# DEVELOPMENT OF LIQUID 1-METHYLCYCLOPROPENE DELIVERY FORMULATIONS FOR MODIFYING ETHYLENE RESPONSE OF FRESH PRODUCE

by

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## ABSTRACT OF THE DISSERTATION

DEVELOPMENT OF LIQUID 1-METHYLCYCLOPROPENE DELIVERY FORMULATIONS FOR MODIFYING ETHYLENE RESPONSE OF FRESH PRODUCE

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Dissertation Director:

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The objective of this dissertation is to develop 1-methylcycopropene (1-MCP) liquid formulations for fresh produce, to manage the undesirable effects of ethylene. The formulation can be used as a sprayable formulation for pre-harvest application, or made into a capsule for post-harvest application. The formulation consists of (1) a cyclodextrin based material (α or modified β-cyclodextrin) as the encapsulant for 1-MCP, (2) a moisture blocking formulation (e.g. polyol/hydrocolloid formulation) to further enclose 1-MCP, and (3) a water based solution to facilitate the release of the formulation. To develop this formulation, five sub-objectives were achieved.

1. Synthesis and characterization of modified β-cyclodextrin
2. Encapsulation of 1-MCP into modified β-cyclodextrin and quantification of 1-MCP inclusion ratio
3. Stabilization of the complex as liquid formulation using materials such as polyol/hydrocolloid blend and mineral oil
4. Evaluation of the sprayability of the liquid formulation and release of 1-MCP
5. Evaluation of biological efficacy of the 1-MCP released from the liquid formulation

The key conclusions of this dissertation are as follows.

- Modification of β-cyclodextrin was optimized to yield 90-95% powder. The powder has better modified pore size and particle size, which are more favorable for 1-MCP encapsulation than β-cyclodextrin.
- 1-MCP can be encapsulated under 0.1 atm headspace concentration both using solid-gas method (inclusion level 0.5%) and in solution-gas method (inclusion level 0.3%).
- A stable formulation was developed consisting of mineral oil, HPC and polysorbate for both α and modified β-cyclodextrins. After 30 days of storage at room temperature, more than 90% of 1-MCP can be retained in the formulation. After 14 days of storage at 55°C (equivalent to 1 year storage at room temperature), more than 80% of 1-MCP can be retained in the formulation.
- After hydration using different concentrations of xanthan gum, liquid formulation

can be sprayed and complete release can be obtained in 6 hours.

- 1-MCP released from the formulation is biologically effective that no dimer or isomer formation at treatment level (1 ppm). It was able to extend the shelf life of bananas (color stage 2-2.5) from 2 days to 5 days at room temperature.

## ACKNOWLEDGEMENT

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I strongly believe that we are not born but made by the people around us. I place on record the unstinted support and encouragement of my parents, in-laws, and friends. I would like to specially express my deepest love and appreciation to my wife Yihua for believing in me.

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# 1. INTRODUCTION

The target produce of the developed technology is apple because 1-MCP is effective on extending its shelf life and 80% of apple in the US market is treated with 1-MCP, indicating the potential possibility of acceptance of this technology by apple industry. In pre-harvest application, 1-MCP can prevent pre-harvest drop which is the result of endogenous ethylene triggering a cascade of enzyme mediated events that accelerates maturity followed by fruit abscission. In postharvest application, 1-MCP is treated in a controlled atmosphere room after a drenching process of DPA, an antioxidant to prevent any oxygen mediated disorder such as superficial scald.

There are both commercially available pre- and postharvest 1-MCP applications but they have their limitations. The pre-harvest application of 1-MCP for apples is in a sprayable form. It has been reported that there is excessive loss of 1-MCP during spraying, resulting in low cost-effectiveness, and certain ingredients in the formulation can react with other pesticides, leading to leave burn. The postharvest application of 1-MCP is in powder or tablet form. Its application is after DPA drenching treatment. These two processes make postharvest treatment of applies tedious and involve multiple rooms and personnel. In addition, the storage rooms are generally large and it takes weeks to fill the entire room with apples, leading to possible existence of maturity variation, i.e., the apples filled earlier are more mature than the ones filled later. The maturity variation in turn results in different 1-MCP responses (1-MCP application is more effective when apples are at early stage).

To overcome these limitations, we propose to develop a liquid formulation containing 1-MCP which can be used as a sprayable formulation for pre-harvest application and can be combined with DPA drenching treatment for postharvest application. The liquid formulation consists of three components: (1) α- or modified β-cyclodextrin based encapsulation material, (2) moisture blocking formulation such as blend of polyol or mineral oil / hydrocolloid, and (3) water based hydrocolloidal solution.

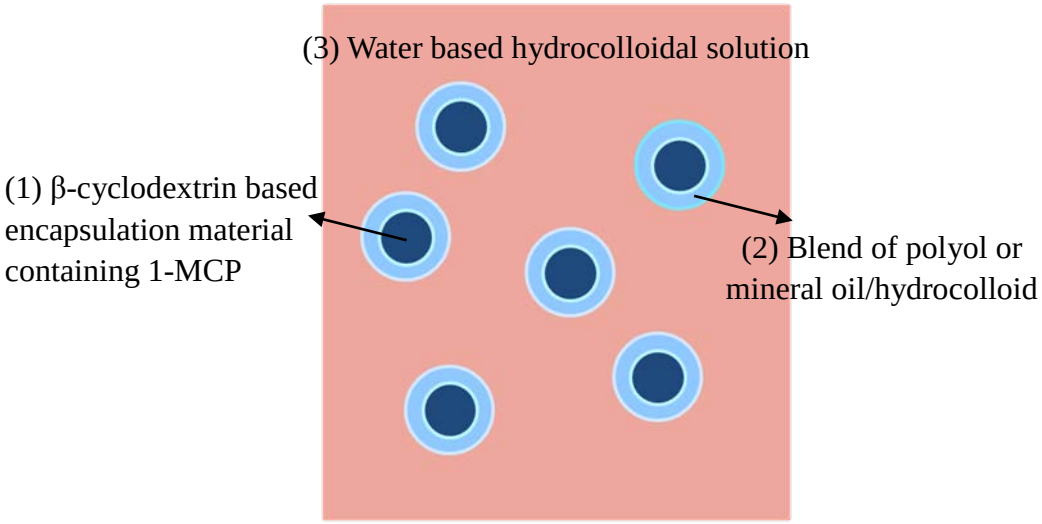


Figure 1: Formulation composition

In component 1, two types of materials were used: (1) α-cyclodextrin, which can trap 1-MCP in its hydrophobic internal cavity and release 1-MCP when contacting with water; (2) modified β-cyclodextrin, which is a cross-linked β-cyclodextrin with a three dimensional polymer network to trap 1-MCP in its intermolecular spaces and internal capvity. In component 2, appropriate polyol or mineral oil / hydrocolloidal solution is formulated to mix with component 1 to retain 1-MCP in the encapsulating material. The dispersion of component 1 in component 2 is envisioned to be the main form for storage of

the formulation. The theory behind this formulation is that the hydrocolloids binds water to limit its contact with α- and modified β-cyclodextrin and creates a torturous path for the diffusion of 1-MCP to restrict its movement. In component 3, low concentration water based hydrocolloid solution is used for pre-harvest application to hydrate the dispersion of component 1 in component 2 to provide the sprayability of the final formulation; and water is used for postharvest application to release 1-MCP.

In summary, the objective is to develop a 1-MCP liquid formulation which can be used for both pre- and postharvest applications. The formulation consists of three components (1) α- and modified β-cyclodextrin encapsulating 1-MCP, and (2) polyol or mineral oil / hydrocolloid blend and (3) water based hydrocolloidal solution. The criteria used to evaluate the successful development of this formulation are (1) the feasibility of encapsulating 1-MCP into cross-linked β-cyclodextrin, (2) the physical and chemical stability of 1-MCP in the formulation, (3) sprayability of the formulation after hydration, and (4) the biological efficacy of the 1-MCP released from the formulation.

# 2. LITERATURE REVIEW

## 2.1. 1-MCP

### 2.1.1. Physical nature and biological function of 1-MCP

1-MCP is a gas (under standard temperature and pressure) with chemical formula of $C_4H_6$ and chemical structure is shown below. Its molecular weight and boiling point are 54.09044 and 12°C, respectively[1].

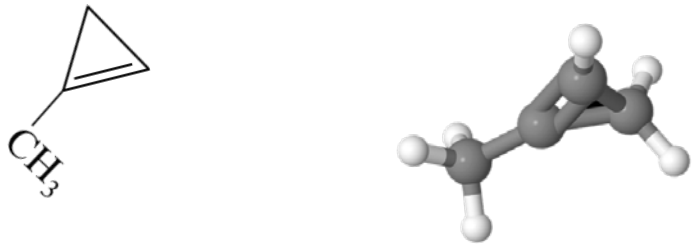


Figure 2: Chemical structure of 1-MCP

Biologically, it is an effective ethylene inhibitor which can delay the ripening of fresh produce. Its mechanism of action is that 1-MCP can bind with ethylene receptor site on fresh produce and so block the binding between ethylene and fresh produce. It was also shown that 1-MCP influences ethylene biosynthesis in some produces through feedback inhibition. It is effective at low concentration (part per billion level) because its affinity is almost 10 times higher to the ethylene receptors than ethylene. It has no odor at effective concentration [2].

### 2.1.2. Determining factors of the effectiveness of 1-MCP

Determining factors of the effectiveness of 1-MCP include application concentration, treatment temperature, treatment time, application cultivar, development stage, time from harvest to treatment, and multiple applications [3]. Literature data of different combinations of were listed in the table below (adopted from [3]).

Table 1: 1-MCP overview (effective treatment on different crops)

| Plant | Concentration | Treatment temperature (°C) | Treatment time | Effects | Reference |
|---|---|---|---|---|---|
| *Ananas comosus* (pineapple) | 0.1 μl $l^{-1}$ | 20 | 18 h | Controlled internal browning; slowed ascorbic acid, ethylene, and soluble solids declines | [4] |
| *Annona squamosa*×*Annona cheimola*(custard apple) | 25 μl $l^{-1}$ | 20 | 14 h | Increased decay, delayed ripening | [5] |
| *Arabidopsis* | 2.2 nl $l^{-1}$ | ? | ? | Allowed normal seedling growth with exogenous ethylene present | [6] |
| *Brassica oleracea*(broccoli) | 1, 12 μl $l^{-1}$ | 5, 10, 20 | 6, 12, 16 h | Delayed yellowing, decreased respiration, decay, extended shelf life | [7]; [8] and [9] |
| *B. rapa var. chinensis* (pak choy) | 12 μl $l^{-1}$ | 10 | 16 h | Increased shelf life if exposed to exogenous ethylene, reduced yellowing | [9] |
| *B. juncea var. foliosa* (Chinese mustard) | 12 μl $l^{-1}$ | 10 | 16 h | Delayed yellowing, increased shelf life if exposed to exogenous ethylene | [10] |
| *B. rapa var. parachinensis* (choy sum) | 12 μl $l^{-1}$ | 10 | 16 h | Delayed yellowing, increased shelf life if exposed to exogenous ethylene | [10] |
| *B. rapa var. nipposinica* (mibuna and mizuna) | 12 μl $l^{-1}$ | 10 | 16 h | Increased shelf life | [10] |
| *B. rapa var. rosularis* (tatsoi) | 12 μl $l^{-1}$ | 10 | 16 h | Delayed yellowing, increased shelf life | [10] |
| *Carica papaya*(papaya) | 25 μl $l^{-1}$ | 20 | 14 h | Increased blemishes slightly, delayed ripening | [5] |
| *Chrysanthemum coronarium* (garland chrysanthemum) | 12 μl $l^{-1}$ | 10 | 16 h | Delayed yellowing, increased shelf life if exogenous ethylene | [9] |
| *Coriandrum sativum*(coriander) | 50 nl $l^{-1}$ | 20 | 24 h | Chlorophyll and protein maintained, senescence delayed | [11] |

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| *Cucumis melo*(melon) plants | 0.1 μl $l^{-1}$ | ? | 10 min | Inhibited ethylene pathway transcripts, wounding response not effected | [12] |
| *Citrullus lanatus*(watermelon) | 5 μl $l^{-1}$ | 20 | 18 h | Reduced internal breakdown | Mao and Huber (personal communication) |
| *Citrus paradisi*(grapefruit) | ? | 25 | 16 h, twice | Prevented degreening and ethylene production | [13] |
| *Citrus* spp. (orange) | 100 nl $l^{-1}$(fruit); 50 μl $l^{-1}$ (petioles) | 25 | 6, 12 h | Increased decay and chilling injury, blocked ethylene induced degreening, inhibited abscission | [14] and [15] |
| *Daucus carota*(carrot) | 1 μl $l^{-1}$ | 20 | 4 h | Prevented acidity loss, respiration increase, and inhibited isocourmarin increase with exogenous ethylene | [7] |
| *Diospyros kaki* | 300 nl $l^{-1}$ | 20 | 3 h | Inhibits fruit softening | [16] and [17] |
| *Fragaria ×ananassa*(strawberry) | 5–15; 250–500 nl $l^{-1}$; 2 μl $l^{-1}$ | 20 | 2–18 h | Increased shelf life, maintained firmness and color, lowered phenolic content, increased decay at high 1-MCP concentrations, lowered ethylene production, effects on respiration vary with maturity | [18], [19] and [20] |
| *Hanconia speciosa* | 90 μl $l^{-1}$ | 31 | Continuous | Induced lateral shoot development of in vitro-grown shoots | [21] |
| *Lactuca sativa*(lettuce) | 1; 0.1 μl $l^{-1}$ | 6 | 4 h | Inhibited ethylene induced russet spotting, postharvest life increased | [7], [22] |
| *Lycopersicon esculentum* fruits (tomato) | 5–7; 10–20, 150 nl $l^{-1}$; 20 μl $l^{-1}$ | 20 | 2–24 h | Postharvest life increased, respiration rate reduced, titratable acidity loss reduced, ACC synthase and ACC oxidase transcripts reduced, delayed ethylene production | [23],[24] [25-27] |
| *L. esculentum* plants | 4 mg EthylBloc | Growing | Overnight | Disease response as regulated | [28] |

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| (tomato) | powder per L | temperature | | by ethylene sensitivity | |
| *Malus domestica*(apple) ‘Anna’, ‘Fuji’, ‘Golden Delicious’, ‘Red Delicious’, ‘Rome’, ‘Gala’, ‘McIntosh’, ‘Granny Smith’, ‘Ginger Gold’, ‘Jonagold’, ‘Empire’, ‘Law Rome’ | 0.6–2 μl $l^{-1}$ | 0, 5, 10, 15, 20–25 | 7–20 h | Maintained firmness, reduced scald, maintained titratable acidity, reduced volatiles, slowed loss of chlorophyll and starch, inhibited ethylene, reduced respiration and decay, prevented/delayed disorders such as scald | [29-36] [37-41] |
| *Mangifera indica*(mango) | 25; 100 μl $l^{-1}$ | 20 | 14 h | Increased shelf life, increased stem rots | [5, 42] |
| *Musa* sp., AAA group, Cavendish subgroup (banana) | 5–500 nl $l^{-1}$; 0.1 μl $l^{-1}$, 45 | 20–24 | 6–24 h | Delayed ripening and peel color change | [15, 43-48] |
| *Nicotiana attenuata*(tobacco) plants | 0.04–0.43 g of 0.43% EthylBloc in 27×40×22.5 cm chamber; 500 mg in a 18.5 L chamber | Growing temperature | 6 h | Prevented ethylene response to injury and nicotine response allowing insects to feed on plant | [48, 49] |
| *Persea americana*(avocado) | 50–300 nl $l^{-1}$; 0.45, 25 μl $l^{-1}$ | 3, 5, 20, 22 | 6–48 h | Inhibited ripening, maintained firmness, reduced cell wall degrading enzymes, delayed and reduced respiration, ethylene production and weight loss; retained green peel color, reduced mesocarp discoloration and decay development | [5, 50] |
| *Pisum sativa*seedlings (pea) | 40 nl $l^{-1}$ | ? | 24 h | Inhibited growth | [2, 15] |
| *Prunus armeniaca*(apricot) | 1 μl $l^{-1}$ | 20 | 4, 20 h | Maintained firmness and titratable acidity, decreased internal flesh browning and decay, inhibited ethylene production in some conditions, reduced respiration, delayed volatile production | [51, 52] |

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| *P. persica* (peach and nectarine) | 20 nl $l^{-1}$; 0.1 µl $l^{-1}$; 0.5 ml $l^{-1}$ | 20–24 | 4, 18, 24 h | Maintained firmness and acidity; delayed ethylene production and reduced respiration; increased internal browning; reduced juice, ethylene associated enzymes and cell wall degrading enzymes | [53-55] |
| *P. salicina* (plum) | 1, 13, 26 or 39 µl $l^{-1}$ | 20 | 6, 20, 24 h | Reduced ethylene and respiration, maintained firmness, slowed color change, decreased internal flesh browning | [51, 56, 57] |
| *Pyrus communis*(pear) | 2, 4 µl $l^{-1}$ | 2 | 16 h | Reduced chilling induced ethylene, slowed softening | [30, 58] |
| *Vigna radiata* leaves (mung bean) | 60 nl $l^{-1}$ | Ambient | 24 h | Inhibited abscission | [23] |
| Summary of 1-MCP treatment conditions and effects on floricultural crops | | | | | |
| Plant | Concentration | Treatment temperature (°C) | Treatment time (h) | Effects | Reference |
| *Alstroemeria spp.* | 20 nl $l^{-1}$ | 20 | 6 | Prevented decrease in vase life due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *Antirrhinum majus* | 20 nl $l^{-1}$ | 20 | 6 | Prevented decrease in vase life due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *Begonia×elatior*‘Najada’ and ‘Rosa’ | 5 or 20 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (1.0 µl $l^{-1}$) abscission of buds, flowers, and leaves, and senescence and increased postharvest life in a non-ethylene environment | [47, 60] |
| *Begonia×tuberhybrida* ‘Non-Stop’ | 5 or 20 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (1.0 µl $l^{-1}$) abscission of buds, flowers, and leaves, and senescence and increased postharvest life in a non-ethylene environment | [47, 60] |

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| *Boronia heterophylla* | 10 nl $l^{-1}$ | 20 | 12 | Prevented exogenous ethylene-induced (10 µl $l^{-1}$) fresh weight loss and flower abscission | [61] |
| *Campanula carpatica*'Dark Blue' | 20, 50 or 100 nl $l^{-1}$ | 20 | 6 | Increased individual flower longevity in the presence of exogenous (3 µl $l^{-1}$) ethylene and in a non-ethylene environment | [23] |
| *C. carpatica*'Dark Blue' and 'Blue Clips' | 20, 50 or 100 nl $l^{-1}$ | 21 | 6 | In the presence of exogenous ethylene (0.5 µl $l^{-1}$), increased display life and flower longevity. Without exogenous ethylene, 1-MCP had no effect on *Campanula* 'Dark Blue' or on display life of *Campanula*'Blue Clips' but did increase flower longevity of *Campanula* 'Blue Clips'. STS increased plant display life and flower longevity greater than 1-MCP for both species regardless of the presence of ethylene | [62] |
| *C. medium*'Champion Pink' | 800 nl $l^{-1}$ | 22 | 4 | Increased vase life in a non-ethylene environment but only in combination with 5% sucrose | [63] |
| *Cassinia adunca* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |
| *Ceratopetalum gummiferum* | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vaselife and increased vaselife without the presence of exogenous ethylene | [64] |
| *Chamelaucium uncinatum* | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of exogenous ethylene (10 µl $l^{-1}$) | [64] |

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| | | | | application including flower abscission and reduced vase life but did not affect normal senescence in a non-ethylene environment | |
| *C. uncinatum* | 200 nl $l^{-1}$ | 21 | 6 or 13 | Prevented the effects of exogenous ethylene (2 µl $l^{-1}$) application including bud and flower abscission. Partially prevented bud, flower, and leaf abscission from dehydration and storage | [65] |
| *Consolido ambigua* | 20 nl $l^{-1}$ | 20 | 6 | Prevented vase life decrease due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *Dendranthema grandiflorum*'Coral Charm' | 200 nl $l^{-1}$ | 20 | 6 | Reduced rooting of both stored and unstored unrooted cuttings | [66] |
| *Dianthus barbatus* | 20 nl $l^{-1}$ | 20 | 6 | Prevented vase life decrease due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *D. caryophyllus*'Sandra' | 0.6, 1.7, 3.3, 5.8 or 20 nl $l^{-1}$ | 20 | 6 | Prevented exogenous ethylene (0.4 µl $l^{-1}$) induced senescence and increase vase life even without exogenous ethylene application | [47, 59] |
| *D. caryophyllus*'White Sim' or 'Sandra' | 5 nl $l^{-1}$ | Not specified, probably 'room temperature' | 12 | Prevented exogenous ethylene (1.0 µl $l^{-1}$) induced senescence and increase vase life even without exogenous ethylene application. Older flowers required higher concentrations to prevent damage | [23] |
| *Epipremnum pinnatum* | 200 nl $l^{-1}$ | 20 | 6 | Decreased leaf yellowing and drop of cuttings after storage of cuttings at 23 °C for 4 days | [67] |
| *Eriostemon scabe* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |

