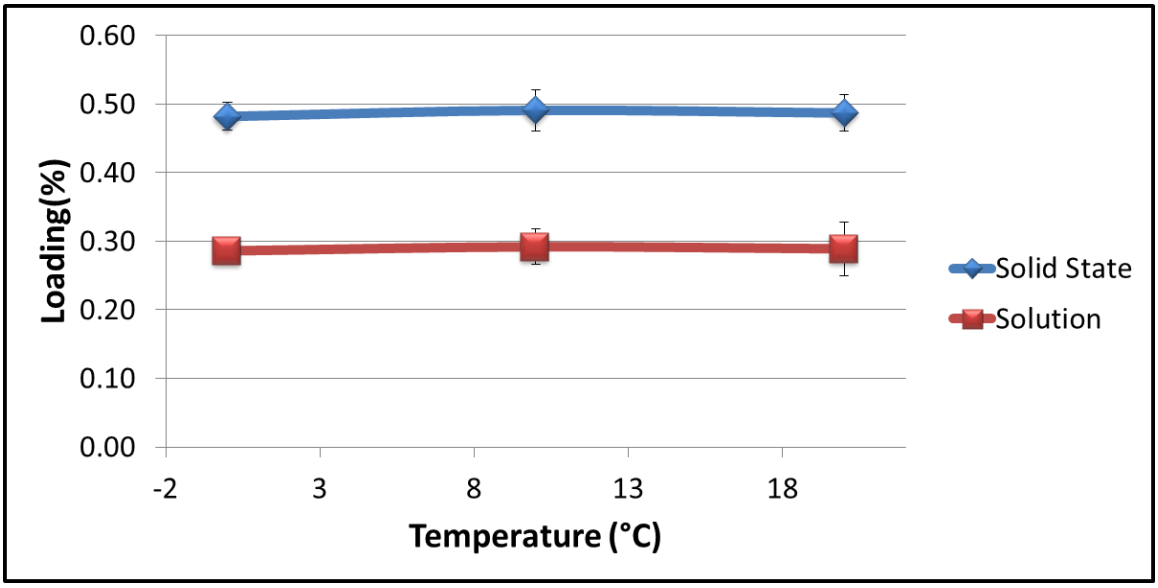

Figure 5.9 Influence of temperature on 1-MCP loading

Possible explanation could be the range of temperature is not high enough to affect the encapsulation process of 1-MCP; the encapsulation process of 1-MCP in MβCD is temperature-dependent but other factors such as polymer structure or encapsulation mechanisms play a much more important role in it.

There could be other reasons that may explain it but the key message is temperature is not the dominant factors we are interested in at this timel.

5.3.1.2 Influence of solution pH

The solution pH here refers to the pH of MβCD encapsulation buffer solution/suspension controlled by citric acid/sodium citrate buffer system. The H^+ concentration could affect the configuration of hydroxyl groups on cyclodextrin rings making rings more open or more closed and this may further change the shape of cavity. H^+ concentration also influences the solubility of the material. 1-MCP loadings at acidic

condition (pH=4), neutral condition (pH=7) and alkaline condition (pH=10) were investigated and compared.

Conditions of pH lower than 4 and pH higher than 10 were not considered because cyclodextrin will undergo hydrolysis under those extreme pH conditions (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Connors, 1997; Tønnesen, Ma´sson, & Loftsson, 2002).

No significant difference of 1-MCP loading under 3 pH conditions was observed as it showed in fig 5.10. The reason could be hydroxyl groups which are supposed to be affected by pH are cross-linked due to the high degree of polymerization between cyclodextrin rings. So H^+ cannot affect opening status of cyclodextrin rings by interacting with hydroxyl groups and thus the sites and cavity are well fixed.

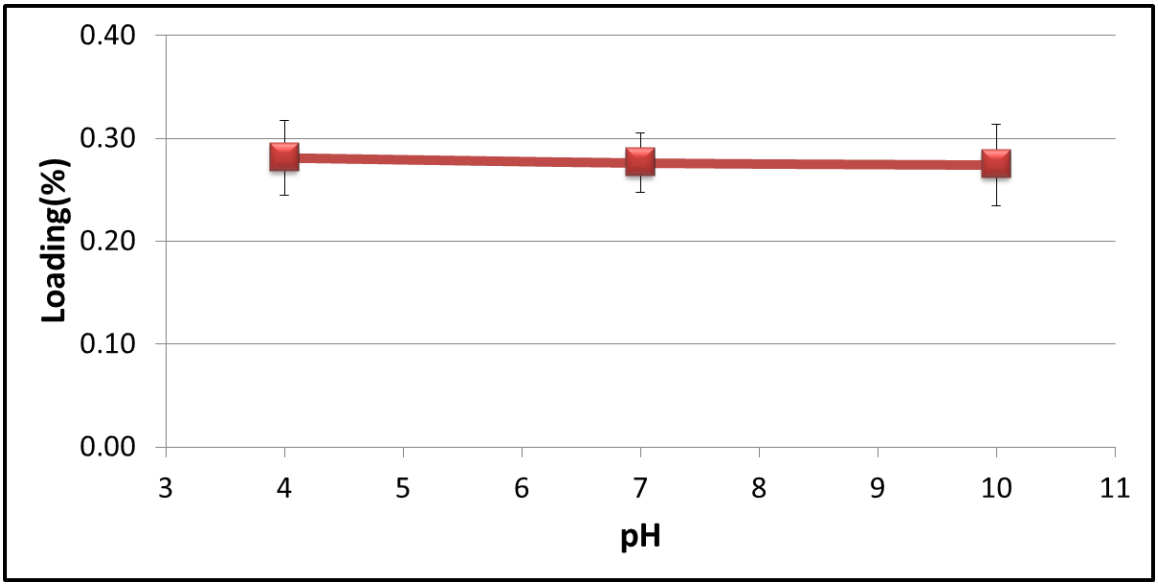

Figure 5.10 Influence of pH on 1-MCP loading

Apparently, pH is not the determine factor that we are looking for either.

5.3.1.3 Influence of MβCD concentration

Similar to pH, MβCD concentration can only be discussed in solution encapsulation method. Higher concentration of MβCD means more encapsulation spots get exposed to 1-MCP molecules and so provides higher chance for 1-MCP molecules to be trapped by MβCD matrix. It is reasonable to say that MβCD concentration may influence the encapsulation process and final loading of 1-MCP.

The solubility of MβCD, based on our test, is between 10-15 g/l, which is about half of original βCD (18.5 g/l). This is another indication of the successful cross-linking.

Back to the influence of MβCD concentration on final loading, 3 concentrations (5 g/l, 10 g/l and 15 g/l) were investigated. Results are showed in fig 5.11.