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| *Grevillea* 'Kay Williams' | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vase life but did not affect normal senescence in a non-ethylene environment | [64] |
| *Grevillea*'Misty Pink' | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vase life but did not affect normal senescence in a non-ethylene environment | [64] |
| *Gypsophila paniculata*'Perfecta', 'Gilboa', and 'Golan' | 200 nl $l^{-1}$ | 20 | 24 | Delayed senescence of open flowers, but not floral buds, exposed to ethylene (0.7 µl $l^{-1}$) while STS delayed senescence of both open flowers and unopen buds | [68] |
| *Hibiscus rosa-sinensis*numerous cultivars | 200 nl $l^{-1}$ | 20 | 6 | Had no effect on open flowers unless flowers were held in 1-MCP continuously in a non-ethylene environment | [69] |
| *H. rosa-sinensis* | 200 nl $l^{-1}$ | 20 | 6 | Retarded storage-induced leaf yellowing of unrooted cuttings but reduced subsequent rooting | [66] |
| *Ixora coccinea*'Big Red' | 100 nl $l^{-1}$ | 20 | 8 | Blocked chilling induced leaf abscission in both an exogenous ethylene (5 µl $l^{-1}$) and a non-ethylene environment | [70] |
| *Kalanchoe blossfeldiana*'Tropicana' | 5 or 20 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (1.0 µl $l^{-1}$) abscission of buds, flowers, and leaves, and senescence and increased postharvest life in a non-ethylene environment | [47, 60] |
| *Kalanchoe*'Alexandria | 200 nl $l^{-1}$ | 20 | 6 | Had no effect on postharvest | [71] |

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| ', 'Debbie', 'Caroline', 'Jaqueline', 'Nadia', 'Pale Jaqueline', and 'Simone' | | | | life | |
| *K. blossfeldiana* | 0.5, 2.5, 5 or 10 nl $l^{-1}$ | 20 | 6 | Increased post production life in the presence of exogenous (3 µl $l^{-1}$) ethylene | [15] |
| *Leptospermum petersonii* | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vase life but did not affect normal senescence in a non-ethylene environment | [64] |
| *L. scoparium* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |
| *Lilium* 'Mona Lisa' | 500 nl $l^{-1}$ | 25 | 18 | Prevented effects of exogenous ethylene (2 or 5 µl $l^{-1}$) application including rapid bud and flower abscission but did not effect normal senescence in a non-ethylene environment | [72] |
| *Lilium*'Stargazer' | 500 nl $l^{-1}$ | 25 | 18 | Prevented effects of exogenous ethylene (2 or 5 µl $l^{-1}$) application including rapid bud and flower abscission but did not effect normal senescence in a non-ethylene environment | [72] |
| *Lilium* hybrids 'Cordelia' and 'Elite' (Asiatic types) | 150 nl $l^{-1}$ | 20 | 6 | Prevented exogenous ethylene (10 µl $l^{-1}$) induced vase life decline but had no effect on vase life, days to bud opening, or percentage of buds opening in a non-ethylene environment | [73] |
| *L. longiflorum*'Lorena' | 150 nl $l^{-1}$ | 20 | 6 | Prevented exogenous ethylene (10 µl $l^{-1}$) induced | [73] |

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| | | | | vase life decline but had no effect on vase life, days to bud opening, or percentage of buds opening in a non-ethylene environment | |
| *Lupinus havardii*‘Texas Sapphire’ | 450 nl $l^{-1}$ | 15 | 12 | Reduced fresh weight loss and reduced flower abscission | [74] |
| *Matthida incana* | 20 nl $l^{-1}$ | 20 | 6 | Prevented vase life decrease due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *M. incana* | 500 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (1 µl $l^{-1}$) abscission of flowers and increased fresh weight and vase life in a non-ethylene environment. STS induced longer vase life and greater fresh weight retention than 1-MCP | [75] |
| *Metrosideros collina* | 0, 1.5, 15, or 150 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (0.1 µl $l^{-1}$) abscission of stamens by 1–2 days. STS delayed stamen abscission by 4 or more days | [76] |
| *Ozothamnus diosmifolius* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |
| *Pelargonium×hortorum* | 0.1 or 1.0 µl $l^{-1}$ | 20 | 3, 6, 12, or 24 | Delayed exogenous ethylene-induced (1.0 µl $l^{-1}$) petal abscission and prevented petal abscission in a non-ethylene environment | [77] |
| *P. zonale*‘Isabel’ | 200 nl $l^{-1}$ | 20 | 6 | Retarded storage-induced leaf yellowing and did not effect rooting of both stored and unstored unrooted cuttings | [66] |
| *P. peltatum*‘Pink Blizzard’ | 1 µl $l^{-1}$ | 22 | 2 | Delayed exogenous ethylene-induced (1.5 µl $l^{-1}$) abscission of petals but the | [78] |

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| | | | | effect diminished and the half-life of the treatment was 2, 3 and 6 days at 25, 20.7 and 12 °C, respectively | |
| *Penstemon*‘Firebird’ | 20 nl $l^{-1}$ | 20 | 6 | Prevented vase life decrease due to exogenous ethylene (1 µl $l^{-1}$) | [59] |
| *Petunia hybrida* ‘Pink Cascade’ | 150 nl $l^{-1}$ | 22 | 6 | Prevented an exogenous ethylene-induced (1–12 µl $l^{-1}$) increase in electrolytic leakage, a decrease of membrane proteins, and a decrease in lipid fluidity. In a non-ethylene environment increased individual flower longevity, fresh weight, and total protein content compared with untreated controls but had no effect on electrolytic leakage, membrane proteins, or lipid fluidity | [79] |
| *Phalaenopsis*‘Herbert Hager’ | 250 nl $l^{-1}$ | 22 | 6 | Prevented the pollination-induced increase in ethylene production and the enhanced senescence of the flowers | [80] |
| *Phlox paniculata*‘Rembrandt’ | 25, 250, or 500 nl $l^{-1}$ | 22±1 | 6 | Prevented exogenous ethylene (0.3, 1 or 3 µl $l^{-1}$) induced flower abscission and vase life decline but had no effect on abscission, number of open flowers, or vase life in a non-ethylene environment | [80] |
| *Platysace lanceolata* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |
| *Rosa* ‘Royal’ and ‘Sunset’ | 100 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (0.5 µl $l^{-1}$) | [81] |

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|---|---|---|---|---|---|
| | | | | abscission of leaves, buds, and flowers, but did not affect leaf yellowing of plants placed in dark | |
| *Rosa* 'Vanilla' and 'Bronze' | 200 nl $l^{-1}$ | 20 | 6 | Delayed but did not prevent ABA (0.1 mM) induced flower senescence in 'Bronze' and had no effect on flower abscission in 'Vanilla'. Did not have any effect on ABA induced leaf abscission | [82] |
| *R. hybrida*'Victory Parade' | 5 or 20 nl $l^{-1}$ | 20 | 6 | Delayed exogenous ethylene-induced (1.0 µl $l^{-1}$) abscission of buds, flowers, and leaves, and senescence and increased postharvest life in a non-ethylene environment | [47, 83] |
| *Schlumbergera truncata* 'Dark Marie' | 20, 50 or 100 nl $l^{-1}$ | 21 | 6 | Increased display life, with or without the presence of exogenous ethylene (0.5 µl $l^{-1}$), of *Schlumbergera*; STS increased plant display life and flower longevity greater than 1-MCP regardless of the presence of ethylene | [62] |
| *Telopea*'Shady Lady' | 10 nl $l^{-1}$ | 20 | 12 | Did not prevent the effects of exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vase life and did not affect normal senescence in a non-ethylene environment | [64] |
| *Thryptomene calycina* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |
| *Tulipa gesneriana*'Apeldoorn' | 1 nl $l^{-1}$ | 20 | 16 | Prevented exogenous ethylene (0.3 Pa) induced gummosis of stored bulbs | [84] |
| *Verticordia nitens* | 10 nl $l^{-1}$ | 20 | 12 | Prevented effects of | [64] |

| | | | | | |
|---|---|---|---|---|---|
| | | | | exogenous ethylene (10 µl $l^{-1}$) application including flower abscission and reduced vase life but did not affect normal senescence in a non-ethylene environment | |
| *Zieria cytisoides* | 10 nl $l^{-1}$ | 20 | 12 | Neither 1-MCP or exogenous ethylene (10 µl $l^{-1}$) application affected senescence | [64] |

Table 2: Climacteric and non-climacteric fruit and vegetables for which responses to 1-MCP have been investigated from references available (Adopted from [85])

| Fruit (climacteric) | | Fruit (non-climacteric) | Vegetables |
|---|---|---|---|
| Apple [*Malus sylvestris* (L) Mill. var.*domestica* (Borkh.) Mansf.] | Melon (*Cucumis melo*L.) | Cherry (*Prunus avium* L.) | Broccoli (*Brassica oleracea*L.) |
| Apricot (*Prunus armeniaca* L.) | Mountain papaya (*Vasconcellea pubescens*) | Clementine mandarin (*Citrus reticulata* L.) | Carrot (*Daucus carota* L.) |
| Avocado (*Persea americana* Mill.) | Nectarine (*Prunus persica* Lindl.) | Cucumber (*Cucumis sativus* L.) | Chinese cabbage (*Brassica campestris* L. spp. *pekinensis*(Lour) Olsson) |
| Banana (*Musa* L.) | Papaya (*Carica papaya* L.) | Grape (*Vitis vinifera*L.) | Chinese mustard (*Brassica juncea* var. *foliosa*) |
| Blueberry, highbush (*Vaccinium corymbosum* L.) | Peach (*Prunus persica* L. Batsch) | Grapefuit (*Citrus paradisi* Macf.) | Choysum (*Brassica rapa* var.*parachinensis*) |
| Chinese bayberry (*Myrica rubra*Siebold and Zuccarni) | Pear (*Pyrus communis* L.) | Lime (*Citrus latifolia*Tanaka) | Chrysanthemum, garland (*Chrysanthemum coronarium*) |
| Chinese jujube (*Zizyphus jujube* M.) | Pear (*Pyrus pyrifolia*Nakai) | Orange (*Citrus sinensis* L. Osbeck) | Coriander (*Coriandrum sativum* L.) |

| Fruit (climacteric) | | Fruit (non-climacteric) | Vegetables |
|---|---|---|---|
| Custard apple (*Annona squamosa*L.) | Persimmon (*Diospyros khaki* L.) | Pepper (*Capsicum frutescens* L.) | Lettuce (*Lactuca sativa* L.) |
| Figs (*Ficus carica* L.) | Plum (*Prunus salicina*L.; *Prunus* x*domestica* L.) | Pineapple (*Ananas comosus* L.) | Mibuna (*Brassica rapa* var.*nipposinica*) |
| Guava (*Psidium guajava* L.) | Tomato (*Solanum esculentum* Mill) | Strawberry (*Fragaria* × *ananassa*Duch.) | Mizuna (*Brassica rapa* var.*nipposinica*) |
| Kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson var. *deliciosa* | | Watermelon (*Citrullus lanatus*) | Pak choy (*Brassica rapa*) |
| Lychee (*Litchi chinensis*) | | | Parsley (*Petroselinum crispum*Mill.) |
| Mamey sapote (*Pouteria sapote*(Jacq.) H.E. Moore and Stearn) | | | Potato (*Solanum tuberosum*) |
| Mango (*Mangifera indica* L.) | | | Tatsoi (*Brassica rapa* var.*rosularis*) |

Table 3: Generalizations regarding the effects of 1-MCP on metabolism of fruit and vegetables (Adopted from [85])

| | Attribute or process affected | Enzyme activity or associated gene expression | Increased (↑), decreased (↓), or unchanged (↔) |
|---|---|---|---|
| Ethylene metabolism | | | |
| | Ethylene perception | ETR1, ERS1 | ↓↔ |
| | Ethylene production | | ↓↑ |
| | | ACC synthase (ACS) expression and activity | ↓ |
| | | ACC oxidase (ACO) expression and activity | ↓ |
| Respiratory metabolism | | | |
| | Respiration rate | | ↓↑↔ |
| | SSC | | ↓↑↔ |

| | Attribute or process affected | Enzyme activity or associated gene expression | Increased (↑), decreased (↓), or unchanged (↔) |
|---|---|---|---|
| | TA | | ↓↑↔ |
| Pigments | Chlorophyll degradation | | ↓ |
| | Lycopene accumulation | | ↓ |
| | Anthocyanin accumulation | | ↓ |
| | | Chlorophyllase activity | ↓ |
| Phenolic metabolism | | | |
| | Total phenolic content | | ↓ |
| | | Phenylalanine ammonia lyase (PAL) activity | ↓ |
| | | Polyphenol oxidase (PPO) activity | ↓ |
| Cell wall metabolism | | | |
| | Soluble polyuronide content | | ↓ |
| | | Polygalacturonase (PG) activity | ↓ |
| | | Pectin methylesterase (PME) | ↓ |
| | | Endo-β-1,4-glucanase (EGase) | ↓ |
| | | Glycosidases | ↓↔ |
| Volatile compound metabolism | | | |
| | Esters | | ↓ |
| | Aldehydes | | ↔ |
| | Terpenoid biosynthesis | | ↔ |
| | Acetaldehyde and ethanol accumulation | | ↓↑ |
| | | Alcohol acyl transferase activity | ↓ |
| | | Alcohol dehydrogenase activity | ↔ |
| Nutritional | Vitamin C loss | | ↓ |
| | Anthocyanin contents | | ↓ |
| | Phenolic contents | | ↓↔ |
| | Antioxidant activity loss | | ↓ |

| | Attribute or process affected | Enzyme activity or associated gene expression | Increased (↑), decreased (↓), or unchanged (↔) |
|---|---|---|---|
| Physiological disorders | | | |
| | Senescent disorders | | ↓ |
| | Chilling injury | | ↓↑ |
| | Superficial scald (apples and pears) | | ↓ |
| | Ethylene-induced disorders | | ↓ |
| | Controlled atmosphere-induced | | ↑ |
| | Abscission | | ↓ |
| Pathological disorders | | | |
| | Susceptibility to pathogens | | ↑↓↔ |
| | Fungal growth | | ↓↔ |

### 2.1.3. Commercialization of 1-MCP

#### 2.1.3.1. Crops allowed for 1-MCP application

The table below summarizes the commercialization of 1-MCP on different crops in different countries (adopted from [86]). As shown in the table, 1-MCP is more acceptable in North America and South America, while in Europe and Asia, the application is still limited. The most popular crop for 1-MCP application is apple.

Table 4: Crops allowed for commercial application of 1-MCP

| Country | Crop |
|---|---|
| Argentina | Apple, pear, plum, tomato |
| Australia | Apple, avocado, melon, tomato and pear |
| Austria | Apple |
| Belgium | Apple |
| Brazil | Apple, avocado, bananas, guava, mango, melon, papaya, tomato |
| Canada | Apple, pear, plum, tomato |
| Chile | Apple, avocado, banana, cherimoya, guava, mango, pear, plum, tomato |
| China | apple, kiwifruit, persimmon |
| Costa Rica, Guatemala and Honduras | Avocado, banana, mango, melon, papaya, pineapple, plantain, tomato |

| | |
|---|---|
| Israel | Apple, avodado, persimmon |
| France | Apple, plum |
| Germany | Apple |
| Israel | Apple, avocado, persimmon |
| Korea | Apple |
| Mexico | Apple, avocado, cucumbers, dates, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, plum, squash, tomato |
| New Zealand | Apple, avocado, kiwifruit, melon, persimmon, tomato |
| Nicaragua | Avocado, banana, mango, melon, papaya, pineapple, plantain, tomato |
| South Africa | Apple, avocado, kiwifruit, plum, tomato |
| Switzerland | Apple |
| The Netherlands | Apple |
| UK | Apple |
| USA | Apple, apricot, avocado, banana, broccoli, kiwifruit, mango, melon, nectarine, papaya, peach, pear, persimmon, plum, tomato |

2.1.3.2. Commercialized powder 1-MCP delivery system

There are two commercialized powder form 1-MCP delivery system, EthylBloc® (0.14% active ingredient) from Floralife and SmartFresh® (3.3% active ingredient) from Agrofresh [3]. Both of them are formulated with α-cyclodextrin, a water soluble compound. The activation of these delivery systems requires moisture as a trigger because α-cyclodextrin is water soluble.

The information below summarizes the technical details of SmartFresh®[87]. The process comprises of generation and purification of 1-MCP, and encapsulation of 1-MCP into α-cyclodextrin.

*Generation and purification of 1-MCP*

This step includes two steps

(1) Generating 1-MCP from two streams of reactants (one stream is an allyl compound and the other stream is a non-nucleophilic strong base) in the first vessel.

Table 5: Condition in 1-MCP reaction vessel

| | |
|---|---|
| Temperature | Room temperature |
| Gas pressure | 1 atm |
| Other reaction condition | Agitated with an inert gas |

(2) Purifying 1-MCP by first passing it through a condenser held at a temperature less than the boiling point of the allyl compound and greater than the boiling point of the 1-MCP, and second passing it through scrubbers.

Table 6: Purification condition of 1-MCP

| | |
|---|---|
| Temperature of condenser | 15°C |
| First gas scrubber | Water (to remove water soluble impurities) |
| Second gas scrubber | 50% water, 30% iso-propanol, 10% ethanolamine, and 10% 2-mercaptoethanol |

*Encapsulation of 1-MCP into α-cyclodextrin*

The encapsulation occurs in a second vessel containing water which is continuously fed into the vessel. Upon formation of 1-MCP/ α-cyclodextrin complex, it precipitates out from the solution. After separating the precipitates from the solution, the complex needs to be dried as quickly as possible to ensure high 1-MCP loading. Depending on the drying method, inclusion ratio of 1-MCP can be in the range between 0.1% to 5%. A continuous belt filter was recommended by the author because it is able to produce 1-MCP/ α-cyclodextrin complex with inclusion ratio of 2-5%.

2.1.3.3. Commercialized liquid 1-MCP delivery system

There are three commercial liquid 1-MCP delivery systems, Harvista$^{TM}$ [88], Invinsa[89], and Lupo Fresh [90]. Harvista is used for field application of apples, it can reduce early fruits drop, maintain fruit firmness on the tree, allows time for additional fruit growth and color development, and eases labor and scheduling of harvest crews[89]. Invinsa is used for agronomic crops such as soybean. Benefits of Invinsa include improvement of flower and pod retention and delay of premature senescence due to stress, and improvement of

yield in soybean; improvement flower and boll retention, improvement of fiber yield and quality in cotton; and improvement of stress tolerance and kernel weight and number in wheat[89]. Harvesta and Invinsa are brands of AgroFresh. Lupo Fresh is a brand of Vankortech and is compressed with $N_2$. There is limited technical information regarding the formulation.

## 2.2. Cyclodextrin

### 2.2.1. Structure of cyclodextrins

Cyclodextrins are cyclic oligosaccharides consisting of six or more α-D-glucopyranose units that are linked through (α-1,4)-glycosidic bonds. The chair conformations of the individual glucose unit in the ring give cyclodextrins their conical toroidal shape with the primary hydroxyl functions of the individual sugar molecule extending from the narrow end of the torus and the secondary hydroxyl groups from the wider end away from the internal cavity into the cone exterior as depicted in the figure below. The internal cavity of the cyclodextrin torus is composed of the skeletal carbons and the ether-linked oxygens of the α-1,4-linked D-glucopyranose units giving the cyclodextrin internal cavity its lipophilic character. Some of the chemical and physical properties of α- and β-cyclodextrins are listed in the Table below.

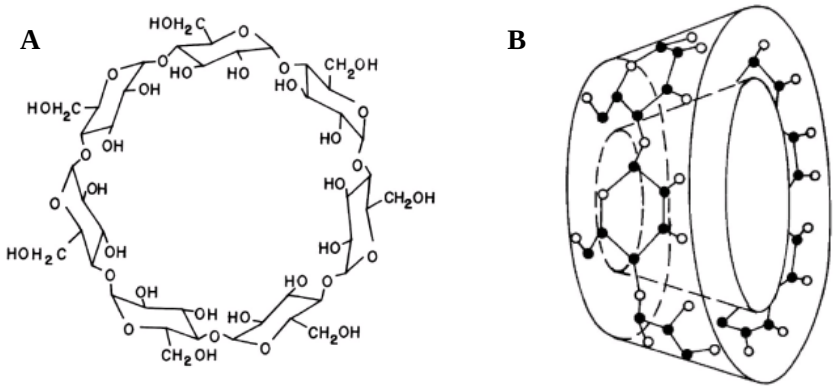


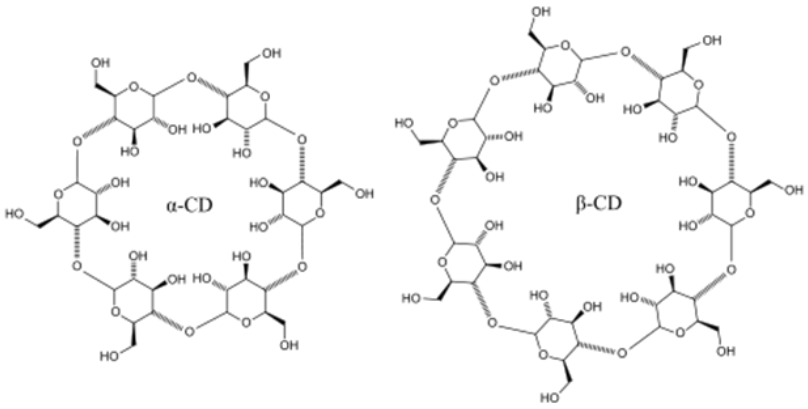

Figure 3: Chemical structures (A) and Conical Toroidal representation (B) of β-Cycloodextrin

Table 7: Some chemical and physical properties of α- and β-cyclodextrins[91, 92]

| | α-Cyclodextrin | β-Cyclodextrin |
|---|---|---|
| Number of Glucopyranose Units | 6 | 7 |
| Molecular Weight | 972 | 1135 |
| Torus Height (Å) | 7.9 | 7.9 |
| Torus Outer Diameter (Å) | 13.7 | 15.3 |
| Internal Cavity Diameter (Å); (Inner rim / Outer rim) | 4.5 / 5.7 | 6.2 / 7.8 |
| Internal Cavity Volume ($Å^3$) | 174 | 262 |
| Hydrate ($x.H_2O$); (Cavity / External) | 2.0 / 4.4 | 6.0 / 3.6 |
| Water Solubility at 25 °C (g/100 mL) | 14.5 | 1.85 |

The structural differences between α-cyclodextrin and β-cyclodextrin are illustrated in the figure below.

A




B

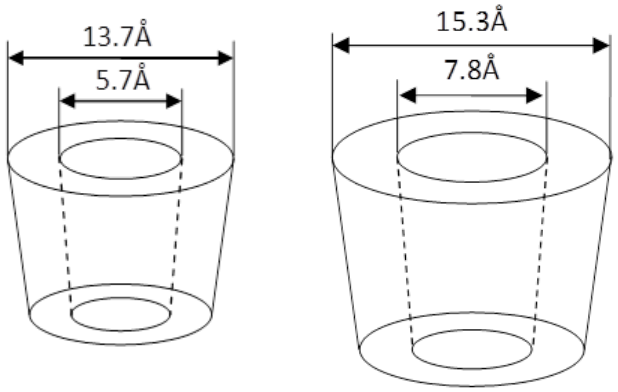



Figure 4: Chemical structures of cyclodextrins (A) and their spatial arrangements (B)

### 2.2.2. Encapsulation using cyclodextrin

This section provides general background information of encapsulation using cyclodextrin. Some names used in literature for encapsulation are adduct, clathrate, molecular compound, cryptate and complex. Considering that no covalent bond is established between the host and guest molecules, the dissociation-association equilibrium in solution is one of the most characteristic features of the host-guest association.

Cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity [citation: book pp80]. Geometrical rather

than chemical factors are decisive in determining the kind of guest molecules which can penetrate into the cyclodextrin cavity.

The structures of cyclodextrin inclusion complexes differ significantly in the crystalline state and in solution. In solution the guest molecule resides in the cavity, and the whole complex is surrounded by a solvate shell of water molecules. In the crystalline state, the guest molecules can be accommodated not only in the cavity of the molecule, but also in the intermolecular cavities formed by the crystal lattice, or sandwich-like between two complex molecules. Some of the cyclodextrin molecules remain unoccupied or they include water. The included molecules are normally oriented in the host in such a position as to achieve the maximum contact between the hydrophobic part of the guest and the apolar cyclodextrin cavity. The hydrophilic part of the guest molecule remains, as far as possible, at the outer face of the complex. This ensures maximum contact with both the solvent and the hydroxyl groups of the host.

Complex formation with molecules significantly larger than the cavity may also be possible. This is done in such a way that only certain groups or side chains penetrate into the carbohydrate cavity.

The extent of the complex formation also depends on the polarity of the guest molecule. Strongly hydrophilic molecules are not, but hydrophobic molecules can form complex in cyclodextrins. Solubility of cyclodextrin: $\gamma > \alpha > \beta$. $\beta$ has the strongest H-bond.

The cavities of cyclodextrins crystallized from water are not empty, but filled with water molecules. Some are included into the cyclodextrin cavity, others are integral parts of the

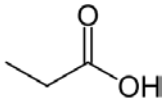
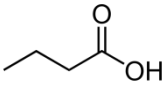
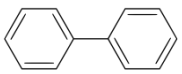
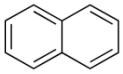
crystal structure (crystal water). The cyclodextrin-inclusion complexes are formed by substitution of included water by the appropriate guest molecule.

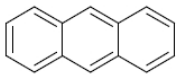
In the following sections, factors influencing cyclodextrin encapsulation are reviewed and discussed.

2.2.2.1. Geometric compatibility

Compounds with compatible size are able to be encapsulated into cyclodextrin cavity. Geometrical factors are more decisive than chemical factors in determining the encapsulation of guest molecules (cyclodetrin technology pp80-84). Since α, β and γ have different cavity sizes, their abilities to complex molecules vary. For example, naphthalene is too bulky to be encapsulated into α-cyclodextrin, but can fit in β and γ-cyclodextrin. The table below lists the complex forming ability of α, β and γ-cyclodextrin with different molecules.

Table 8: Encapsulation compatibility between different chemicals with cyclodextrins

| | Chemical structure | Molecular weight | α-CD | β-CD | γ-CD |
|---|---|---|---|---|---|
| Propionic acid ($C_3H_6O_2$) |  | 74.08 | + | - | - |
| Butyric acid |  | 88.11 | + | + | - |
| Biphenyl |  | 154.21 | + | + | + |
| Cyclohexane |  | 84.16 | + | + | + |
| Naphthalene |  | 128.17 | - | + | + |

| | | | | | |
|---|---|---|---|---|---|
| Anthracene |  | 178.23 | - | - | + |
| $Cl_2$ | | 34 | + | - | - |
| $Br_2$ | | 70 | + | + | - |
| $I_2$ | | 106 | + | + | + |

2.2.2.2. Cyclodextrin complex formation in solid and in solution

The formation of cyclodextrin complex differs significantly according to whether it is processed in solid or in solution. In solution, the guest molecule is encapsulated into the cavity, while in solid form, the guest molecule is encapsulated both in the cavity and intermolecular cavity. The reason behind it is that in solution, the intermolecular space is large and occupied by solution, so it is not available to guest molecule.

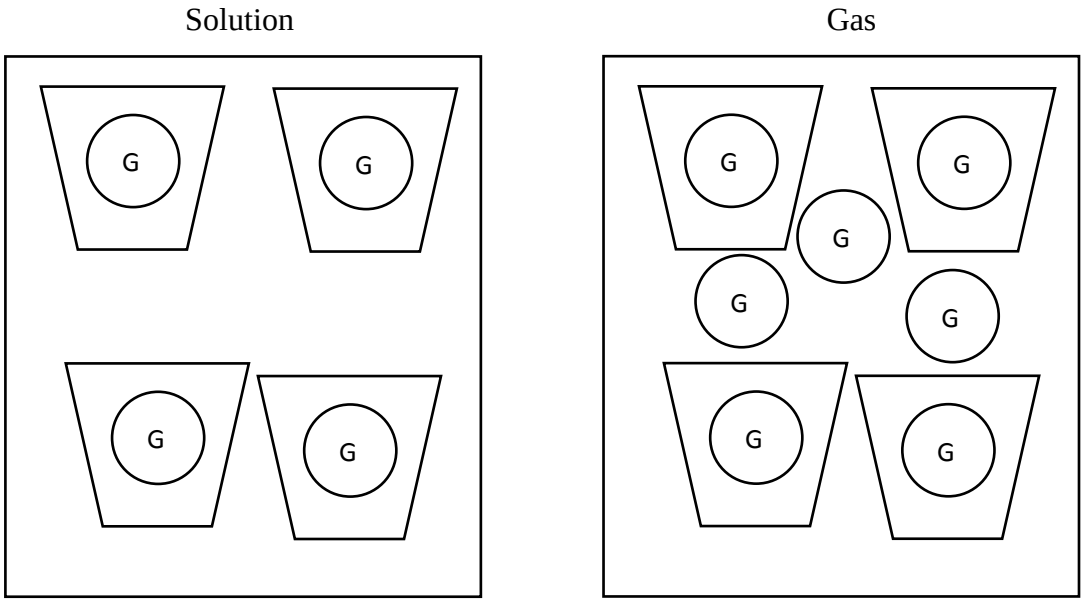


Figure 5: Difference between encapsulation in solution and in solid

2.2.2.3. Hydrophobicity of guest molecule

The inner cavity of cyclodextrin is hydrophobic, therefore the hydrophobic part of a guest molecule resides in the cavity of cyclodextrin and the hydrophilic part is at the intermolecular cavity. Hydrophobicity of the guest molecule also determines the stability of the formed complex—the more hydrophobic the guest molecule is, the more stable the complex is.

2.2.2.4. Medium for encapsulation in solution

Medium are not necessary for cyclodextrin complex formation. Formulating cyclodextrin with guest molecule along is able to form the complex. Complexation of solid phase guest molecule has to be in solution because the process is extremely slow without medium. In solution, the complexation is fast, and the degree of complexation depends on the hydrophobicity of the guest molecule as mentioned in 2.6.3. In some situations, the solution cannot be removed, and thus a cyclodextrin-solution-guest complex is formed. For example, ethanol is often used to dissolve cyclodextrin, but it bounds into the cavity of cyclodextrin with the guest molecule. Formation of the cyclodextrin complex using DMF is not recommended for the preparation of cyclodextrin complexes (CD technology book pp85).

2.2.3. The mechanism of 1-MCP inclusion in α-cyclodextrin in solution

The mechanism of 1-MCP inclusion in α-cyclodextrin in water is essentially the

replacement of included water molecule in the α-cyclodextrin cavity by 1-MCP molecule because 1-MCP is more hydrophobic than water and therefore thermodynamically it is more favorable to reside in the hydrophobic cavity of α-cyclodextrin.

The replacement occurs following the steps of

- Water molecule escapes from the α-cyclodextrin cavity and its energy level shifts as it is in gaseous state. Hydrogen bonding and van der Waals interactions decrease as a consequence
- The replaced water becomes an integral part of the hydrated α-cyclodextrin. The α-cyclodextrin structure relaxes and the cavity opens to be available for 1-MCP
- 1-MCP molecules enter the empty α-cyclodextrin cavity and the inclusion complex rearranges and stabilized by van der Waals interactions

## 2.3. Properties of different hydrocolloidal solutions

### 2.3.1. Xanthan gum

#### 2.3.1.1. Chemical structure

Xanthan gum is a microbial generated heteropolysaccharide whose primary structure consists of pentasaccharide units that formed by two glucose, two mannose, and one glucuronic acid (paper). The chemical structure of the backbone of xanthan consists of β-D-glucose units linked at the 1 and 4 positions and is identical to that of cellulose, and a trisaccharide side chain links at the O-3 position of every other glucose unit in the

backbone (shown figure below).

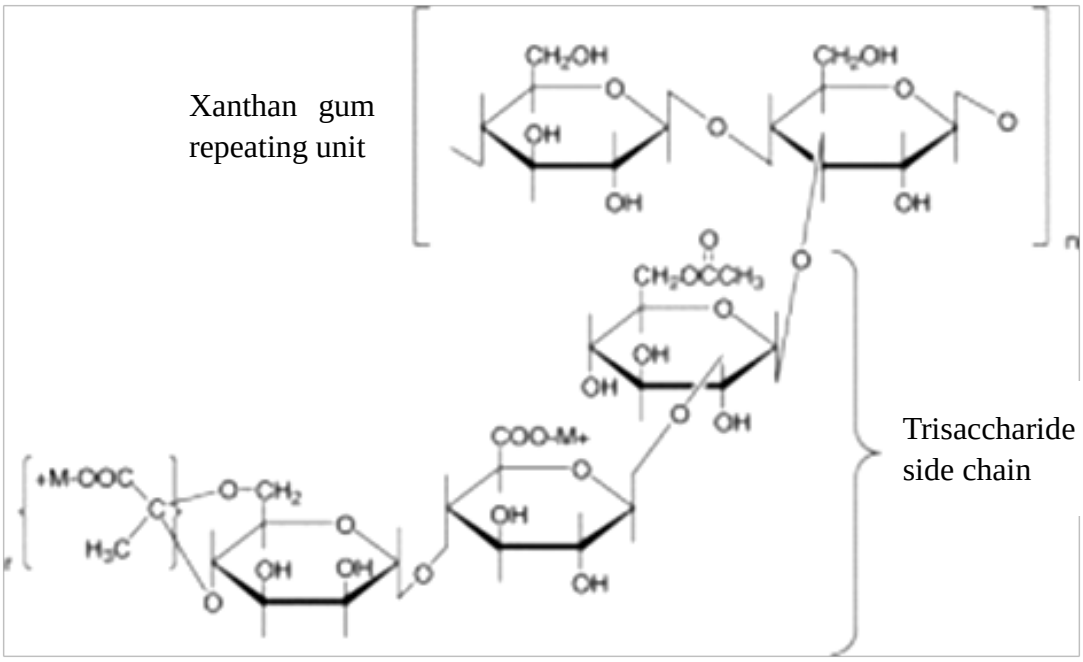



Figure 6: Chemical structure of xanthan gum

2.3.1.2. Application

Xanthan gum can dissolve in water at various temperatures. Solution of xanthan gum is quite viscous at low temperature. Depending on different temperatures, xanthan molecule can have two conformations, i.e. helix and random coil. The solubility property of xanthan is due to its polyelectrolyte nature, and provides different industries a good candidate as a thickener and stabilizer of suspensions and emulsions.

Xanthan gum solution is non-Newtonian fluid whose viscosity decreases as shear rate increases. This property is suitable to develop sprayable solution because shear force is applied during spraying. An initial yield stress is needed to be overcome for xanthan gum solution to flow.

Its applications involve emulsion stabilization, temperature stability, compatibility with food ingredients, and its pseudoplastic rheological properties. In pharmaceutical and cosmetics products (similar to our application in this dissertation), it is used as dispersing agent, and stabilizer of emulsions and suspensions.

### 2.3.2. Cellulose based materials

#### 2.3.2.1. Chemical structure of carboxymethylcellulose (CMC)

CMC is cellulose ether, obtained by substituting hydroxyl groups of cellulose (Figure below). Based on number of hydroxyl groups been substituted (degree of substitution, or DS), the property of CMC varies. Because CMC has a long chain with negative charges given by the carboxyl groups leading to electrostatic repulsion in solution, CMC solution is viscous and stable.

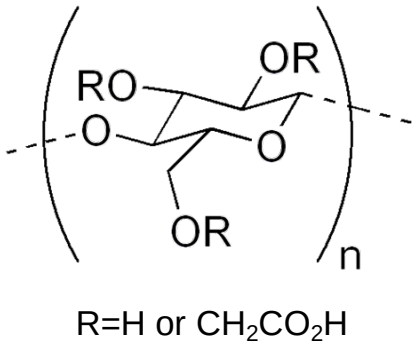



Figure 7: Chemical structure of CMC

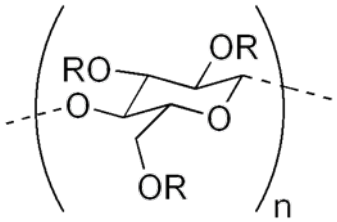
#### 2.3.2.2. Application of caroxymethylcellulose

CMC acts as a thickener, binder, stabilizer, protective colloid, suspending agent, and rheology control agent. It also dissolves in both cold and hot water. The rheology

property of CMC can be pseudoplastic or thixotropic, both of which are shear-thinning that is suitable for developing sprayable solution as high shear force is applied when spraying.

2.3.2.3. Chemical structure of hydroxypropylmethylcellulose (HPMC)

HPMCs are made by reacting alkali cellulose with propylene oxide and methyl chloride, and are cold water soluble because the methyl and hydroxypropyl ether group prevents the association of cellulose units.




R=H or $CH_3$ or $CH_2CH(OH)CH_3$

Figure 8: Chemical structure of HPMC

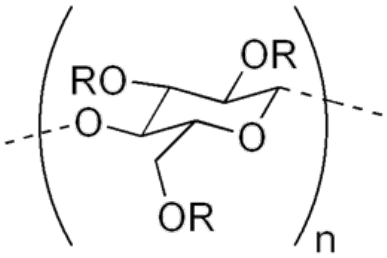
2.3.2.4. Application of hydroxypropylmethylcellulose

Because HPMCs are surface active due to their ether groups, they often used as stabilizer for emulsions and foams. When heat is applied, gelation occurs in HPMC solution because water molecules dissociate from HPMC and HPMC chain associates and van der Waals interaction forms. As temperature lowers, the gel reverts and HPMC redissolves.

2.3.2.5. Chemical structure of Hydroxypropyl cellulose (HPC) [93]

Hydroxypropylcellulose is nonionic hydroxypropyl ether of cellose. It is soluble in water

below 40°C, and hot and cold organic solvents.




R=H or $CH_2CH(OH)CH_3$

Figure 9: Chemical structure of hydroxypropyl cellulose

2.3.2.6. Application of HPC

Water solution of HPC is Newtonian at low shear rates and thixotropic at high shear rates. It is surface active and is used as O/W emulsifier. Applications include whipped toppings, salad dressings and edible films.

2.3.3. Other ingredients

2.3.3.1. Polysorbates

Polysorbates are formed by reacting sorbitan esters with ethylene oxide. Different degrees of polymerization and esterification produce polysorbates with different properties. Common types of polysorbates include polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65, and polysorbate 80, but only the last three are permitted in food formulation.

Polysorbates are commonly used as emulsifiers and stabilizers. Polysorbate 60 is particularly used as oil-in-water emulsifier in whipped toppings, coffee whiteners, icings,

and confectioner’s coatings. Polysorbate 65 is used in ice cream and frozen desserts, mellorine, ice milk, and bakery products. Polysorbate 80 is used in various dietary foods, gelatin desserts, pickles and bakery products [94].

2.3.3.2. Glycerol

Glycerol is a polyol compound with three hydroxyl groups (Figure below) which contribute to its ability to hold water from surroundings [95]. It is also used as an emulsifier in food industry. These two features (water holding ability and emulsifier) are useful to develop our sprayable formulation.

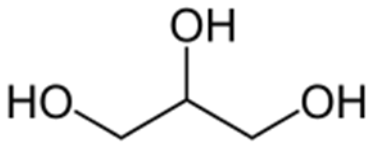



Figure 10: Chemical structure of glycerol

## 2.4. Difference between 1-MCP encapsulation in α-cyclodextrin and adsorption in MOFs

Molecular encapsulation agent is defined as a compound that has a lock and key structure similar to an enzyme whereby a substrate selectively fits into the encapsulation site. A lock and key structure refers to one guest molecule or part of the guest molecule is anchored in the host substrate due to the stereometrical compatibility of the guest or part of the guest with the host molecule, which is true for 1-MCP encapsulation in CD.