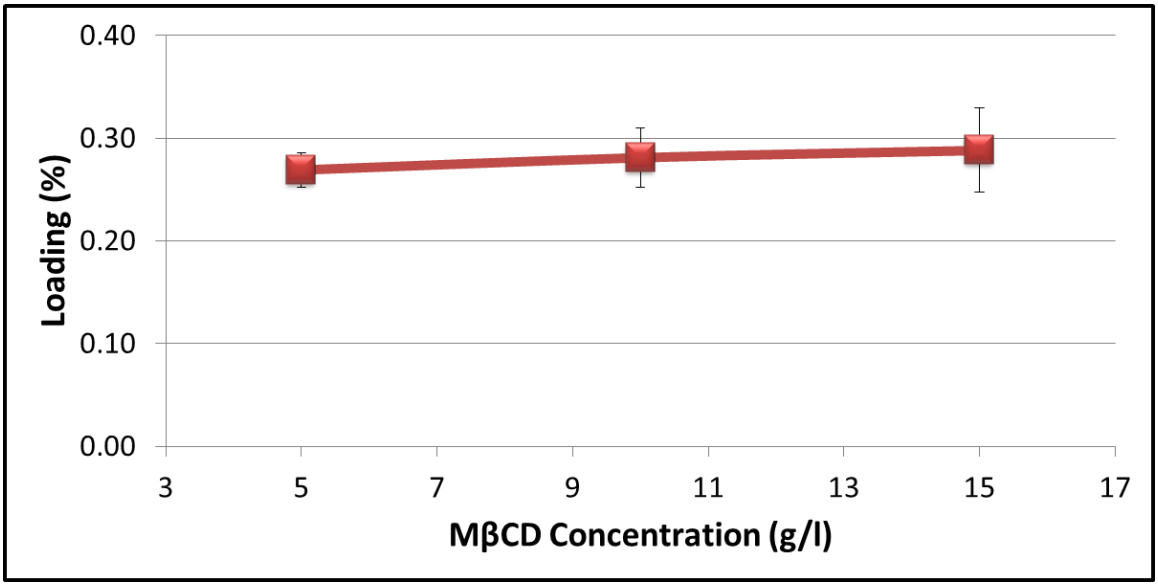

Figure 5.11 Influence of MβCD concentration on 1-MCP loading

According to the result, MβCD concentration again does not play a major role in encapsulation process in terms of final loading even though we see slight increase of

loading as the concentration increases. This may be explained by the saturation of all the available encapsulation sites or cavities. As long as 1-MCP molecules have occupied all the available sites and cavities, no matter how high the concentration of MβCD is in the solution, the loading cannot be further improved.

However, higher concentration of MβCD does not help with 1-MCP loading in MβCD, but it does increase the yield of 1-MCP- MβCD complex. The yield is defined as below.

$$\text{Yield} = \frac{weight\ of\ final\ dried\ MCP - \text{MβCD complex}}{weight\ of\ \text{MβCD initially put in solution}}$$

The relationship between yield and MβCD concentration is showed in fig 5.12 below.

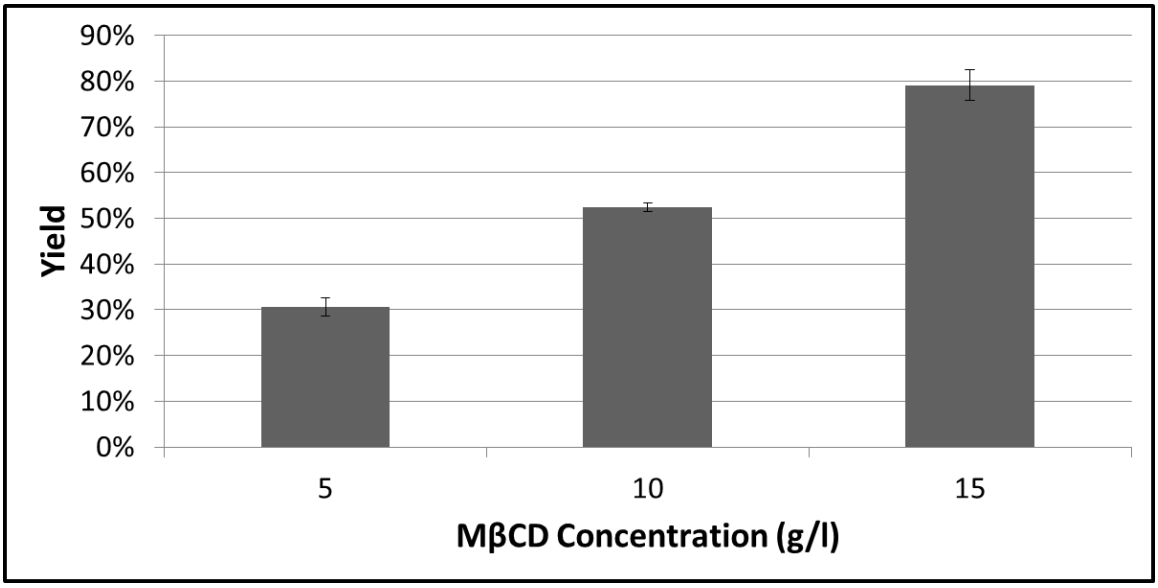

Figure 5.12 Influence of MβCD concentration on the yield of MCP- MβCD complex

It can be clearly observed that the yield is significantly increased as MβCD concentration increases. When MβCD concentration is 15 g/l, the yield is about 80%, which is 2.7 times of the yield at 5 g/l. There still is space to improve the yield by adding more MβCD in the

solution and this can be further optimized to achieve the highest yield if it is needed in mass production.

5.3.1.4 Influence of encapsulation duration

Time is so powerful that it changes everything if it is long enough. Apparently, this is also true to the encapsulation process. Longer time allows more 1-MCP molecules find suitable encapsulation spots to stay and so gives 1-MCP more chances to interact with MβCD and reach equilibrium (Kono, Nakamura, Hashimoto, & Shimizu, 2015; Pinho et al., 2014).

The influence of encapsulation duration on loading for each method was investigated and results are showed in fig 5.13.

In solution method, maximum 30 hours was tested and turning point at 20 hours was identified. Result shows that loading increases from 0.02% to 0.23% as time goes from 5 hours to 20 hours. At 20 hours, loading reaches its maximum and drops to 0.11% at 30 hours.

In solid method, maximum 15 hours was tested with turning point at 9 hours. Loading increases from 0.09% to 0.49% as time goes from 3 hours to 9 hours. At 9 hours, loading reached its maximum and drops to 0.29% at 15 hours.