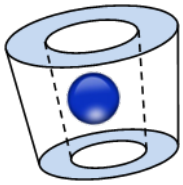


Figure 11: Encapsulation of 1-MCP in CD

However, in 1-MCP adsorption in modified β-cyclodextrin and Zeolites type of materials, unlike the lock and key structure, where a guest is anchored in a host, the entrapment occurs for a group of 1-MCP molecules. The pore structure in modified β-cyclodextrin and Zeolites can be cage-like and tube-like, and the size of the pore opening determines the type of gas that can be trapped. The group of molecules is aligned on the inner surface of the pore.

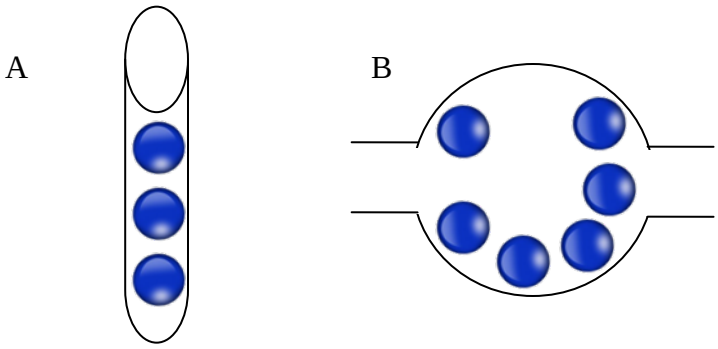



Figure 12: Adsorption of 1-MCP in zeolites
(A: tube-like structure, B: cage-like structure)

# 3. OBJECTIVES

The objective of this dissertation is to develop a 1-MCP liquid formulation consisting of (1) α- and modified β-cyclodextrin encapsulating 1-MCP, and (2) polyol or mineral oil / hydrocolloid blend and (3) water based hydrocolloidal solution. To develop this formulation, the following sub-objectives were achieved.

1. Synthesis and characterization of modified β-cyclodextrin
2. Encapsulation of 1-MCP into modified β-cyclodextrin and quantification of 1-MCP inclusion ratio
3. Stabilization of the complex as liquid formulation using polyol or mineral / hydrocolloid blend
4. Evaluation of the sprayability of the liquid formulation and release of 1-MCP
5. Evaluation of biological efficacy of the 1-MCP released from the liquid formulation

# 4. EXPERIMENTAL DESIGN

## 4.1. Experimental design overview

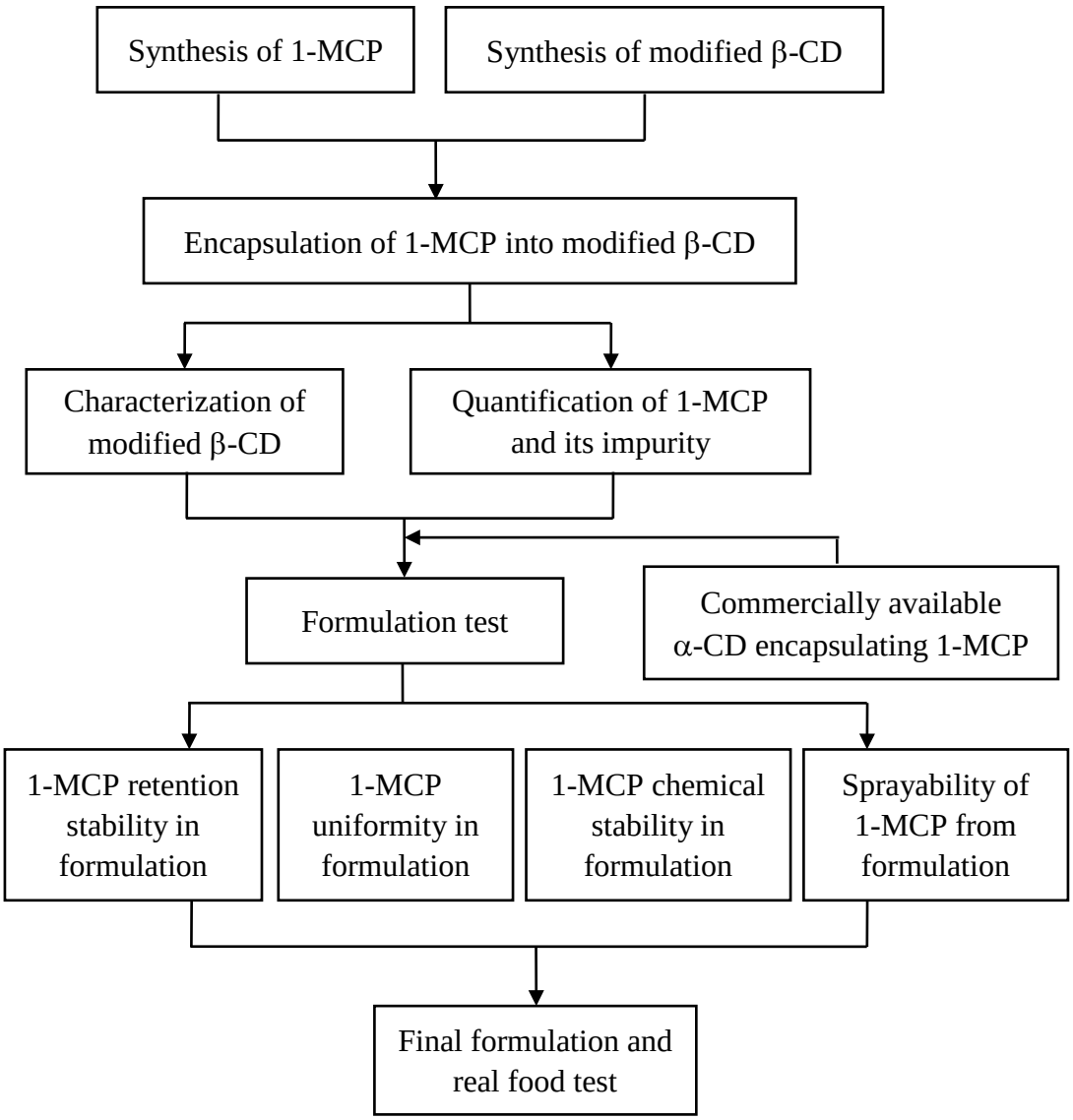



Figure 13: Flow chart of experimental design

### 4.1.1. Tasks for sub-objective 1

To achieve sub-objective 1, the following tasks were performed: (1) modified

β-cyclodextrin synthesis, and (2) FTIR and NMR analyses to confirm the formation of crosslinking, and (3) SEM and TEM analyses to confirm the structural change after modification.

4.1.2. Tasks for sub-objective 2

The purpose of this sub-objective is to quantify the loading of 1-MCP in modified β-cyclodextrin. To achieve sub-objective 2, different encapsulation methods were evaluated to identify the method that results in the highest inclusion ratio.

4.1.3. Tasks for sub-objective 3

The purpose of sub-objective 3 is to screen and identify the polyol/hydrocolloid material and test the physical and chemical stability of 1-MCP in this formulation. The tasks involve (1) identifying the optimal combination of polyol/hydrocolloid for dispersion, (2) optimizing the loading of the modified β-cyclodextrin in the formulation, and (3) testing the uniformity of the formulation.

4.1.4. Tasks for sub-objective 4

Tasks to achieve sub-objective 5 involve testing the release profiles of 1-MCP using different hydrocolloidal solutions.

4.1.5. Tasks for sub-objective 5

Tasks to achieve sub-objective 6 involve testing the biological efficacy of 1-MCP

released from formulation.

## 4.2. Materials

Table 9: Chemicals used in this dissertation

| Chemical | Supplier |
|---|---|
| β-cyclodextrin | Fisher Scientific and Wacker |
| Carbonyldiimidazole | Fisher Scientific and SigmaAldrich |
| Glycerol | Fisher Scientific |
| DMF | Sigma Aldrich and Fisher Scientific |
| Triethylamine ($Et_3N$) | Fisher Scientific |
| Maltodextrin | GPC M040 |
| 3-chloro-2-methylpropene | Fisher Scientific |
| Phenyl lithium | Fisher Scientific |
| n-butanol | Fisher Scientific |
| Acetone | Sigma Aldrich |
| Acetic acid | Sigma Aldrich |
| Xanthan gum | Fisher Scietific |
| 1-chloro-2-methylpropene (1-CMP) | Sigma Alderich |
| Mineral oil | Sigma Alderich |
| Methylhydroquinone | Sigma Alderich |
| TWEEN®20 | Sigma Alderich |

| | |
|---|---|
| 2-Mercaptoethanol | Sigma Alderich |
| Ethanolamine | Sigma Alderich |
| Ethanol | Decon Laboratories Inc. |
| Mineral oil | Sonneborn |

## 4.3. Methods

### 4.3.1. 1-MCP synthesis

The methodology of synthesizing 1-MCP is based on literature. Briefly, 1.5 ml of 3-chloro-2-methylpropene in 15 ml of diethyl ether (99%) was added drop wise to 15 ml of phenyl lithium at room temperature. The duration of this step was about 30 minutes. After addition, the mixture was stirred using a magnetic stirrer and the reaction was continued for 1 hour. The mixture was then placed in a vacuum drier to eliminate volatile impurities. The resulting product, the lithium salt, was stored at -20°C until use. Lithium salt is the precursor for synthesizing 1-MCP, to synthesize 1-MCP, water was added to the lithium salt and 1-MCP was released.

### 4.3.2. 1-MCP purification

There are two impurities, 1-chloro-2-methylcyclopropene and 3-chloro-2-cyclopropene (1-CMP and 3-CMP), that are carcinogenic and are strictly regulated. The purification of 1-MCP was done by using two bottles of washing solutions. Washing solution 1 is water and washing solution 2 contains 50% water, 30% iso-propanol, 10% ethanolamine, and 10%

2-mercaptoethanol [96]. The impurities were quantified using the following GC method.

GC type: Agilent 6890

Column: DB-624 30 m length x 0.25 mm i.d. x 1.4 µm film thickness for hp 6890

Injection system

Injector mode: spilt injection

Injector insert: 4 mm i.d., straight through glass (no glass wool)

Injection volume: 0.50 mL

Split flow: 20 mL/min

Detector: flame ionization

Temperatures

Injection port: 75 °C

Detector: 185 °C

Oven program: temp 1, 40 °C, hold 0 min, ramp rate 25 °C/min, temp2 165 °C, hold 0 min

Gas flow rates

Helium (carrier): 2 mL/min; approximately 40 cm/sec (run at constant flow)

Detector:

Air: 400 mL/min

Hydrogen: 45 mL/min

Nitrogen (make up): carrier flow + make up flow = 30 mL/min

Retention times:

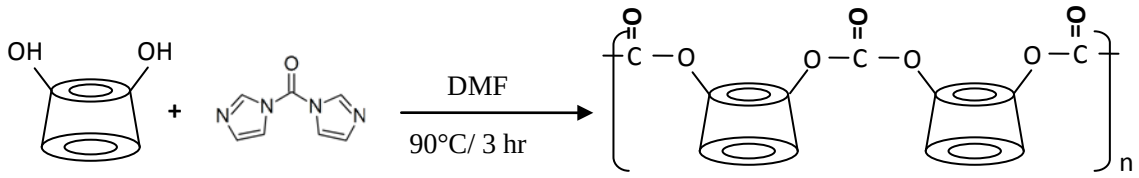
1-CMP: 3.65-3.71 min

3-CMP: 3.75-3.81 min

4.3.3. Modification of cyclodextrin

4.3.3.1. Crosslinking reaction using carbonyldiimidazole as crosslinker

The modification method is essentially to cross-link individual cyclodextrin molecules using carbonyldiimidazole as a cross-linker to create a three dimensional network structure which can accommodate more 1-MCP. Different molar ratios of cyclodextrin to carbonyldiimidazole were used (1:2, 1:4, 1:8). The cross-linking reaction is as follows:




Molar ratio of 1:2 was used as an example to describe the detailed methodology. 0.5 g of CD and 0.125 g of carbonyldiimidazole were measured and mixed in 10 ml of anhydrous DMF. The mixture was mixed by a magnetic stirrer for different durations and under different temperatures. After mixing, the mixture was cooled to room temperature and excess amount of different chemicals were added to precipitate cross-linked cyclodextrin. Then, the mixture was further washed for different durations and collected using a vacuum filtration method. The purified cyclodextrin was placed in an aluminum tray and dried in vacuum oven for different durations under different temperatures. The collected material was grounded into powder.

Table 10: Reactions conditions for modifying β-cyclodextrin

| | |
|---|---|
| Reactants ratio (CD: CDI) | 1:2, 1:4, 1:8 |
| Reaction temperature | 60°C, 80°C, 90°C |
| Reaction time | 1 hr, 3 hrs, 7 hrs |
| Washing agent | Water, ethanol (100%), toluene + water |
| Washing time | 2 hrs, 16 hrs, 24 hrs |
| Drying temperature | 80°C, 100°C |
| Drying time | 8 hrs, 12 hrs |

The experimental setup is shown below.

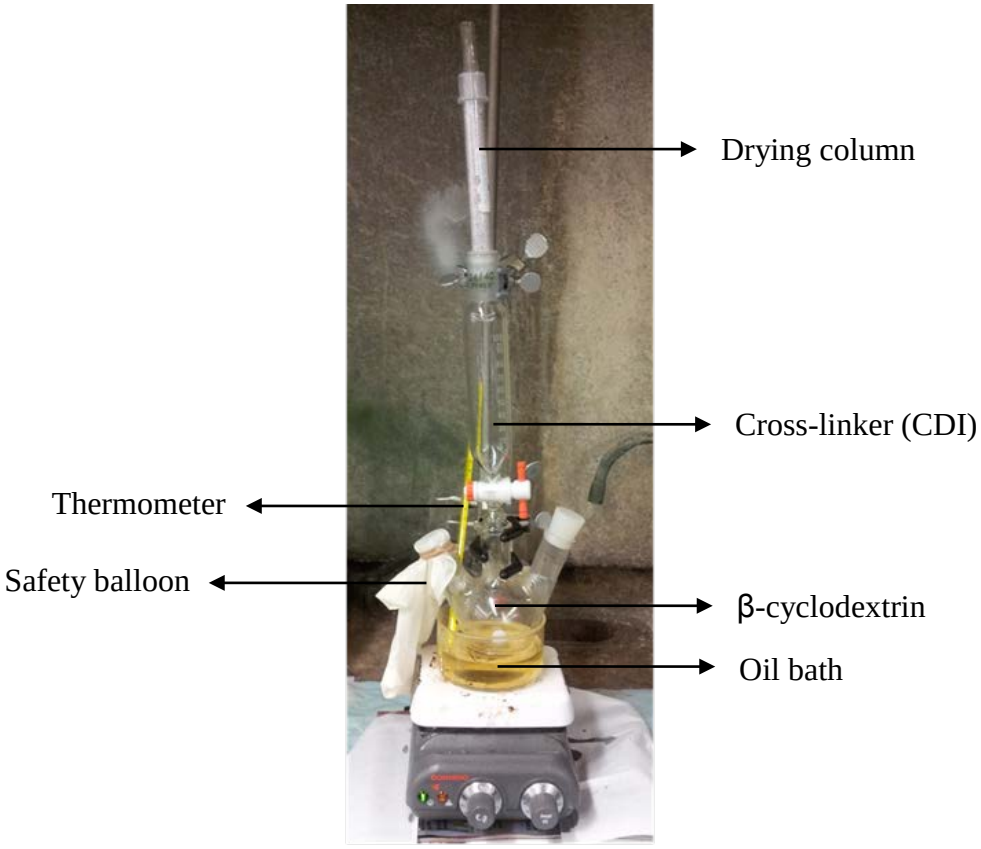



Figure 14: Experimental setup for modifying β-cyclodextrin

4.3.3.2. Thin layer chromatography (TLC)

TLC was used to confirm the modification. Mobile phase solution consists of 80 ml of n-butanol, 20 ml of acetone, 10 ml of $H_2O$, and 0.3 ml of acetic acid. Samples (5µl) were withdrawn using a disposable micropipette (Fisher Scientific) every hour from the reaction flask and spotted on silica plates (Polygram SIL G, layer 0.25 mm). The distance between spots was around 2 cm. Control samples (pure β-cyclodextrin and CDI) were also spotted for comparison purpose.

4.3.3.3. Particle size modification

A NETZSCH milling machine was used to reduce the particle size to obtain more surface area and more exposure of the powder to 1-MCP. Samples were prepared by suspending modified β-cyclodextrin in water (6 grams of powder in 300 mL water). The milling machine was operated at 100 rpm pump speed and 3000 rpm milling speed. Operation time was 12 hours. Samples were withdrawn in between to measure the reduction of particle size. The particle size was measured using BTC 90 Plus particle size analyzer (Brookhaven Instruments Coporation). Around 1.5 mL of sample was used to each measurement.

#### 4.3.4. Encapsulation

Different procedures of encapsulation are performed as shown below. Two categories were encapsulation in liquid and solid. The methods tested below did not include washing step for convenience purpose. After identifying the optimal condition, washing steps were added into the procedure for encapsulation. Further optimization of the encapsulation method including encapsulation time, 1-MCP headspace concentration, and encapsulation temperature, was conducted by Han Zhang, a graduate student at Food Science Department, Rutgers University. Detailed methods can be found in his thesis.

##### 4.3.4.1. Procedure A

β-CD is modified and purified to obtain dry powder. 0.02 g of dry powder was dispersed in 2 mL of water in a 75 mL glass jar. Lithium salt was added in water in a small aluminum cup to generate 1-MCP (headspace concentration > 100,000 ppm), the cup was

then placed in the glass jar. The glass jar was closed and placed on magnetic stirrer for 15 hours. This method is to encapsulate 1-MCP into cyclodextrin solution, the 1-MCP released from the smaller jar gets in contact with solution and encapsulation takes place in the solution.

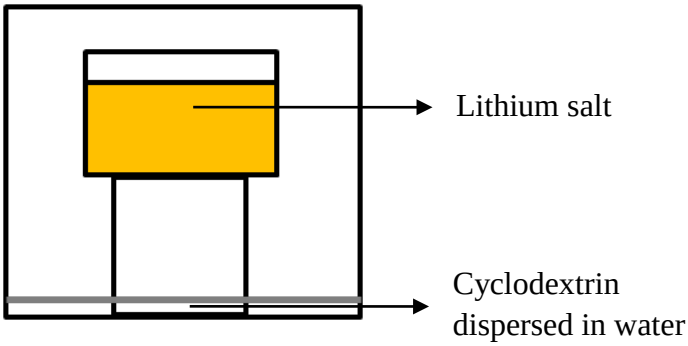



Figure 15: Schematic diagram of Procedure A

4.3.4.2. Procedure B

1-MCP gas was first generated and injected into the reaction vessel of β-CD modification. The purpose of this method is to encapsulate 1-MCP as β-CD is modified. 1-MCP headspace concentration was measured as function of time to monitor the encapsulation process. This method is to encapsulate 1-MCP during the formation of cross-linking of β-cyclodextrin, therefore combining the two steps of modified β-cyclodextrin synthesis and 1-MCP encapsulation into one step.

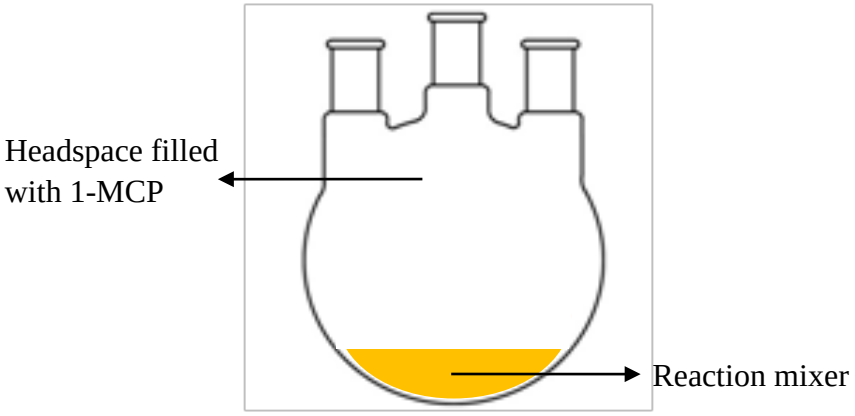



Figure 16: Schematic diagram of Procedure B

4.3.4.3. Procedure C

After modifying β-CD (without any washing or purification), the same procedure as A (instead of dispersed powder, the resulted solution of modifying β-CD) was performed to encapsulate 1-MCP. After encapsulation, ethanol was added to the solution to wash out the encapsulated powder. The purpose of this method is to simplify the encapsulation procedure. The temperature of encapsulation was under room temperature and freezing temperature.