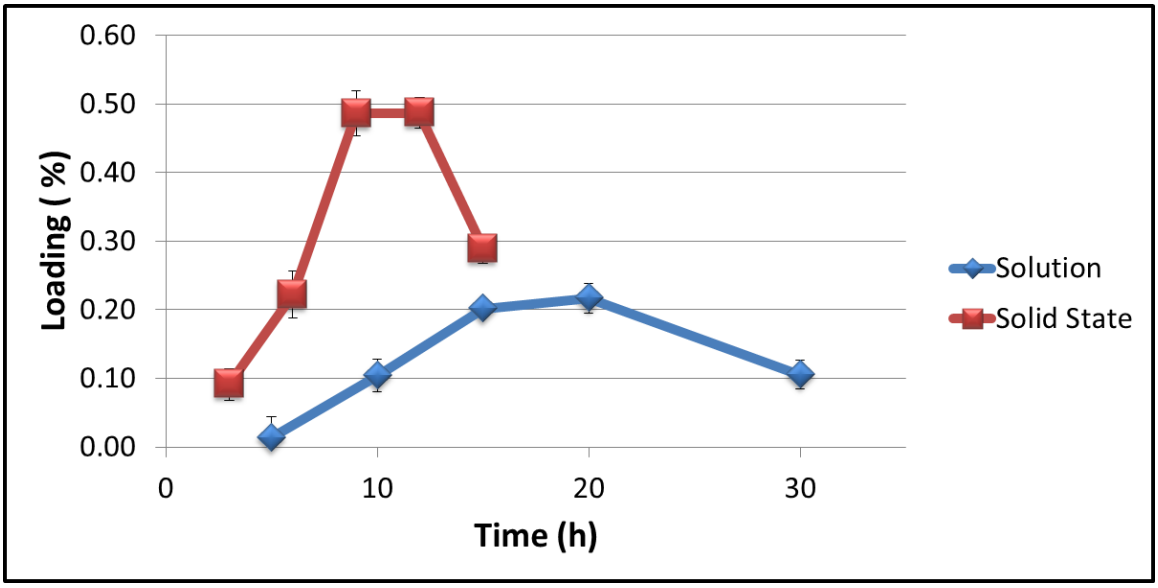

Figure 5.13 Influence of encapsulation duration on 1-MCP loading

When comparing solid method and solution method, we find that encapsulation by solid method takes less time to achieve maximum loading than solution method and the maximum loading of solid method is about 2 times higher than loading of solution method.

So encapsulation duration is the first factor we identified with significant influence on 1-MCP loading. The optimization boundary for solid method is 5 to 15 hours and 15 to 25 hours for solution method according to results above.

5.3.1.5 Influence of 1-MCP concentration

1-MCP concentration affects the final equilibrium between 1-MCP in headspace and 1-MCP in MβCD (Arumugam, Kaanumalle, & Ramamurthy, 2007; Haidong, Fang, Zhihong, & Changle, 2011; Piel et al., 2006). Ideally, high 1-MCP concentration is able to push the

equilibrium to the point that more MCP molecules enter and stay in cavities and thus promote the encapsulation and improves loading level of 1-MCP in MβCD.

1-MCP concentration at the range from $2X10^4$ ppm to $12X10^4$ ppm was investigated for both methods. Later concentration was the highest 1-MCP concentration we can safely achieve in the lab. Results are showed in fig 5.14 below.

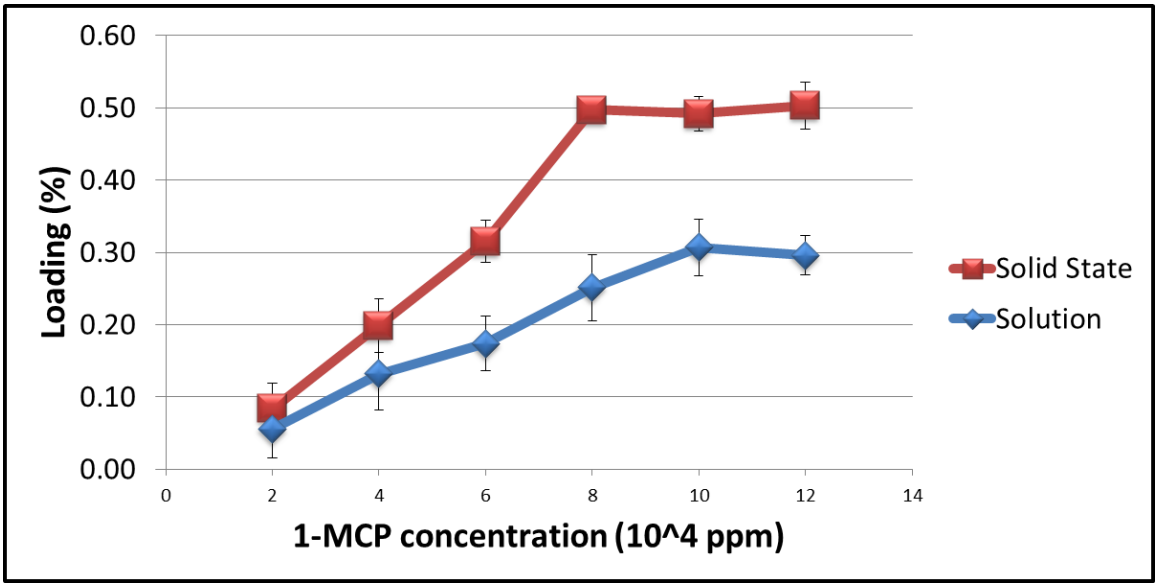

Figure 5.14 Influence of 1-MCP concentration in headspace on loading

In solution method, loading increased from 0.06% to 0.31% as 1-MCP concentration increased from $2X10^4$ ppm to $10X10^4$ ppm and reached maximum loading level at $10X10^4$ ppm. Loading remained at the same level when 1-MCP concentration kept increasing to $12X10^4$ ppm.

In solid method, similar pathway was also observed. 1-MCP loading increased from 0.08% to 0.50% as 1-MCP concentration increased from $2X10^4$ ppm to $8X10^4$ ppm and reached

maximum loading level at $8X10^4$ ppm. Loading remained at same level when 1-MCP concentration was further increased to $12X10^4$ ppm.

In this series of experiments, solid method was able to give higher 1-MCP loading level with lower 1-MCP concentration compared with solution method. Saturation points were found in both methods, after which no increase of 1-MCP loading was detected. In solid method, it was at $8X10^4$ ppm of 1-MCP concentration and it was at $10X10^4$ ppm in solution method.

So 1-MCP concentration was the second dominant factor we found in both methods. The boundary 1-MCP concentration for both methods was set from $5X10^4$ ppm to $15X10^4$ ppm for optimization in next step.

5.3.2 Response Surface Methodology

All the statistical work in this section is done using program Design Expert. Experimental runs are designed based on central composite design with 3 center points.

5.3.2.1 RSM for solid encapsulation method

Experiments of solid encapsulation method were conducted according to table 5.3 given by Design Expert as showed below.

Table 5.3 Experimental runs of solid encapsulation (coded and actual)

Runs	Time		1-MCP Con.		Loading
	Coded	Actual	Coded	Actual	
1	-1	5	-1	5	
2	-1	5	0	10	
3	-1	5	1	15	
4	0	10	0	10	
5	0	10	-1	5	
6	0	10	1	15	
7	1	15	-1	5	
8	0	10	0	10	
9	1	15	0	10	
10	1	15	1	15	
11	0	10	0	5	

2 key factors were coded for use in RSM. 3 levels of encapsulation duration (5 hours, 10 hours, 15 hours) were coded as -1, 0 and 1. 3 levels of 1-MCP concentration ($5X10^4$ ppm, $10X10^4$ ppm, $15X10^4$ ppm) were also coded following the same rule.

1-MCP Loading results under each condition are showed in table 5.4 below and those data were utilized to build the response surface in Design Expert.

Table 5.4 loading results of solid encapsulation under each condition

Runs	Time	1-MCP Con.	Loading
1	-1	-1	0.221±0.03
2	-1	0	0.336±0.06
3	-1	1	0.345±0.04
4	0	0	0.482±0.11
5	0	-1	0.304±0.02
6	0	1	0.501±0.05
7	1	-1	0.318±0.17
8	0	0	0.491±0.09
9	1	0	0.495±0.04
10	1	1	0.509±0.07
11	0	0	0.488±0.12

The second-order polynomial model was used to fit the results and regression equation was obtained as showed below in equation 5.2.

$$\text{Response} = 0.483 + 0.07 \times \text{A} + 0.085 \times \text{B} + 0.017 \times \text{AB} - 0.062A^2 - 0.075B^2$$

Equation 5.2 Regression equation for solid method

Response = 1-MCP loading in MβCD

Factor A = encapsulation duration, hour

Factor B = 1-MCP concentration, $1X10^4$ ppm

In figure 5.15, a good fitting of this equation was showed indicating the reliability of this model.

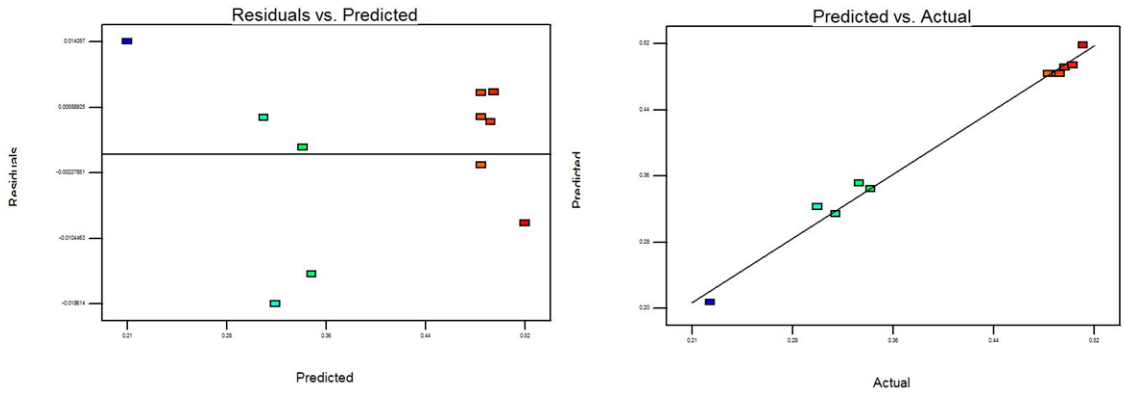

Figure 5.15 Residual vs. Predicted and Predicted vs. Actual

The fitness of the model was also confirmed by ANOVA results in table 5.5.

Table 5.5 ANOVA of solid method regression model

Source	Sum of Squares	Mean squares	F value	p-value
Model	0.11	0.021	105.02	<0.0001
A	0.029	0.029	144.01	<0.0001
B	0.044	0.044	214.01	<0.0001
AB	0.0011	0.0011	5.50	0.066
A2	0.0098	0.0098	48.35	0.0009
B2	0.014	0.14	70.59	0.0004
Lack of Fit	0.00098	0.00033	15.54	0.0611

Based on the results given by Design Expert, the model itself is significant while the lack of fit is not significant. It proved the effectiveness of model.

In this equation, coefficient of A, A^2, B and B^2 is 0.07, -0.062, 0.085 and -0.075 respectively. When comparing the coefficients of 2 first order items which were A and B, we found that coefficient of B was higher than coefficient of A (0.085>0.07). Same results are observed in second order items (0.075>0.062). It tells us that factor B (1-MCP

concentration) has greater impact on final 1-MCP loading compare to factor A (encapsulation duration) in solid encapsulation method.

According to the regression equation, response surface was built and showed in figure 5.16. In the graph, Y axis represents loading while X1 and X2 axis represent encapsulation duration and 1-MCP concentration respectively. Blue area on the surface represents low 1-MCP loading while red area represents high 1-MCP loading. A clear trend has been observed that loading increases as 1-MCP concentration and encapsulation duration increases and reaches a maximum point somewhere within red area and then drops. The optimum point which is the point with maximum 1-MCP loading locates at red area.

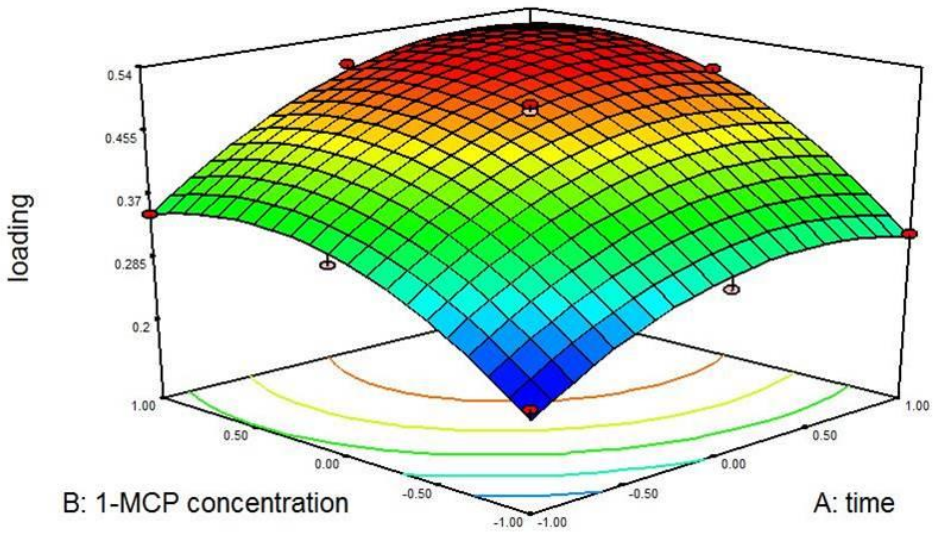

Figure 5.16 3D response surface of solid method

Figure 5.17 shows the 2D response surface on which optimum point is clearly demonstrated. In the graph, those curves are contour and each of them represents one level of loading. The optimum point predicted by RSM is at the coordinate of (0.45, 0.78)

corresponding to (duration, 1-MCP concentration) with the maximum loading of 0.529%, which is higher than any of the loading results in previous experiments.