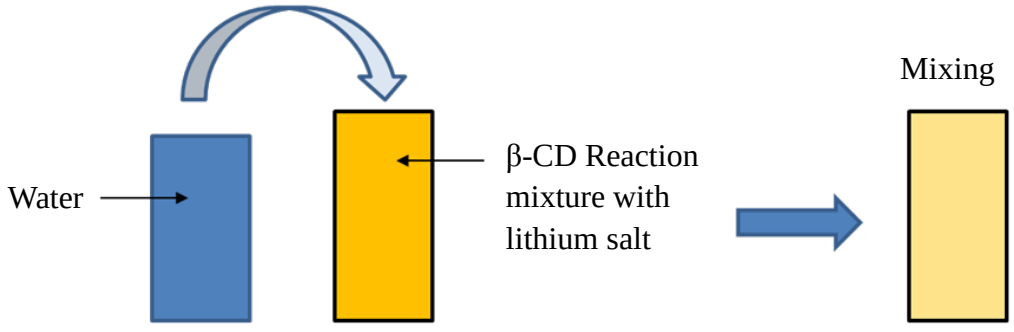



Figure 17: Schematic diagram of Procedure C

4.3.4.4. Procedure D

After modifying, purifying and drying of β-cyclodextrin, the powder was dispersed into water to form a uniform solution, and lithium salt was also added to perform *in situ* encapsulation for 15 hours.

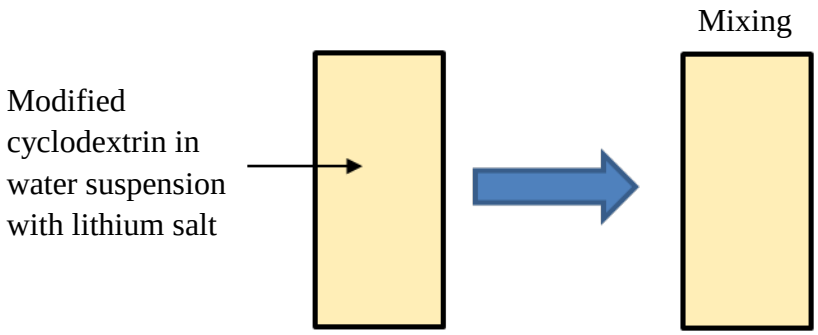



Figure 18: Schematic diagram of Procedure D

4.3.4.5. Procedure E

This procedure conducts the encapsulation in solid phase. Solid modified β-cyclodextrin powder was placed in a closed jar with 1-MCP filled in the headspace. The encapsulation took place for 6 hours.

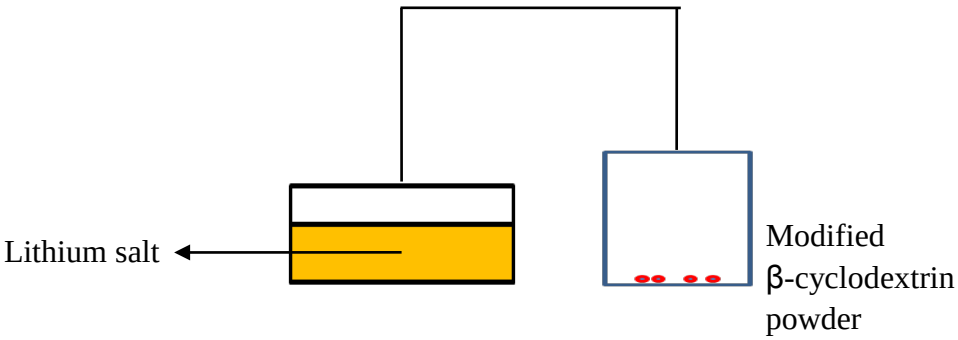
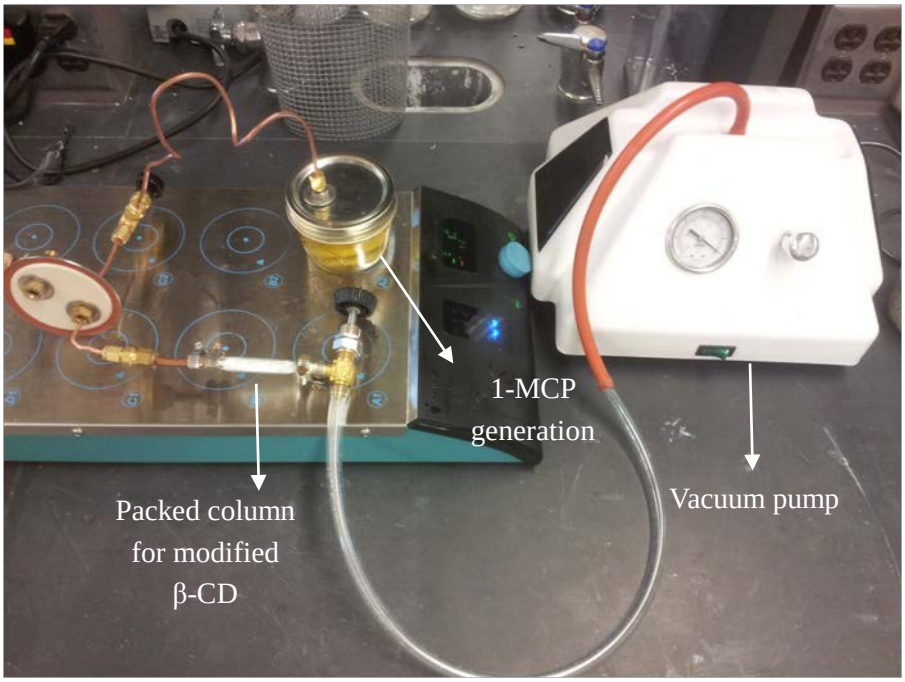



Figure 19: Schematic diagram of Procedure E

4.3.4.6. Procedure F

This procedure is similar to Procedure E where encapsulation takes place in solid phase, but instead of using a jar, the powder are in a glass column packed with glass beads. The headspace of in the column is minimal and more contact between 1-MCP gas and cyclodextrin powder is expected.



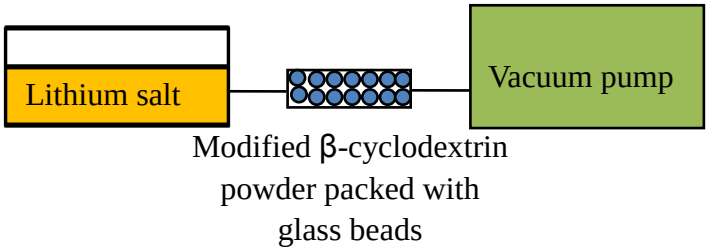




Figure 20: Experimental setup of Procedure F and schematic illustration

4.3.4.7. Procedure G

In procedure G, the basic difference is that there is a pressure control syringe to pump 1-MCP into the encapsulation vessel. This operation is expected to control the pressure as

needed to study the inclusion ratio under various pressures. The schematic diagram and the real setup are shown as below.

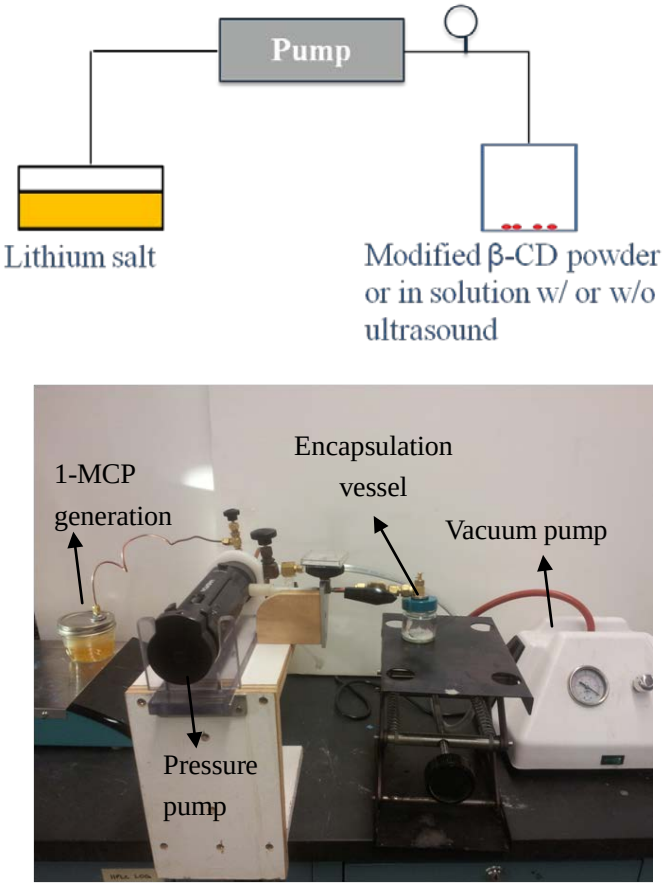



Figure 21: Experimental setup of Procedure F and schematic illustration

4.3.5. Characterization analysis

4.3.5.1. Solubility test

Solubility of modified β-cyclodextrin was tested by mixing the powder with water until precipitation was observed. Certain amount (unit in mL) of supernatant was collected and dried. The residue powder was weighed and the weight was divided by the amount (mL) of supernatant to calculate solubility.

#### 4.3.5.2. FTIR analyses

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 conduct the experiment. The spectra were collected at room temperature within the wavelength range of 400 $cm^{-1}$ to 4000 $cm^{-1}$. The results were analyzed based on standard FTIR database [97, 98].

#### 4.3.5.3. Theoretical analysis of encapsulation

Jmol, an open-source Java viewer for chemical structure, was used to build the molecular model and measure the distances between atoms of 1-MCP and thus to estimate the size of 1-MCP molecule. The molecular volume and dimension were calculated based on van der Waals surface. Assumption was made that the shape of the surface is approximately a square box. The size of 1-MCP molecule is used compared to the size of the cavities and pores of cyclodextrins to analyze the geometric compatibility. This analysis is complementary to porosity characterization (4.3.5.4).

#### 4.3.5.4. Porosity characterization

The porous structure of modified β-cyclodextrin (pore size, void volume, and pore surface) was characterized. The information helps understand the structure and estimate the inclusion ratio of 1-MCP. The high resolution $N_2$ gas adsorption-desorption-measurements were performed on an automated micropore gas analyzer Autosorb-1 MP (Quantachrome

Instruments).

4.3.5.5. Thermogravimetric analysis

Thermogravimetric analysis was conducted to test the thermo-stability of modified β-cyclodextrin. It was conducted during the optimization of modification process, so the results also helped design the modification process. 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.

4.3.5.6. TEM and SEM

TEM and SEM analyses were done to investigate the structure of modified β-cyclodextrin and MOFs.

4.3.5.7. Inclusion ratio quantification

GC-FID (Agilent 6890 series Plus+ GC system) was used to quantify the inclusion ratio of 1-MCP in the encapsulation complex. The detailed GC conditions are listed in the table below. The quantification was performed by dissolving the modified β-cyclodextrin into alkaline solution to destruct the encapsulation complex and allow 1-MCP to release into the headspace of a closed container.

The GC method for 1-MCP quantification is as follows.

Column: CP-PoraBOND Q, 25 m length, 0.25 mm i.d., 3 μm film thickness

Temperature:

Injector port: 75°C

Detector: 200°C

Oven: 75°C for 1 min, ramp 5°C/min, 110°C (total 8 min)

Carrier gas: Helium

Flow rates (mL/min):

Helium: 30

Detectors

Air: 400

Hydrogen: 45

Retention time:

Isobutene: 4.5-4.6 min

1-MCP: 4.7-4.9 min

### 4.3.6. Liquid formulation using α-cyclodextrin

The concept of liquid formulation was first tested using α-cyclodextrin because α-cyclodextrin is water soluble in water, and the retention of 1-MCP is only achieved by other ingredients in the formulation, not the encapsulation material. In addition, 1-MCP encapsulation in α-cyclodextrin is well established and its product is commercially available. Therefore, it is a perfect system to test our concept.

#### 4.3.6.1. Effect of glycerol

25 mg of α-cyclodextrin encapsulating 1-MCP was mixed with 1 ml of glycerol in a jar

which was placed in a closed chamber (295 L). The reason for testing glycerol is because of its multiple –OH groups which can be used as a water binding agent to block the contact between cyclodextrin with moisture. The mixing was done using a spatula and no additional mixing was done for headspace analysis. Headspace 1-MCP concentration was tested as function of time.

4.3.6.2. Effect of xanthan gum

Different concentrations of xanthan gum were added to the formulation. 12.5 mg of α-cyclodextrin encapsulating 1-MCP was mixed with 37.5 ml of xanthan gum solution. The reason for testing xanthan gum is because it is a GRAS material with wide usage in food products as texture thickener and it is a shear thinning hydrocolloid which can facilitate the spraying. The mixing was done using a spatula and no additional mixing was done for headspace analysis.

Table 11: Formulation to test the effect of xanthan gum

| 1-MCP (mg) | Xanthan gum (37.5 ml) |
|---|---|
| 12.5 | 0.5% |
| 12.5 | 0.05% |
| 12.5 | 0.005% |

4.3.6.3. Effect of combination of glycerol and xanthan gum

Xanthan gum and glycerol were mixed together. Different concentrations of xanthan gum were tested including 1%, 0.5%, 0.25%, 0.1% and 0.05%. The mixing was done using a

spatula and no additional mixing was done for headspace analysis.

Table 12: Formulation to test the combination of glycerol and xanthan gum

| 1-MCP (mg) | Glycerol (ml) | Xanthan gum (37.5 ml) |
|---|---|---|
| 12.5 | 11.5 | 1% |
| 12.5 | 11.5 | 0.5% |
| 12.5 | 11.5 | 0.25% |
| 12.5 | 11.5 | 0.1% |
| 12.5 | 11.5 | 0.05% |

4.3.6.4. Effect of combination of sorbitol and xanthan gum

D-Sorbitol was tested to replace glycerol. α-cyclodextrin encapsulating 1-MCP was first dispersed into 70% of D-sorbitol and then the mixer was dispersed into varying concentrations of xanthan gum. The mixing was done using a spatula and no additional mixing was done for headspace analysis.

Table 13: Formulation to test the effect of the combination of D-sorbitol and xanthan gum

| 1-MCP (mg) | 70% D-sorbitol (ml) | Xanthan gum (37.5 ml) |
|---|---|---|
| 12.5 | 11.5 | 1% |
| 12.5 | 11.5 | 0.5% |
| 12.5 | 11.5 | 0.25% |
| 12.5 | 11.5 | 0.1% |
| 12.5 | 11.5 | 0.05% |

4.3.6.5. Effect of CMC

Varying concentrations of CMC was tested. α-cyclodextrin encapsulating 1-MCP was first dispersed into 70% of D-sorbitol and then the mixer was dispersed into varying concentrations of CMC. The mixing was done using a spatula and no additional mixing was done for headspace analysis.

Table 14: Formulation to test the effect of the combination of D-sorbitol and CMC

| 1-MCP (mg) | 70% D-sorbitol (ml) | CMC (37.5 ml) |
|---|---|---|
| 12.5 | 11.5 | 0.5% |
| 12.5 | 11.5 | 0.1% |
| 12.5 | 11.5 | 0.005% |

4.3.6.6. Combination of glycerol, polysorbate, HPC and maltodextrin

A two-step mixing was performed. Firstly, a combination of 9 mL glycerol, 1 mL polysorbate, 0.5 g HPC, and 0.5 g maltodextrin was mixed with 25 mg of α-CD encapsulating 1-MCP as a base. Secondly, the base mixture was mixed with 90 mL of 0.05% of xanthan gum. The reason for testing polysorbate is to improve the uniformity of the formulation. The mixing was done using a spatula and no additional mixing was done for headspace analysis.

4.3.6.7. Spraying Study

Two formulations, (1) 1-MCP 12.5 mg +70% sorbitol 11.5 ml+0.05% xanthan gum 37.5 ml and (2) 1-MCP 12.5 mg+glycerol 11.5 ml+0.05% xanthan gum 37.5 ml, were tested

before and after spraying which was achieved using a plastic sprayer to understand the effect of shearing on accelerating the release of 1-MCP from the formulation.

### 4.3.7. Liquid formulation using modified β-cyclodextrin

In this section, different formulations were designed based on the results obtained from the previous section where α-CD was used. The purpose of these tests was to confirm the observation obtained from α-CD. The use of xanthan gum was to test its efficacy to hold 1-MCP and therefore indicate its ability to slow down the release of 1-MCP.

#### 4.3.7.1. Xanthan gum

Two concentrations of xanthan gum (5% and 0.5%) were tested. 5% xanthan gum solution has a thick gel-like texture. 0.5% xanthan gum solution is a viscous solution. 25 mL of xanthan gum was mixed with certain amount of encapsulated powder in a 25 mL glass vial. The release of 1-MCP was measured every day. The test was done under elevated temperature 50°C.

#### 4.3.7.2. Combination of glycerol and xanthan gum

The formulation has 5 ml of glycerol, 10 ml 0.5% xanthan gum, and 0.0085 g modified β-cyclodextrin powder. The formulation was in a 25 ml glass vial (15 ml headspace) under room temperature. Headspace concentration of 1-MCP was measured every day to track the retention of 1-MCP in the solution.

#### 4.3.7.3. Combination of glycerol, poly sorbate, HPC, and maltodextrin

The formulation has 18 ml glycerol, 3 ml poly sorbate, 0.5 g of HPC, and 0.5 g of maltodextrin. The formulation was in a 25 ml glass vial (2 ml of headspace) under three temperatures: room temperature (23°C), refrigeration temperature (5°C), and elevated temperature (50°C). Headspace concentration of 1-MCP was measured every day to track the retention of 1-MCP in the solution.

4.3.7.4. Combination of glycerol, HPC, and maltodextrin

The purpose of this test is to evaluate the effect of poly sorbate as a by eliminating it from the formulation. The formulation has 21 ml glycerol, 0.5 g of HPC, and 0.5 g of maltodextrin. The formulation was in a 25 ml glass vial (2 ml of headspace) under three temperatures: room temperature (23°C), refrigeration temperature (5°C), and elevated temperature (50°C). Headspace concentration of 1-MCP was measured every day to track the retention of 1-MCP in the solution.

4.3.7.5. Combination of glycerol, poly sorbate, HPC, maltodextrin, and xanthan gum after spraying

The combination of the previously mentioned formulation (referred as Base A: glycerol, poly sorbate, HPC, maltodextrin) and 0.5% xanthan gum was tested after spraying. Small amount of modified β-cyclodextrin 1-MCP complex (0.0078g) was mixed with 3 mL of Base A, and then mixed with 15 mL of 0.5% xanthan gum solution in a 25 mL atomizer. The solution was sprayed into a 500 mL glass jar which was then closed immediately to characterize the release.

4.3.8. Optimize the right combination of polyol/hydrocolloid for dispersing modified β-cyclodextrin in the formulation

Based on the formulations mentioned above, four combinations were selected as shown the table below.

Table 15: Formulation composition and function of each ingredient

| Ingredients | Function of each ingredient | Formulations | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Glycerol (ml) | Water holding ability and emulsifier | 21 | 18 | 18 | 21 |
| Hydroxypropyl cellulose (HPC) (g) | Thickener, sprayability at high shear force, surface active as emulsifier | 2 | 2 | 1 | 1 |
| Polysorbate (ml) | Emulsifier and surfactant to provide uniformity of the formulation | 0 | 3 | 3 | 0 |
| Maltodextrin (g) | Texture thickener | 0 | 0 | 1 | 1 |

The final volume of the formulations was 23 ml, which was contained in a jar of 25 ml. Around 2.3 mg of modified β-cyclodextrin containing 1-MCP was mixed into these formulations and the headspace concentration of it was measured every day to track the loss of 1-MCP and calculate the retention of it.

4.3.9. Optimize the initial loading of modified β-cyclodextrin in the formulation

The test was done by varying the loading (2.5 mg and 5 mg) of modified β-cyclodextrin in the formulations. The formulations used in the above section were tested. The duration of the test was 30 days.

4.3.10. Test the uniformity of the formulation

Each jar of samples from Tests 1 and 2 will be divided into several equal portions (1.5 ml) which are placed into 2.5 ml containers. The headspace concentration of each container If the amount of 1-MCP in each container is quantified to be the same, it can be inferred that the formulation is uniform. Formulations with more surfactants and emulsifiers are expected to have better uniformity.