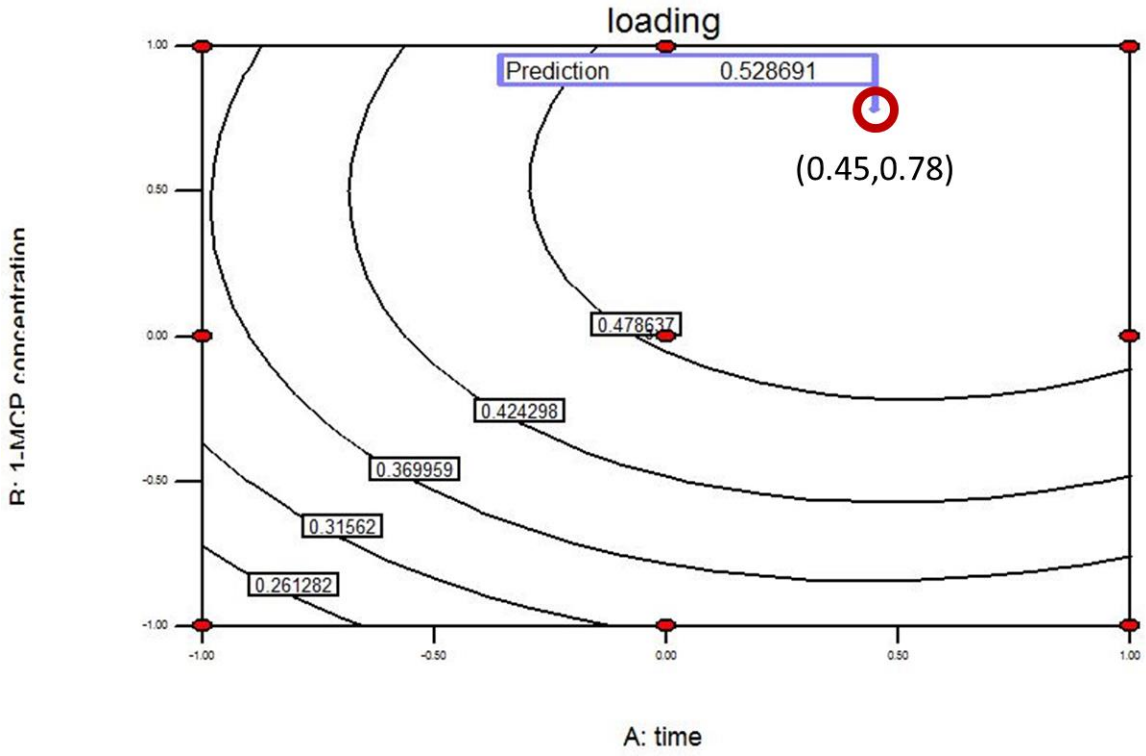

Figure 5.17 2D response surface of solid method (contour view)

5.3.2.2 RSM for solution encapsulation method

Table 5.6 gives all the experimental runs needed to collect data for solution encapsulation. Totally 11 runs were done with triplication for each run.

Similar to what is done in solid method, 3 levels of encapsulation duration (15 hours, 20 hours, 25 hours) were coded as -1, 0 and 1. 3 levels of 1-MCP concentration ($5X10^4$ ppm, $10X10^4$ ppm, $15X10^4$ ppm) were also coded as -1, 0 and 1 following the same rule.

Table 5.6 Experimental runs of solution encapsulation (coded and actual)

Runs	Time		1-MCP Con.		Loading
	Coded	Actual	Coded	Actual	
1	-1	15	-1	5	
2	-1	15	0	10	
3	-1	15	1	15	
4	0	20	0	10	
5	0	20	-1	5	
6	0	20	1	15	
7	1	25	-1	5	
8	0	20	0	10	
9	1	25	0	10	
10	1	25	1	15	
11	0	20	0	10	

Loading results are showed in table 5.7 below. Collected data was used in Design Expert to build the response surface following the same procedure as discussed in solid method.

Table 5.7 loading results of solution encapsulation under each condition

Runs	Time	1-MCP Con.	Loading
1	-1	-1	0.098±0.09
2	-1	0	0.200±0.01
3	-1	1	0.216±0.05
4	0	0	0.293±0.08
5	0	-1	0.232±0.08
6	0	1	0.302±0.18
7	1	-1	0.226±0.21
8	0	0	0.296±0.13
9	1	0	0.293±0.04
10	1	1	0.306±0.07
11	0	0	0.298±0.11

Equation 5.3 is the regression equation of the polynomial model used to fit the data.

$$\text{Response} = 0.297 + 0.052 \times \text{A} + 0.045 \times \text{B} - 0.010 \times \text{AB} - 0.052A^2 - 0.032B^2$$

Equation 5.3 Regression equation for solution method

Response = 1-MCP loading in MβCD

Factor A = encapsulation duration, hour

Factor B = 1-MCP concentration, $1X10^4$ ppm

In residual graphs showed in figure 5.18, the model fits the data very well which indicating the reliability of the regression model.

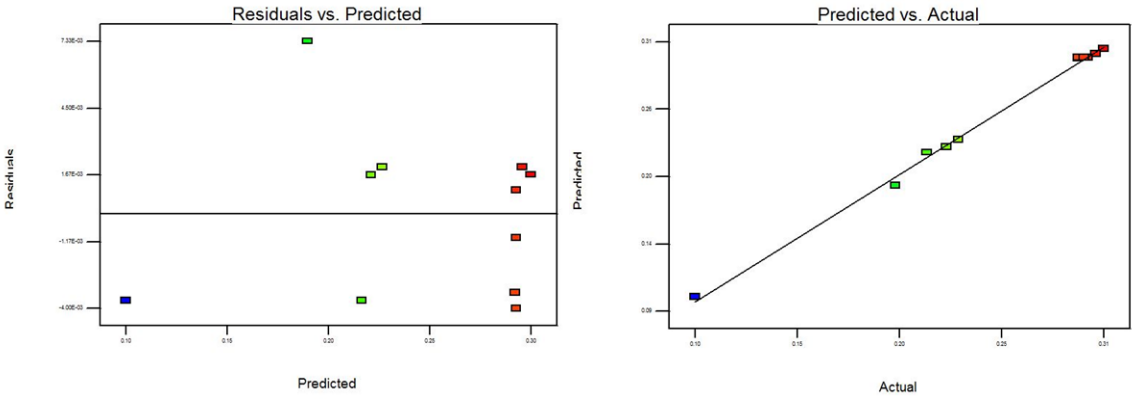

Figure 5.18 Residual vs. Predicted and Predicted vs. Actual

ANOVA results showed in table 5.8 also confirm the fitness of the model with the data by showing that the model itself is significant while Lack of Fit is not significant.

Table 5.8 ANOVA of solution method regression model

Source	Sum of Squares	Mean squares	F value	p-value
Model	0.041	0.0069	224.25	<0.0001
A	0.016	0.016	522.82	<0.0001
B	0.00245	0.0024	79.46	0.0009
AB	0.00036	0.000036	11.71	0.0267
A2	0.0069	0.0069	226.46	0.0001
B2	0.0026	0.0026	84.13	0.0008
Lack of Fit	0.00011	0.000055	8.74	0.1027

In this regression equation, coefficient of A, A^2, B and B^2 is 0.052, -0.052, 0.045 and -0.032 respectively. Comparing the coefficients of 2 first order items which are A and B, we find coefficient of A is bigger than coefficient of B (0.052>0.045). Same results are observed in second order items (0.052>0.032). It tells us that factor A (encapsulation duration) has greater impact on the loading compare to factor B (1-MCP concentration) in solution encapsulation method.

This result is different with solid method and it can be explained by the saturation of 1-MCP in aqueous solution. In the case of solid encapsulation, 1-MCP directly interacts with MβCD. While 1-MCP concentration increases, it increases the chance of 1-MCP molecules getting into MβCD without any limitation on the equilibrium until MβCD gets saturated. However, in the case of solution encapsulation, aqueous solution is used as media to deliver 1-MCP molecules to MβCD. However, the solubility of 1-MCP in water is fixed (137 mg/L) (FAO, 2010). Once 1-MCP gets its saturation in the solution, further increase of 1-MCP concentration in headspace will not affect the encapsulation process anymore. That is why 1-MCP concentration has smaller impact compared to encapsulation duration in solution method while it has higher impact in solid method.

3D response surface is built based on equation 5.3 is built and showed in figure 5.19. In the graph, Y axis represents loading while X1 and X2 axis represent encapsulation duration and 1-MCP concentration respectively. Blue area on the surface represents low 1-MCP loading while red area represents high 1-MCP loading. A clear trend has been observed that loading increases as 1-MCP concentration and encapsulation duration increases and reaches a maximum point somewhere within red area and then drops. The optimum point which is the point with maximum 1-MCP loading locates at red area.

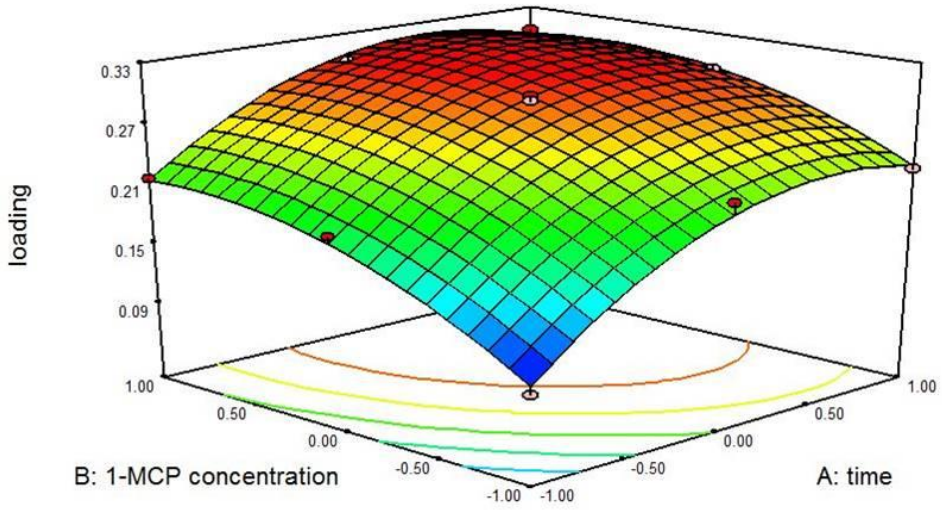

Figure 5.19 3D response surface of solution method

Figure 5.20 shows the 2D response surface on which optimum point is clearly demonstrated. In the graph, contours of same level of loading are exhibited. The optimum point predicted by RSM is (0.24, 0.66) corresponding to (duration, 1-MCP concentration) with the maximum loading of 0.320%. The predicted loading at optimum point is higher than any previous loading results.

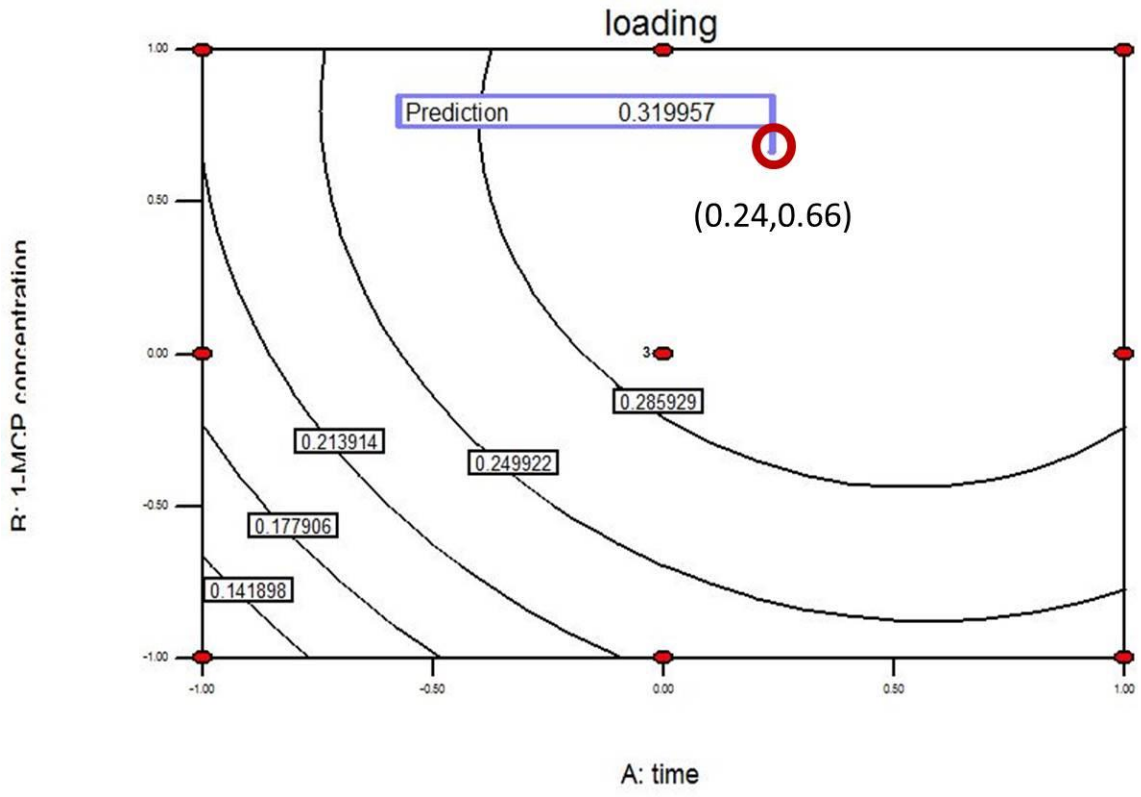

Figure 5.20 2D response surface of solution method (contour view)