4.3.11. Evaluation of the stability of the liquid formulation

Stability and release of 1-MCP was evaluated at different stages: (1) stability of 1-MCP encapsulated in modified β-cyclodextrin powder, and (2) stability of 1-MCP after the encapsulation complex is dispersed in the polyol/hydrocolloid formulation.

4.3.11.1. Stability of 1-MCP in the encapsulation complex

The stability of 1-MCP encapsulation complex powder was tested by quantifying the 1-MCP inclusion level as function of time. 0.2 g encapsulation complex (solid encapsulation method because of the higher inclusion level) was stored in a 1.5 ml vial. Vials were prepared for an 8-day trial. One vial was opened every day and the inclusion level was measured.

4.3.11.2. Improved stability of 1-MCP encapsulated in modified β-cyclodextrin and suspended in liquid formulation

The test was done at three temperatures (refrigeration temperature, room temperature,

and 50°C) in polyol/hydrocolloid solution. Formulation 3 was selected in the test as a model formulation because it has more surfactant and emulsifier to stabilize the system. The formulation was placed into three jars at three temperatures and the headspace concentration of 1-MCP was measured over time.

4.3.12. Evaluation of biological efficacy of developed formulation

4.3.12.1. GC-MS evaluation

GC-MS analysis was conducted to identify 1-MCP and other impurities. Different amounts of formulation (0.6% 1-MCP concentration) were mixed with water in a 25 mL glass jar to create different headspace concentrations of 1-MCP. The headspace sample was withdrawn and tested using the same method as 1-MCP impurity method.

4.3.12.2. Real food analysis

Bananas were used in this test as our model produce due to their availability. They were purchased from local grocery store at color stage 2.5 (green figures) and stored at room temperature. One cluster was treated with 1-MCP at 1 ppm concentration for overnight, and the other cluster was left on the bench as control. The tests were continued until the observation of sugar spot.

Figure 22: Bananas in a treatment chamber (1 ppm treatment concentration)

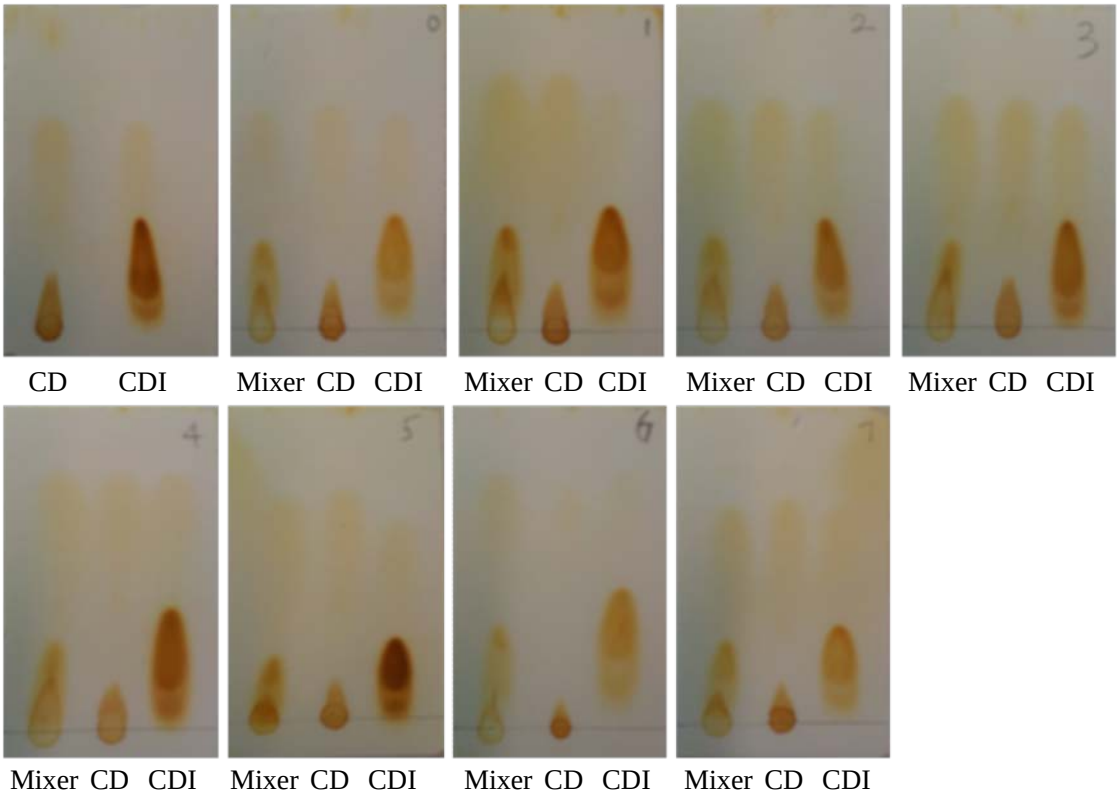
# 5. RESULTS & DISCUSSIONS

## 5.1. Modification of cyclodextrin

### 5.1.1. TLC

The TLC results indicate that the structure of β-cyclodextrin was modified as shown in the figure below. As modification occurs, there is a dark It may also suggest that the reaction time may be 3 hrs under 90°C, which are consistent with literature. As shown in the Figure below, during the first 3 hrs of reaction, $R_f$ value of the reaction mixer increased and reached plateau. After 4 hrs, $R_f$ value decreased, possibly because of generation of side products or occurrence of side reactions.

A: Chromatograph




B: $R_f$ calculation

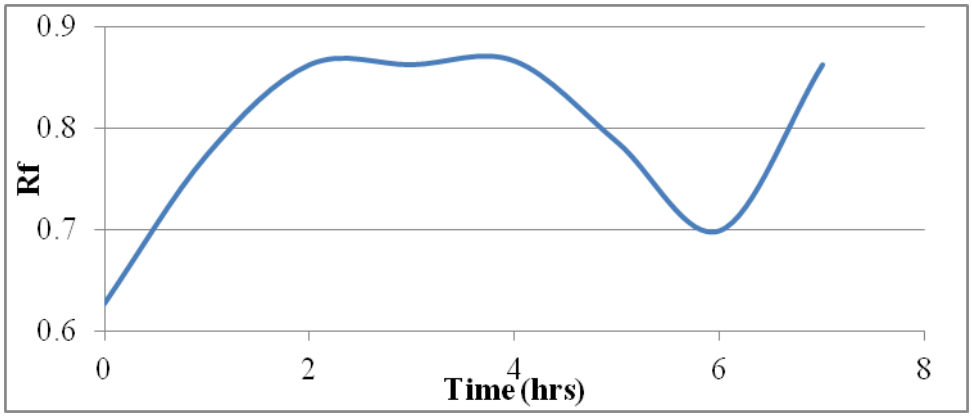



Figure 23: TLC results

5.1.2. Particle size modification

After milling, the average particle size reduced dramatically from 100 micron to 300-400

nm after 7 hours. Further milling beyond 7 hours did not reduce the particle size. Later, we found that milling process does not improve the encapsulation efficiency of modified β-cyclodextrin.

## 5.2. Characterization Analysis

### 5.2.1. Solubility test

The results of the solubility of modified β-cyclodextrin with different CD to CDI ratio are summarized as follows. Results show that as CD: CDI ratio increases, the solubility increases, indicating that higher cross-linker ratio results in more polymer network formation and water molecule is less accessible to it.

Table 16: Results of solubility

| Sample (CD: CDI) | 1:2 | 1:4 |
|---|---|---|
| Solubility (mg/mL) | 7.4 | 1.88 |

### 5.2.2. FTIR analysis

FTIR results confirm the formation of cross-linking. In the spectra below, the green spectrum is for modified β-cyclodextrin, the red spectrum is for pure β-cyclodextrin (control). As shown, there are two distinct peaks at 1258.8 $cm^{-1}$ and 1745.3 $cm^{-1}$ that are the only difference between the two spectra after modification. The peak at 1745.3 $cm^{-1}$ represents ester formation due to the cross-linking reaction.

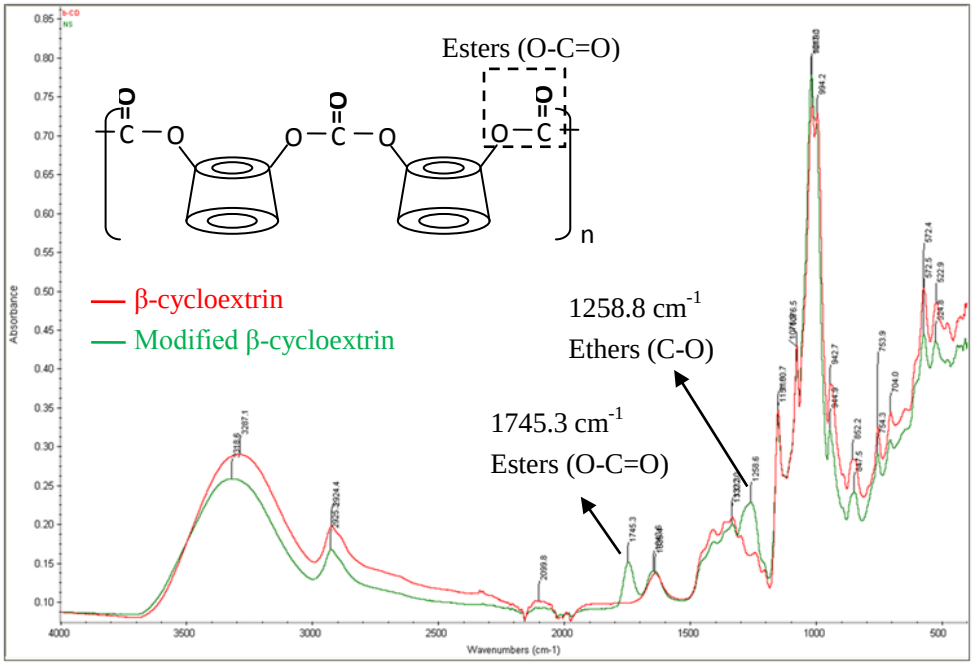



Figure 24: FTIR results (β-CD vs modified β-CD)

The results of two CDI samples (Figure below) show identical spectrum, indicating that the samples they have same qualtiy.

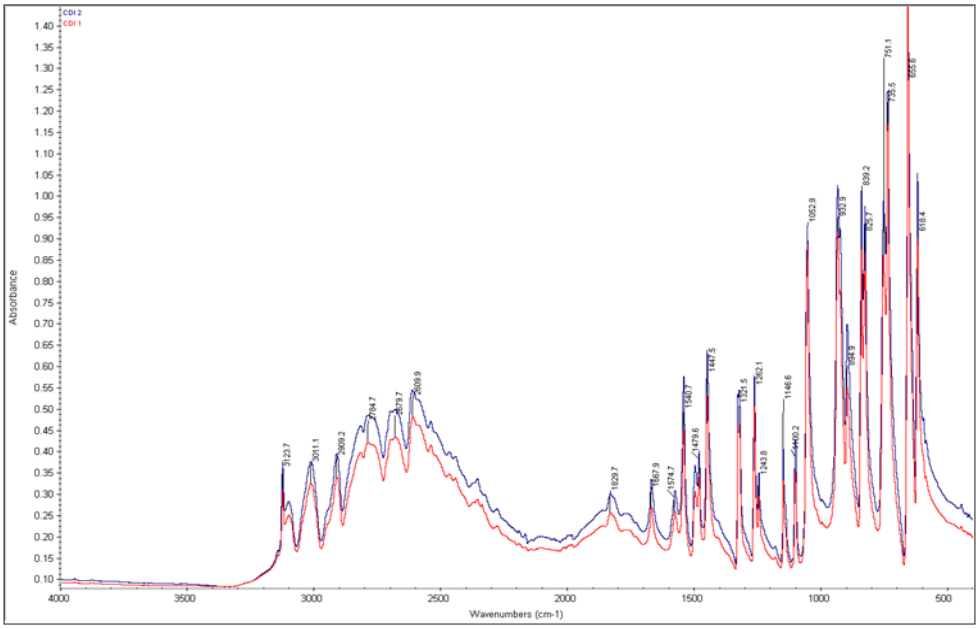



Figure 25: FTIR results of CDI (from two suppliers)

5.2.3. Theoretical analysis of encapsulation of 1-MCP

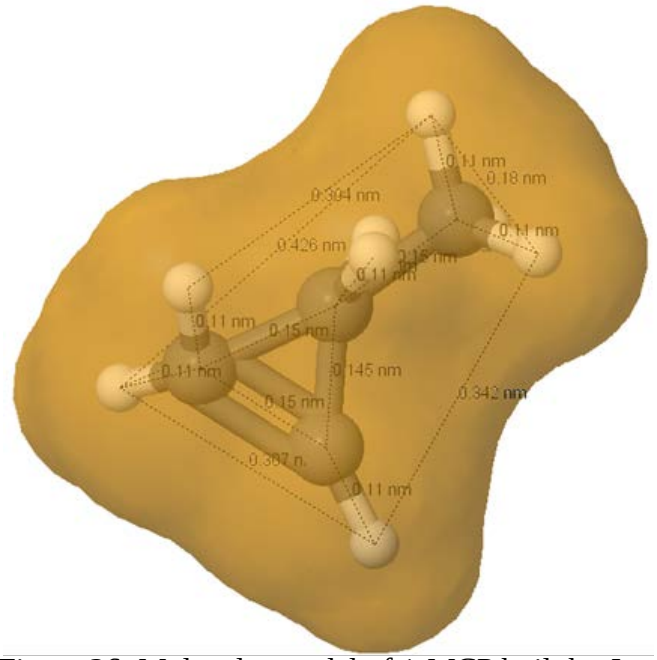
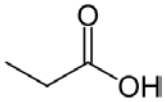
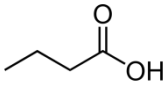
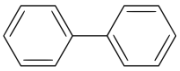
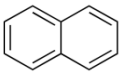
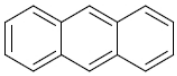



Figure 26: Molecular model of 1-MCP built by Jmol

The table below summarizes the relationship between the encapsulation of different cyclodextrins for different guest molecules. Additional information of molecular volume (calculated using Jmol, same method as mentioned above) and molecular weight of different guest molecules is provided in the table.

Table 17: Geometrical compatibility between molecular volume of guest molecules and internal cavity of cyclodextrin

| | Chemical structure | Molecular weight | Molecular volume ($A^3$) | α-CD | β-CD | γ-CD |
|---|---|---|---|---|---|---|
| Propionic acid ($C_3H_6O_2$) |  | 74.08 | 175.9 | + | - | - |
| Butyric acid |  | 88.11 | 181.4 | + | + | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Biphenyl |  | 154.21 | 338.8 | + | + | + |
| Cyclohexane |  | 84.16 | 185.6 | + | + | + |
| Naphthalene |  | 128.17 | 287.1 | - | + | + |
| Anthracene |  | 178.23 | 342.9 | - | - | + |
| $Cl_2$ | | 34 | 72.6 | + | - | - |
| $Br_2$ | | 70 | 78.9 | + | + | - |
| $I_2$ | | 106 | | + | + | + |

The above table was also converted into the figure below. The purpose of it is to summarize the geometrical compatibility between the molecular volume and internal cavity of cyclodextrins in a visual friendly manner. The first role is the type of cyclodextrin that are geometrically compatible with the molecules listed in the table, the second role is the molecular volume of the molecules, the third role is the internal cavity of cyclodextrins (the position matches the order of the size of molecular volume in the second role), the third role is molecular volume of 1-MCP (the position matches the order of the size of molecular volume in the second role). Considering that the calculated molecular volume is based on the assumption that the simulated molecular model is a square box, so it is a little larger than the true molecular value. For example, when comparing molecular volume with internal cavity, it may happen that molecular volume is a little larger than internal cavity, but they are still geometrically compatible.

For α-CD (174 $Å^3$), its internal cavity volume is compatible with molecular volume of 72.6 ($Cl_2$), 78.9 ($Br_2$), 175.9 (propionic acid), 181.4 (butyric acid), 185.6 (cyclohexane) and 338.8 (biphenyl) $Å^3$. It is interesting that biphenyl, a large molecule, can fit in α-CD. The reason is that only one phenyl group is trapped in the cavity (the size of one phenyl group is similar to cyclohexane). The range of compatible molecular volume of α-CD should be between 72.6 and 185.6 $Å^3$. 1-MCP is in this range, and so it is compatible with α-CD.

For β-CD (262 $Å^3$), its internal cavity volume is compatible with molecular volumes of 181.4 (butyric acid), 185.6 (cyclohexane), 287.1 (naphthalene), and 338.8 (biphenyl) $Å^3$. The range of compatible molecular volume of β-CD should be between 181.4 and 278.1 $Å^3$. For γ-CD, the range is 185.6 to 342.9 $Å^3$. 1-MCP is out of the range, and so it is not compatible with β-CD.

The goal of modifying β-CD is to reduce its cavity volume into the range below 185. The section below provides more information about the change in porosity after modification.

| Type of CD | α | α, β | α | α, β | α, β, γ | β, γ | α, β, γ | γ |
|---|---|---|---|---|---|---|---|---|
| Molecular volume of different guests | 72.6 | 78.9 | 175.9 | 181.4 | 185.6 | 287.1 | 338.8 | 342.9 |
| Internal cavity of CDs | 174, α-CD | | | | 262, β-CD | | | |
| Molecular volume of 1-MCP | 157.5, 1-MCP | | | | | | | |

Figure 27: Molecular volume vs cyclodextrin encapsulation

5.2.4. Thermogravimetric analysis

Results show that the modification did not change the thermo-stability of β-CD, however, there were residue chemicals, probably residual DMF, after the modification. These residues may block the available sites for 1-MCP. Later, the washing process after the modification reaction was prolonged to remove the residual chemicals.

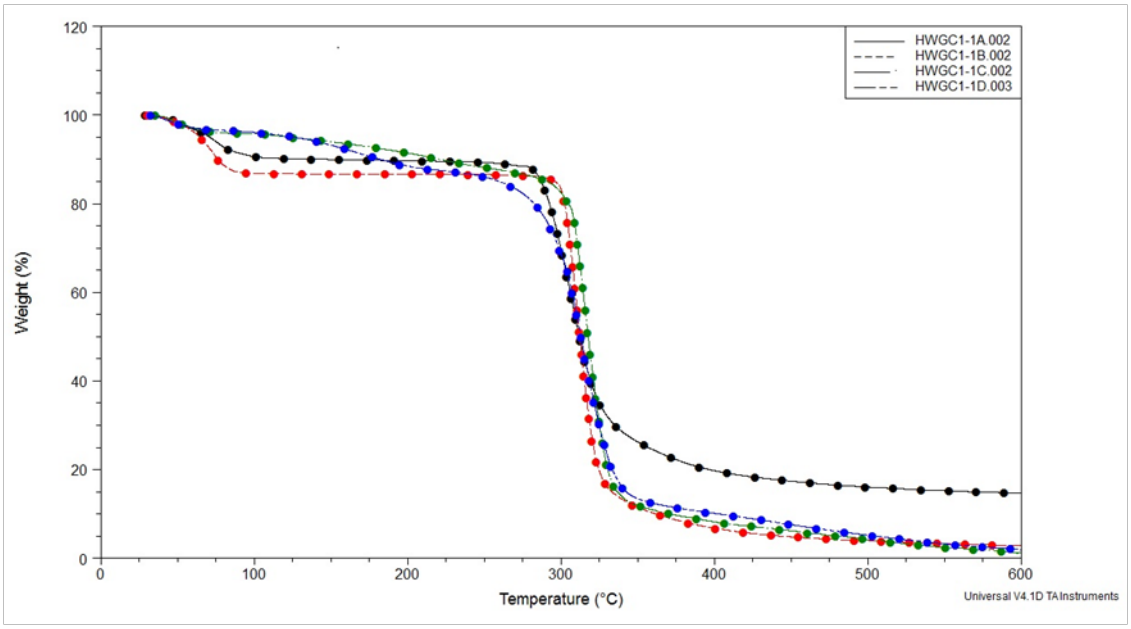



Figure 28: TGA results (A, black: α-CD; B, red: β-CD; C, green: β-CD washed by ethanol; D, blue: β-CD washed by acetone)

### 5.2.5. Porosity Characterization

The table below shows the porosity of α-CD, β-CD and modified β-CD. The average pore size of α-cyclodextrin is 7 Å (suitable for encapsulating 1-MCP), β-cyclodextrin is 32 Å. After modification, the pore size of β-cyclodextrin reduces to 14 Å which is considerably smaller than β-cyclodextrin, indicating that pores were cross-linked. Pore volume after modification also reduced but higher than α-cyclodextrin. The same trend occurred for surface area (ideally, surface area should increase after crosslinking, the reason why it

decreased may be that the crosslinking also covered some pores and made it unavailable).

Table 18: Pore size characterization

| Sample name | α-CD | β-CD | Modified β-CD |
|---|---|---|---|
| BET surface area ($m^2/g$) | 3.8 | 7.2 | 6.7 |
| Pore volume (cc/g) | 0.0051 | 0.025 | 0.014 |
| NLDFT Average pore size (Å) | 7 | 32 | 14 |

It is important to correlate the pore volume shown in the table above with encapsulation capacity. To meet this purpose, pore volume needs to be converted to encapsulation pore size, e.g. internal cavity is the encapsulation pore size for α and β-CD. The equation below was used to estimate the encapsulation pore size for modified β-CD. It does not represent the true value, but provides a crude estimation because pore volume shown in the table is the combination of internal cavity and intermolecular space.

$$\frac{262-172}{0.025-0.0051}=\frac{(encapsulation\ size\ of\ modified\ \beta-\mathrm{CD})-172}{0.014-0.0051}$$

Results show that the encapsulation pore size for modified β-CD is 208. It is a little larger than the ideal size for 1-MCP encapsulation (185, see section above). Therefore, further modification is needed for the modification reaction to reduce the pore size.

The figure below shows the pore size distribution of α-CD (HWGC1-1A), β-CD (HWGC1-1B), and modified β-CD (HWGC1-1C washed by ethanol, and HWGC1-1D washed by acetone). The highest peak of each curve shows NLDFT average pore size listed

in the table above.

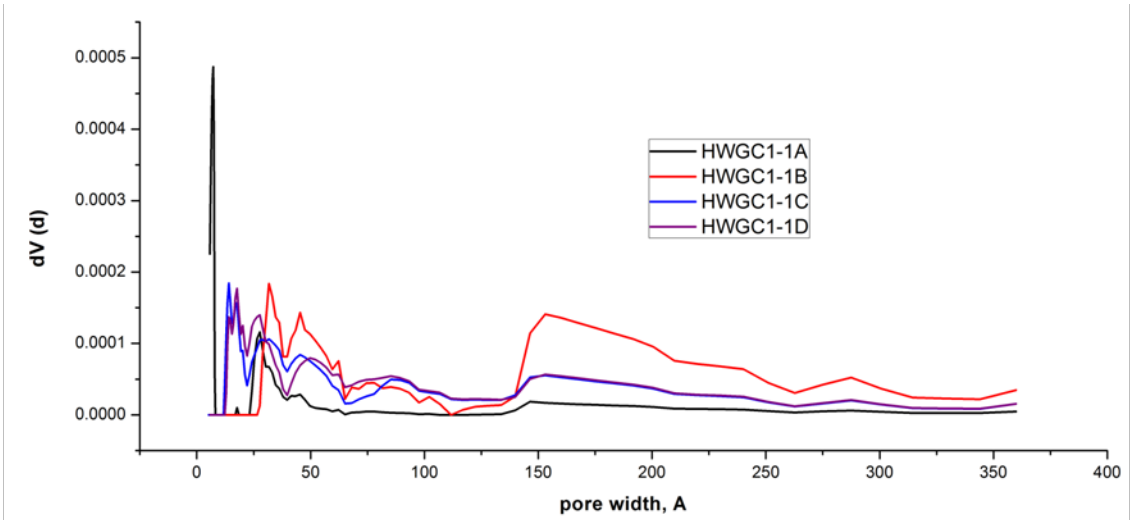



Figure 29: Pore size distribution

### 5.2.6. TEM and SEM

TEM shows the image of modified β-cyclodextrin clusters in water. The round dark zone is the cluster, the lighter zone is background. As shown in the magnified image, there are dark and light spots in a cluster in which dark spots are modified β-cyclodextrin polymers and light spots are the pores and inherent cyclodextrin cavities. 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 size measured here is smaller than the size obtained from particle size analyses, possibly because the sample underwent sonication before TEM analyses and the particles were further broken down.

Compared to TEM images of pure α (A) and β-cyclodextrin (B), whose cluster sizes are smaller than modified β-cyclodextrin, the cluster of β-cyclodextrin has more organized

structure and possesses a sponge-like shape.

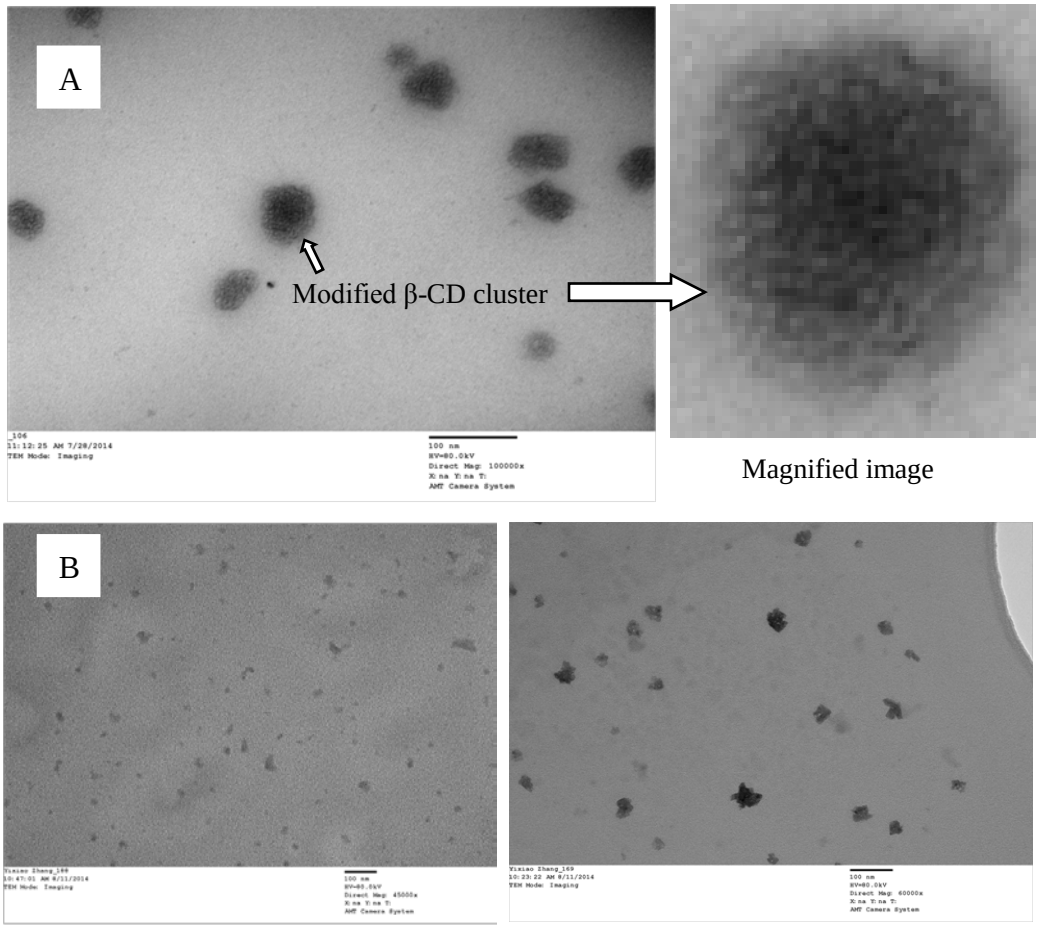



Figure 30: TEM results (A: modified β-cyclodextrin, B: α-cyclodextrin, C: β-cyclodextrin)

SEM results (figure below) also show that there is distinct difference before and after modification. Pure α and β cyclodextrins are block-like, but modified β-cyclodextrin are spong-like with a porous structure. The results were expected as modification reaction bridges individual cyclodextrin molecules to form a network.

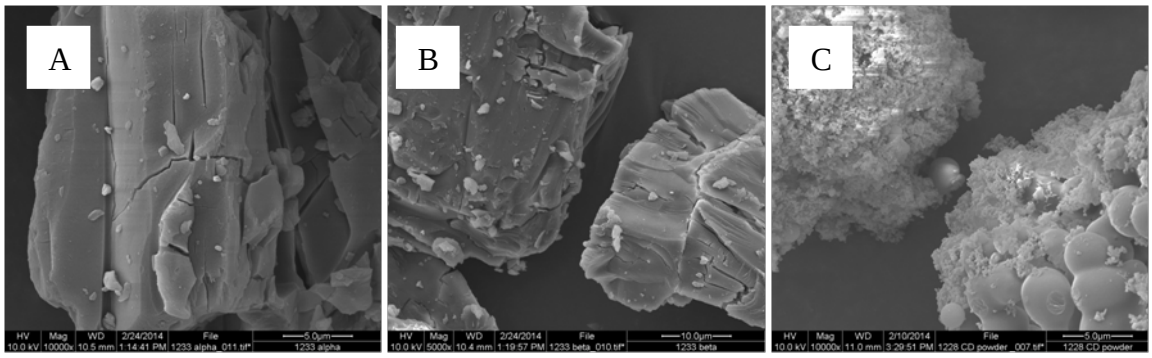



Figure 31: SEM results (A: α-cyclodextrin, B: β-cyclodextrin,
C: modified β-cyclodextrin)

## 5.3. Encapsulation

Results show that only Procedure A was able to encapsulate 1-MCP at 0.31%.

For procedure B, there was no headspace concentration of 1-MCP decrease observed, indicating 1-MCP was not encapsulated. For procedure C, no 1-MCP was encapsulated.

The reason for the failure of Procedure B and C was that DMF was not an ideal candidate for cyclodextrin encapsulation. In addition, the recovery process and drying process may also be detrimental to retain 1-MCP.

For procedure D, no 1-MCP was able to be encapsulated, possibly because that the chemical composition in the mixed solution (including the reaction products from lithium salt and water) may block the access of 1-MCP, and the washing process may also wash the 1-MCP away if any 1-MCP is encapsulated.

For procedure E, 0.46% of 1-MCP was encapsulated. Therefore, procedure A is the best for encapsulation in liquid, and procedure E is the best for encapsulation in solid.

## 5.4. Liquid formulation using α-cyclodextrin

### 5.4.1. Effect of glycerol

The figure below shows that glycerol is effective to hold 1-MCP in cyclodextrin matrix. It demonstrates that 1-MCP gas is released rapidly when encapsulated 1-MCP powder is dissolved in water (control). The 100% 1-MCP gas release is complete in 1 hour. When the encapsulated 1-MCP powder was dispersed in glycerol, the release rate of 1-MCP gas from encapsulated powder is drastically reduced, allowing only 7.6% to be released over a 16 hour holding period at room temperature of 22° C.

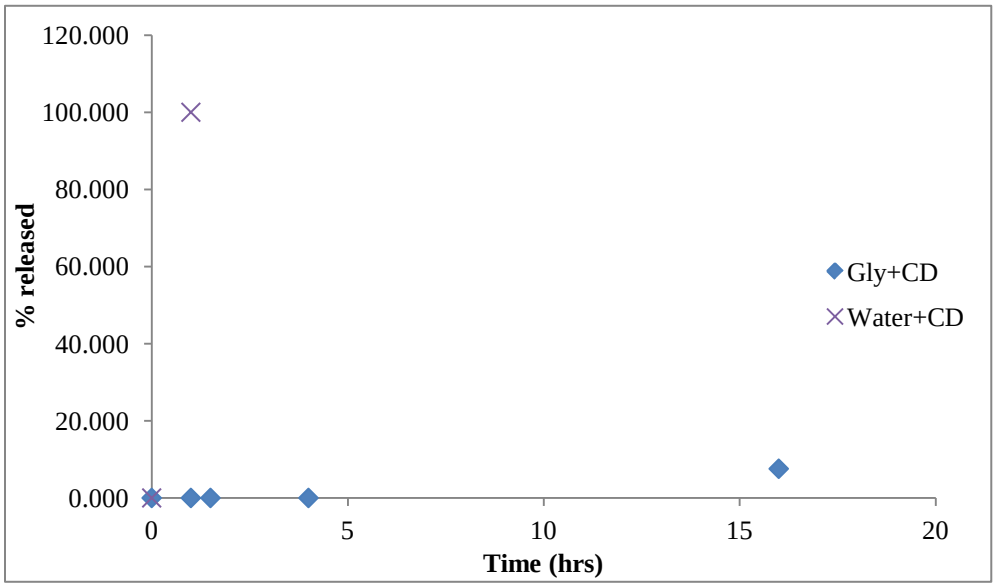



Figure 32: Effect of glycerol on retaining 1-MCP (α-cyclodextrin encapsulating 1-MCP)

### 5.4.2. Effect of xanthan gum

Results show that xanthan gum is effective to retain 1-MCP. After 1 hour, only around 6% of 1-MCP released, which is considerably less than water. Different concentrations, from 0.005% to 0.5%, did not make much difference, indicating that a low concentration of

xanthan gum is sufficient to retain 1-MCP.

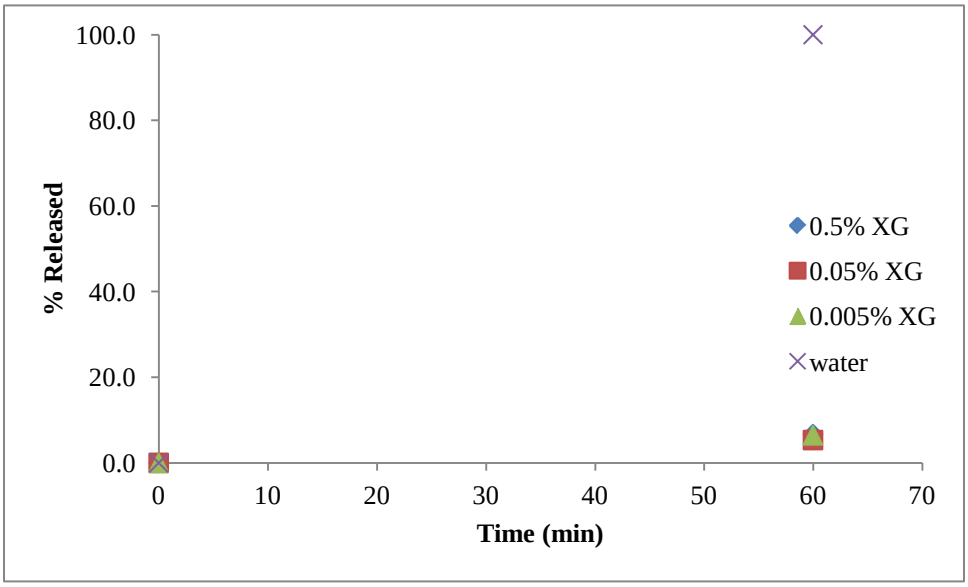



Figure 33: Effect of xanthan gum on retaining 1-MCP (α-cyclodextrin encapsulating 1-MCP)

### 5.4.3. Effect of combination of glycerol and xanthan gum

Results show that combination of glycerol and xanthan gum is effective to retain 1-MCP. There was no release of 1-MCP from high concentrations of xanthan gum solution (1%, 0.5%, and 0.25%) until 60 minutes. 1-MCP lost around 3% and 9% from 0.1% and 0.05% of xanthan gum formulation, respectively.

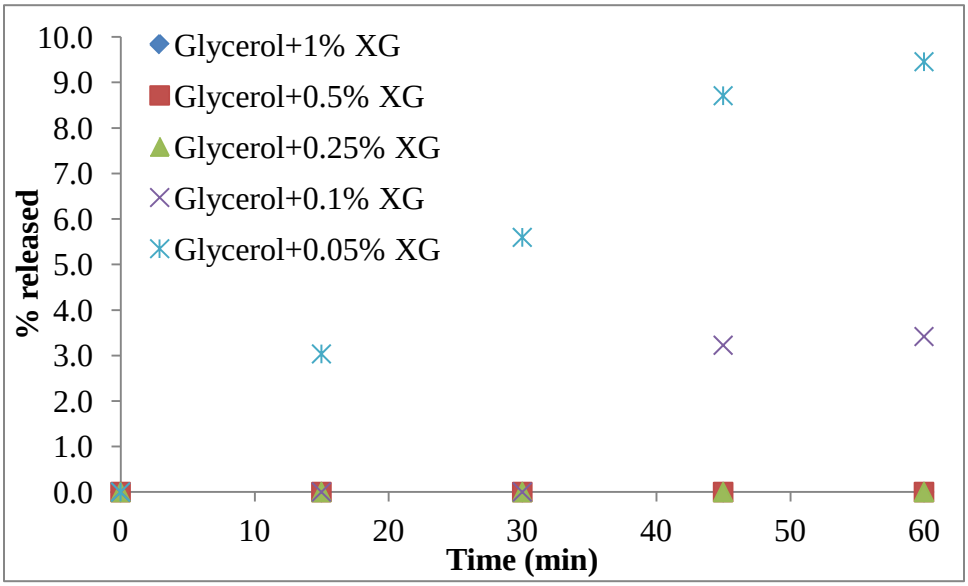



Figure 34: Effect of combination of glycerol and xanthan gum on retaining 1-MCP (α-cyclodextrin encapsulating 1-MCP)

5.4.4. Effect of combination of sorbitol and xanthan gum

Different concentrations of xanthan gum dispersed in water, ranging from 0.005% to 1% were evaluated for their efficacy in holding the release of encapsulated 1-MCP dispersed in D-sorbitol. The results were also compared to the effect of water alone (0% xanthan gum). The results show that increasing xanthan gum concentration in water from 0.00% to 1% shows down the release of encapsulated 1-MCP dispersed in D-sorbitol from 23.24% to 0% in 1 hour. Dispersing xanthan gum with 100% water (0% xanthan gum) results in 100% (complete release) of 1-MCP in 1 hour.

Results show that sorbitol is as effective as glycerol to retain 1-MCP. Higher concentrations (1%, 0.5%, and 0.25%) are more effective than 0.1% and 0.05%. 1-MCP lost around 1% and 3.5% from 0.1% and 0.05% xanthan gum formulation, respectively.

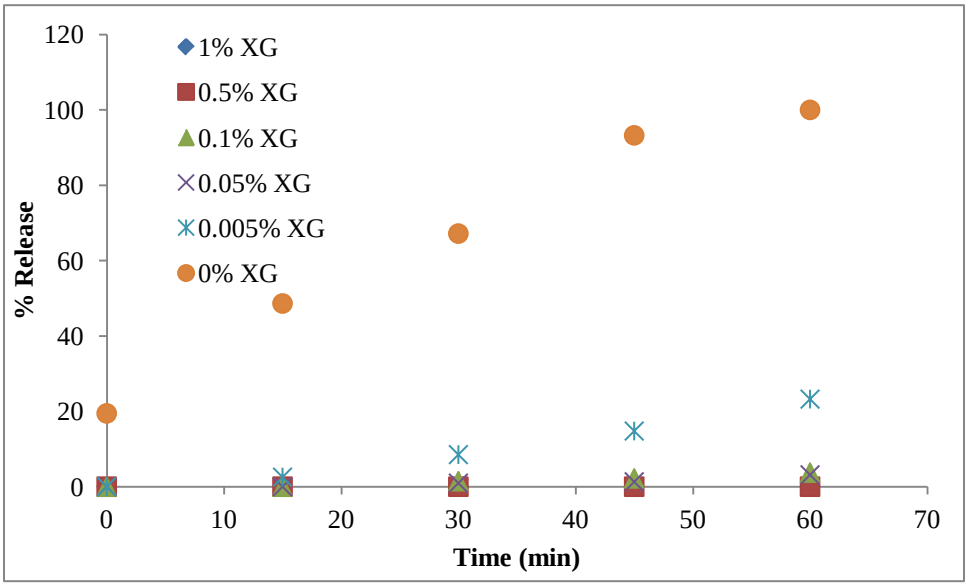



Figure 35: Effect of combination of sorbitol and xanthan gum on retaining 1-MCP (α-cyclodextrin encapsulating 1-MCP)

5.4.5. Comparison of glycerol and mineral oil

Results show that both glycerol and mineral oil are effective to retain 1-MCP. In the first 4 hours, there was no 1-MCP release detected. After 16 hours, 7.6% of 1-MCP released from glycerol. After 13.5 hours, 1.6% of 1-MCP released from mineral oil.