5.3.3 Confirmation of optimum point

According to the results discussed at section 5.3.2, optimum point of solid method is (0.45, 0.78), representing the condition of 12.25 hours and $13.9X10^4$ ppm, with predicted maximum loading of 0.529%. Optimum point of solution method is (0.24, 0.66), representing the condition of 21.2 hours and $13.3X10^4$ ppm, with predicted maximum loading of 0.320% (table 5.9).

Encapsulation was done under identified optimum conditions for each method to check the difference between actual loading and predicted loading. Table 5.9 shows the result of validation.

Table 5.9 Confirmation of optimum points

	Solution method	Solid method
Optimum condition	21.2 h, $13.3X10^4$ ppm	12.25 h, $13.9X10^4$ ppm
Predicted loading	0.320%	0.529%
Actual loading	0.317%	0.535%
Difference	-0.937%	+1.13%

The difference between predicted value and actual value is -0.937% for solution method and +1.13% for solid method under each of their optimum conditions. The difference shows that predicted values and actual values are close to each other and confirm that identified optimum points are reasonable and valid.

5.4 Comparison of two methods

5.4.1 Encapsulation kinetics

According to the optimization results, it took solution method 21.2 hours to get the maximum loading while it took only 12.25 hours to achieve the maximum. When converted to the encapsulation rate with the unit of loading per hour, solution method gets 0.0149%/h while solid method gets 0.0437%/h. The encapsulation rate of solid method is almost 3 times higher than the encapsulation rate of solution method (figure 5.21). The difference in encapsulation rate is also indicated in figure 5.13, in which the slopes of the two curves are rough estimation of the encapsulation rate.

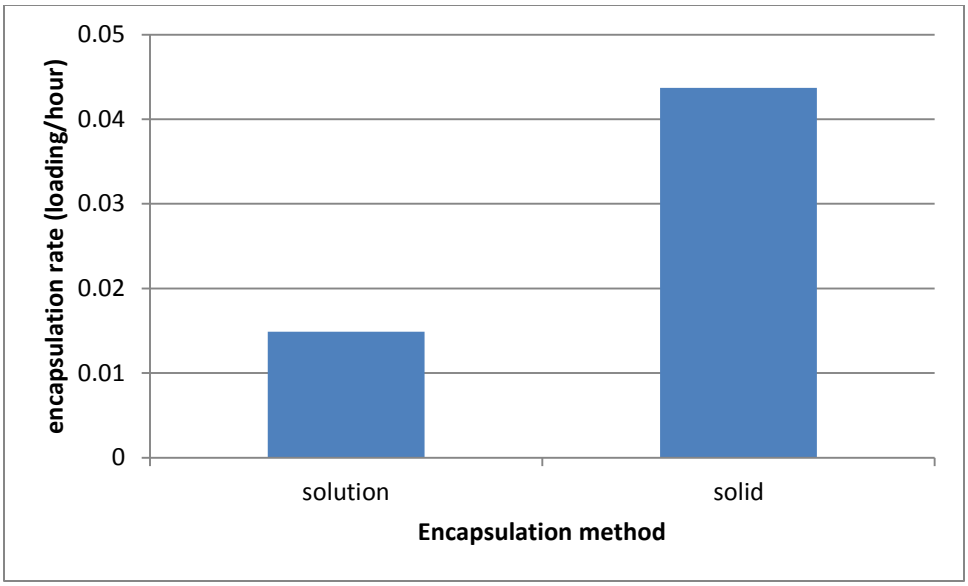

Figure 5.21 Encapsulation rate of solution and solid method

The difference could be explained by different encapsulation mechanisms followed by the two methods.

In the case of solution method, 1-MCP molecules are surrounded by water molecules. 1-MCP molecules, which are hydrophobic, would try to enter the cavities of MβCD which are also hydrophobic. However, they can only stay in those cavities with suitable size which means if those cavities are too small, 1-MCP molecules cannot go in while if the cavities are too large, 1-MCP molecules can easily go through them or get pushed out by water molecules instead of staying. Only the cavities with right size are able to tightly trap 1-MCP molecules. This is actually a type of equilibrium. It takes certain time for 1-MCP molecules to access those available cavities and find one to occupy. At the same time, water molecules also tend to push 1-MCP molecules in cavities out if they are not encapsulated.

In solid method, adsorption takes place as major effect. 1-MCP molecules are able to be adsorbed on any exposed surface of MβCD matrix to low the surface energy. 1-MCP adsorption does not necessarily require the size of cavities matches the size of 1-MCP molecules. So adsorption can also occur in large cavities even though the adsorption is loose that 1-MCP can easily escape. Adsorption process occurs much faster than the encapsulation process occurs in solution method as 1-MCP molecules move more quickly and freely in headspace compared to solution. And this is also because the double bond gives 1-MCP molecules more binding potential to the surface.

In section 5.3.2, we discussed that encapsulation duration plays a more important role in solution method while 1-MCP concentration in the headspace is more important in solid method. This can also be explained by the difference of mechanisms. The diffusion or movement of 1-MCP in solution as well as the access process of 1-MCP to cavities are relatively slow, so it limits the progress of encapsulation process even with high 1-MCP concentration in the headspace. The process always needs certain amount of time to complete. Also, 1-MCP molecules need to find size-fitted cavities to stay and this process also takes time. That is why encapsulation duration has more impact in solution method. In solid method, increase of 1-MCP concentration directly provides more 1-MCP for cavities to absorb. Considering the adsorption process and the diffusion is very fast and does not need much time to get fully adsorbed. So 1-MCP concentration affects more on the solid encapsulation process.

5.4.2 Release profile

Different release profile of 1-MCP that encapsulated by the two encapsulation methods was also observed and showed in figure 5.22.

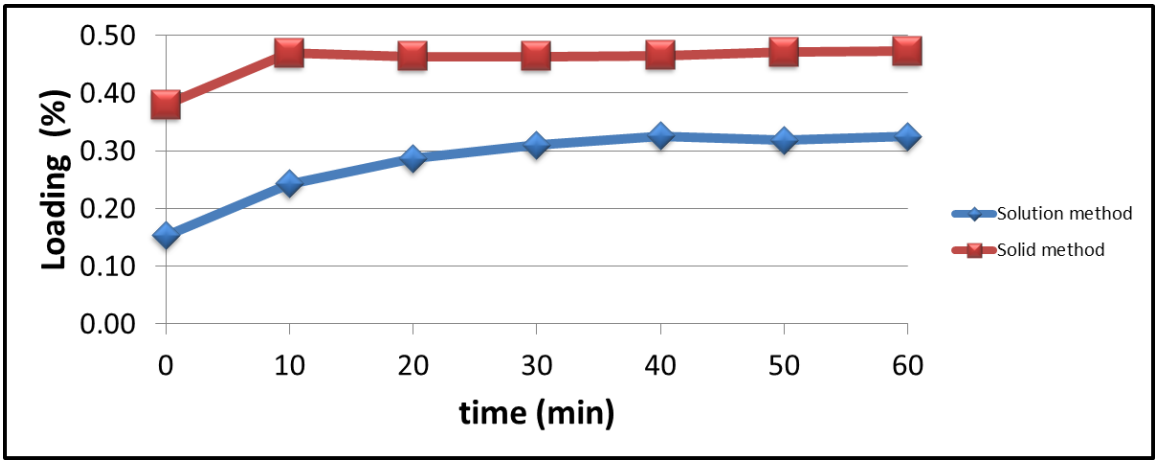

Figure 5.22 Release profile of 1-MCP encapsulated using different methods

In solution method, it took 30 to 40 minutes to fully release all the encapsulated 1-MCP from MβCD matrix. At time 0, about 50% of the encapsulated 1-MCP had already been released. Most of the released 1-MCP at time 0 was encapsulated in the cavities of out layer so they could be easily attacked by water molecules to trigger the release. After that, the release rate was relatively high at first 10 minutes which is about 0.01% per minute from 0 to 10 minute. The rate kept decreasing during the release process and dropped to 0.003% per minute at last 10 minutes from 30 to 40 minute.

In solid method, the release was much faster compared to solution encapsulated 1-MCP. At time 0, about 80% of 1-MCP had already been released and the rest 20% got completely released within 10 minutes.

The different release profiles also match the different mechanisms we discussed before. In solution method, all the 1-MCP molecules are tightly trapped (means encapsulated) by cavities, so it takes time for water molecules to push those encapsulated 1-MCP molecules out from the cavities. Only 50% of encapsulated 1-MCP is released at time 0 because water molecules also need time to diffuse into the inner part of MβCD and 1-MCP also needs time to move out of the matrix. In solid method, most of 1-MCP is loosely adhered on the exposed surface of cavities, so the release is quite fast compared to the case in solution method. This is also the reason why at time 0, more than 80% of 1-MCP is released.

In other word, release is the reverse process of encapsulation. Fast encapsulation gives fast release (solid method) and slow encapsulation gives slow release (solution method). Both of the encapsulation and release profile could be useful under certain conditions and need to be further explored for application.

6. Conclusion

As mentioned before, the objective of this research is to 1) compare solid encapsulation method and solution encapsulation method and 2) optimize encapsulation conditions for each method to identify the optimum method and conditions with maximum 1-MCP loading in modified β-cyclodextrin.

In this study, solution method gives the maximum loading of 0.320% while solid method gives 0.529%. So the optimum method of encapsulating 1-MCP into MβCD is solid

method. The maximum 1-MCP loading is 0.529% achieved at the optimum condition of 12.25 hours (encapsulation duration) and $1.39X10^5$ ppm (1-MCP concentration).

The result proves that the chemical modification of β-cyclodextrin is effective to improve 1-MCP loading level from 0.1% in original β-cyclodextrin to 0.529% in modified β-cyclodextrin by creating more encapsulation cavities in the structure. The chemical modification process could also be further optimized to provide more cavities with suitable cavity size and so to further improve the loading level of 1-MCP.

The result also indicates different mechanisms of encapsulation occur in the two encapsulation methods. In solution method, 1-MCP molecules are in water. They are able to enter those hydrophobic cavities due to weak hydrophobic interaction, but surrounded water molecules can still wash them out by its movement. To keep 1-MCP in the cavity, the cavity size has to fit the size of 1-MCP molecule so it can provide additional physical entrapment, which means only size-fitted cavities can encapsulate 1-MCP while other unfitted cavities have to remain empty. In solid method, 1-MCP molecules are in vacuum. They can stay in any available cavities only relying on the hydrophobic interaction regardless the size of the cavity because there is no other molecule such as water to disturb the interaction. Also in solution case, the aqueous condition slows down the diffusion and the encapsulation process compared to the vacuum condition in solid case. That is why solution method takes longer to complete the encapsulation but gives lower loading than solid method.

7. Future work

Below list some of the questions that need to be answered or further investigated.

1. Get more clarity about MβCD structure and further improve it to increase loading capacity of 1-MCP

   1-MCP loading has already been significantly improved from 0.1% in original β-CD to 0.529% in MβCD. The detailed structure especially high level of structure is still unknown for MβCD even though general characterization has already provided a lot of information. Clarify detailed structure and further improve the MβCD structure is necessary and feasible by adding more cavities and making cavity size more compatible with 1-MCP molecule to finally improve the loading capacity of 1-MCP.

2. Optimize MβCD concentration in solution to maximize encapsulated complex yield

   It has been observed and discussed in 5.3.1.3 that complex yield increases as MβCD concentration increases. The relationship of yield and MβCD concentration can be further explored to maximize complex yield in the process.

3. Understand encapsulation mechanisms and processes

   Different encapsulation mechanisms have been proposed and discussed in previous section. However, all the discussion is based on the speculation from current results and none of them can be the direct evidence. To better understand the mechanisms and the process, key parameters such as binding

constant and surface energy need to be measured to support the proposed theory. The study from the chemistry point of view may provide a brand new angel to look at the process and potentially a new method or technology for encapsulation.

4. Improve stability of solid encapsulated MβCD

   According to figure 5.22, the release of 1-MCP encapsulated by solid method is very fast. We also have data to show that 1-MCP encapsulated by solid method will completely escape from MβCD within 2 hours when exposed to the open air under room temperature. Quick depletion of 1-MCP makes a huge problem if we want to develop a product based on it. The solution of this problem could be a protection layer on the encapsulated MβCD or MβCD with more compatible cavities so more 1-MCP can be trapped longer.

5. Application on fruits and vegetables

   A delivery system including a formulation with consistent 1-MCP content needs to be developed to use MβCD to carrier 1-MCP. The application conditions such as humidity and temperature also need to be investigated. When applied to a specific fruit or vegetable, specific release profile may be needed to generate enough amount of 1-MCP to interact with those plants. How long it takes to treat the plants; how much concentration is needed to inhibit ethylene response and under what conditions. All of those questions will need to be answered in application.

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