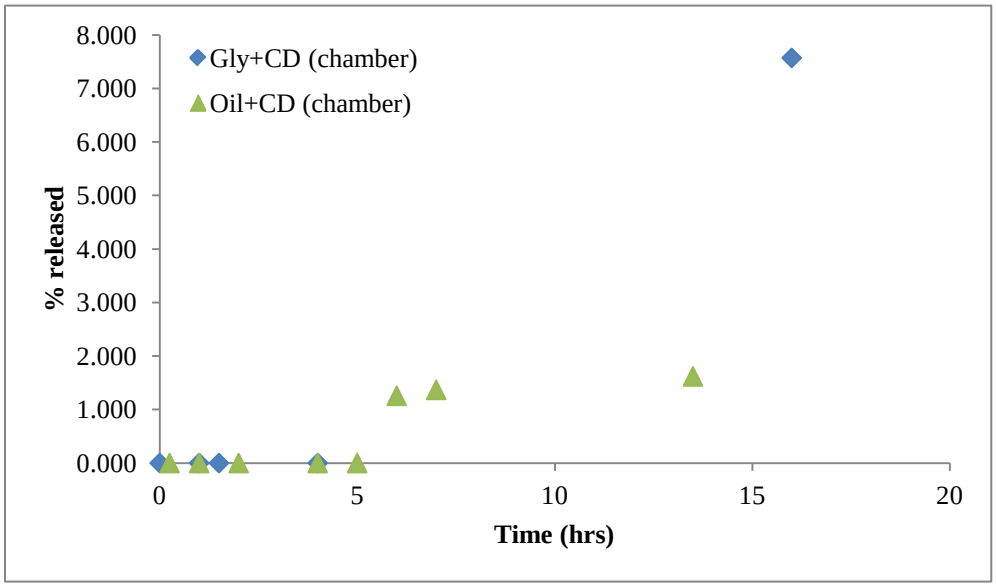


Figure 36: Retention of 1-MCP in glycerol and mineral oil

5.4.6. Combination of glycerol, polysorbate, HPC and maltodextrin

Table 19: Effect of combination of glycerol, polysorbate, HPC and maltodextrin (α-cyclodextrin encapsulating 1-MCP)

| **9 ml gly + 1 ml PS + 0.5 g HPC + 0.5 g MAL** | |
|---|---|
| Base A | |
| hr | % released |
| 0 | 0.302 |
| Base B (Base A + 0.05% XG 90 ml) | |
| 0 | 9.180 |
| 0.5 | 9.093 |
| 1 | 9.745 |
| 1.5 | 11.200 |
| 2 | 11.999 |

This formulation is effective to retain 1-MCP by using the combination of base A and B. Base A creates a viscous surrounding for α-CD and prevent its contact with water when base B (0.05% xanthan gum solution) is added. Because of the protection from base A, it is possible to use a relatively low concentration of xanthan gum.

## 5.5. Liquid formulation using modified β-cyclodextrin

5.5.1. Combination of glycerol and xanthan gum

Table 20: Effect of glycerol and xanthan gum on retaining 1-MCP (modified β-cyclodextrin encapsulating 1-MCP)

| Day | % released |
|---|---|
| 1 | 1.227 |
| 3 | 1.227 |
| 5 | 1.221 |
| 7 | 1.215 |
| 9 | 1.348 |
| 14 | 1.452 |
| 18 | 1.712 |

| | |
|---|---|
| 23 | 1.762 |
| 25 | 1.704 |

As shown in the table above, there was only a small amount of 1-MCP (~1.2%) released initially, but negligible release (~0.5%) was observed up to 25 days. The initial release is possibly caused by the shearing during mixing powder with the formulation. The results show that the 1-MCP encapsulated in modified β-cyclodextrin can be retained in this liquid formulation effectively.

### 5.5.2. Combination of glycerol, polysorbate, HPC and maltodextrin

Table 21: Effect of glycerol, polysorbate, HPC and maltodextrin on retaining 1-MCP (modified β-cyclodextrin encapsulating 1-MCP)

| | % released | |
|---|---|---|
| Day | Refrigeration Temperature | 50°C |
| 0 | 0.10 | 0.12 |
| 2 | 0.07 | 0.07 |
| 4 | 0.05 | 0.05 |
| 6 | 0.06 | 0.06 |
| 8 | 0.05 | 0.06 |
| 12 | 0.11 | 0.19 |
| 14 | 0.14 | 0.22 |
| 16 | 0.17 | 0.22 |
| 19 | 0.21 | 0.33 |
| 21 | 0.20 | 0.32 |
| 23 | 0.20 | 0.38 |

As shown above, similar results as the first formulation (glycerol and xanthan gum) were obtained that only a small amount of 1-MCP released initially, but limited release was observed afterwards. Because the headspace of this test was smaller than the first one, the amount released was also smaller. It is also interesting that the concentration in the headspace decreased in the first several days, probably because the headspace was too

small and the loss of withdrawn 1-MCP samples causes the fluctuation in % of release. In summary, 1-MCP can be retained in the above formulations in container with minimal headspace.

### 5.5.3. Stability of 1-MCP in the encapsulation complex

The stability of 1-MCP encapsulation complex powder was tested by quantifying the 1-MCP inclusion level as function of time. 0.2 g encapsulation complex (solid encapsulation method because of the higher inclusion level) was stored in a 1.5 ml vial. Vials were prepared for an 8-day trial. One vial was opened every day and the inclusion level was measured. Results show that there was a loading drop only in the initial two days and stable afterwards. The results indicate that certain amount of 1-MCP was only trapped on the surface, which can also be desorbed easily, and storage temperature did not make much difference.

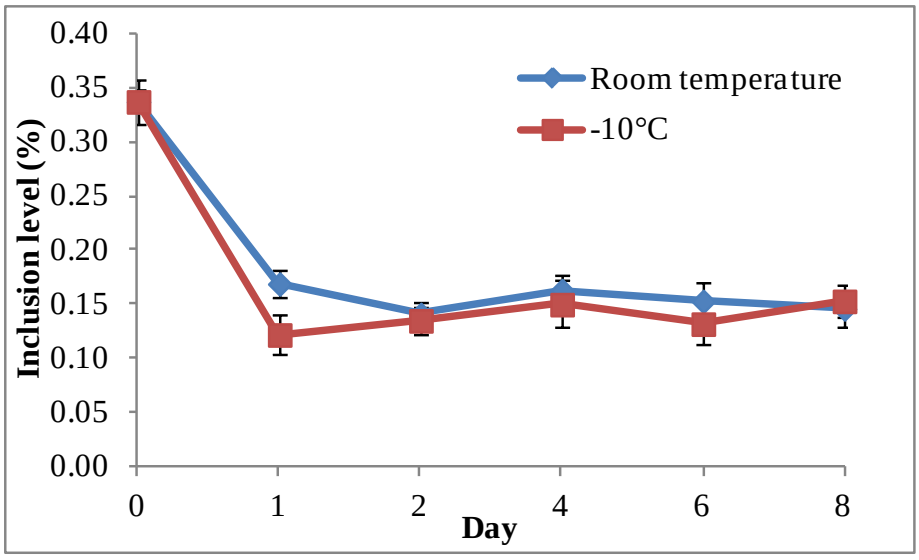



Figure 37: Stability test of 1-MCP in modified β-CD

5.5.4. Stability of 1-MCP in the formulation

Although the modified β-cyclodextrin encapsulation complex may not be ideal for retaining 1-MCP, addition of glycerol/HPC/polysorbate can dramatically improve the stability. The test was conducted at three temperatures (refrigeration, room temperature, and 50°C) with encapsulated 1-MCP suspended in formulation. Results show that increasing temperature did not cause any significant additional loss than at room temperature. More than 95% retention was still achieved after 30 days.

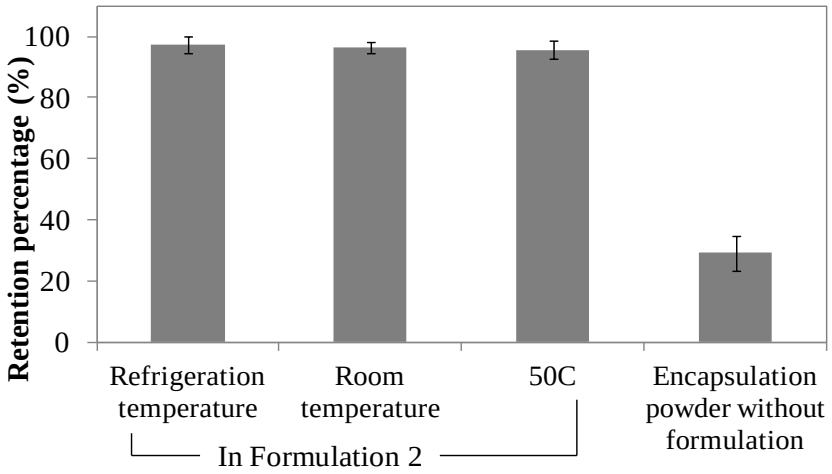



Figure 38: 1-MCP retention in Formulation 2 (glycerol/HPC/polysorbate) at 3 temperatures (after 30 days) and in encapsulation complex powder (no formulation after 2 days)

5.5.5. Optimize the initial loading of modified β-cyclodextrin in the formulation

2.5, 5, 10, and 15 mg of encapsulation complex were formulated with 23 ml of Formulation 2 and stored in 25 ml of closed vials. The headspace concentration of 1-MCP in each vial was measured over time. Results show that the initial loading within this range did not affect the retention. The retention ratio was between 93% and 98% after 30 days of room temperature storage.

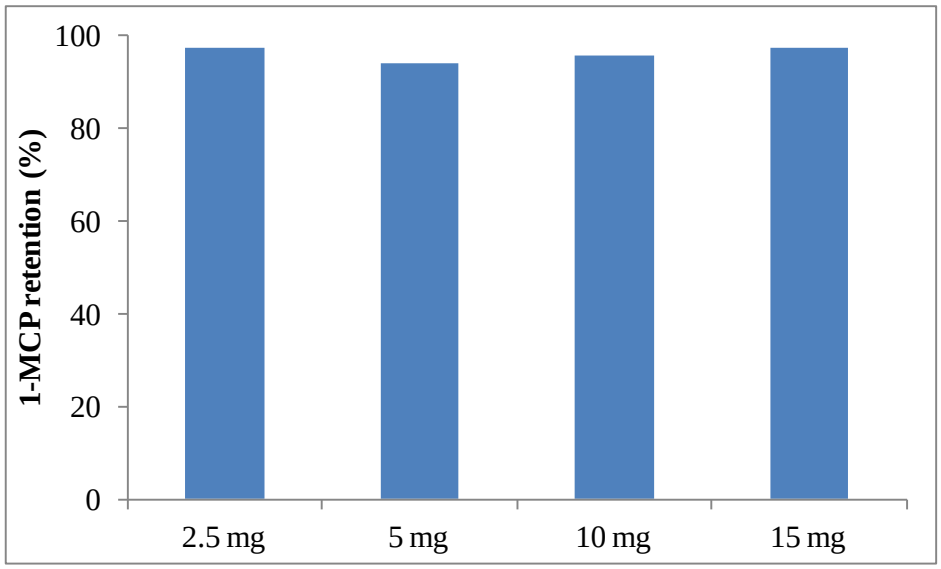



Figure 39: 1-MCP retention in liquid formulation with different initial loading

5.5.6. Test the uniformity of the formulation

Samples of Formulation 2 was shaken and divided into 8 equal portions which were placed into 8 containers with water. The mixture of formulation and water was stirred to release 1-MCP. The amount of 1-MCP in each container was then quantified. Results showed that the amount of 1-MCP in the 8 portions were approximately the same, and matched the theoretical amount calculated based on the total amount of 1-MCP β-cyclodextrin loaded.

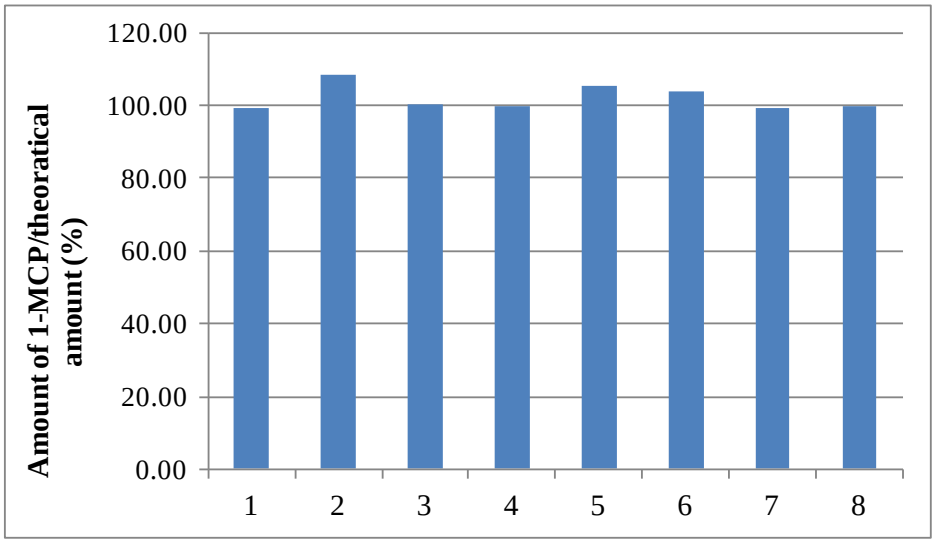



Figure 40: Uniformity of Formulation 2

5.5.7. Combination of glycerol, poly sorbate, HPC, and xanthan gum after spraying

A spraying test at room temperature was done to characterize the release of 1-MCP after spraying. Formulation 2 was used to disperse modified β-cyclodextrin containing 1-MCP, and 0%, 0.025%, and 0.05% of xanthan gum solution were used to dilute the formulation. The solution was sprayed into a 250 ml jar which was closed immediately after spraying. The headspace concentration of 1-MCP was measured over time. The spraying was performed using a handheld finger sprayer. Results show that 1-MCP released within 60 minutes from all the three concentrations of xanthan gum solution. As the concentration increases, the release was extended.

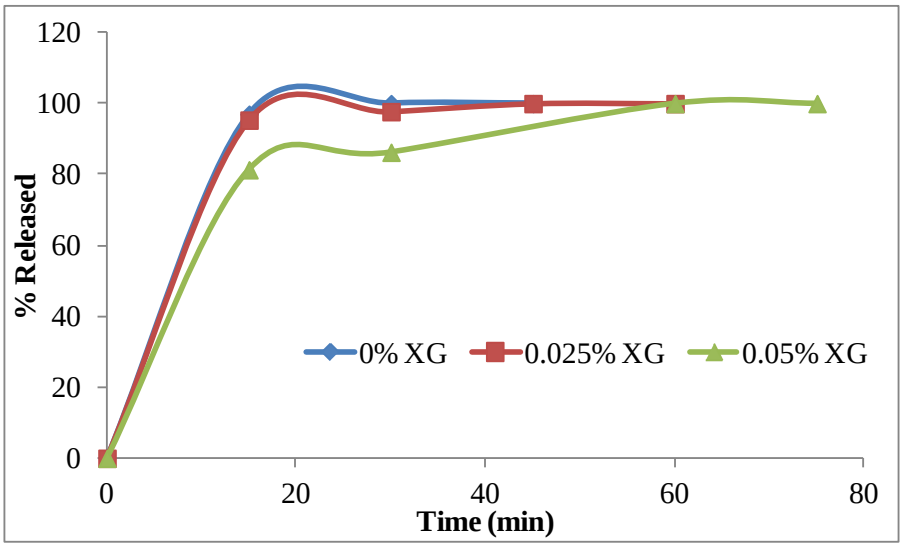



Figure 41: Release profile of 1-MCP from liquid formulation after spraying

### 5.6. 1-MCP stability in the formulation

It has been proven that 1-MCP can be retained in the glycerol based liquid formulation according to the results in the earlier section, however, it is also critical to confirm if there is no other chemical reaction occurred to it during storage.

The figure below shows that (A) 1-MCP can form isomer during storage in lithium salt, and (B) degrade in the liquid formulation. To solve these two challenges, during lithium salt synthesis, triethylamine was added as a stabilizer and/or intermediate whose function is to push the reaction to favor generation 1-MCP [99, 100]. During the vacuum drying process after lithium salt synthesis, methylhydroquinone was added as an antioxidant to prevent any reaction happening to 1-MCP lithium complex. The antioxidant mechanism of methylhydroquinone is free radical scavenging[102, 103] . As shown in C, isomerizaition also occurs in commercial available products, indicating that it is a

common issue for 1-MCP products.

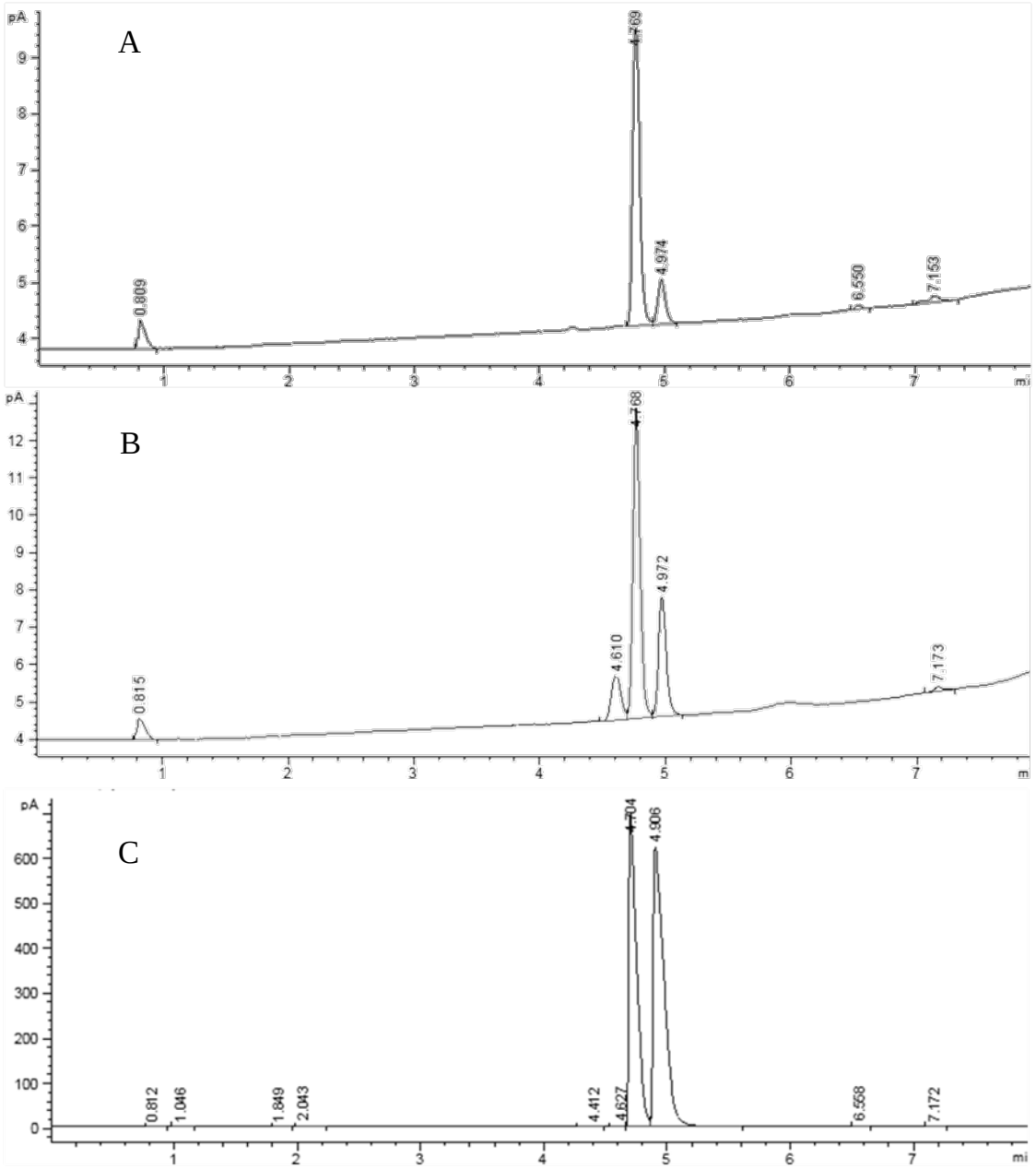



Figure 42: 1-MCP isomerization (A), degradation (B), and isomerization in commercial product (C)

5.6.1. Effect of level of heavy metal in glycerol

To solve the challenge of 1-MCP degradation, the individual compound of the liquid formulation was analyzed. It was found that in glycerol, there is 5 ppm heavy metal presence (MSDS and product specification did not specify the type of metal). Because 1-MCP is not stable with the presence of transition metal [104-106], glycerol was hypothesized to be the issue causing 1-MCP degradation. To prove it, different glycerol samples with different levels of heavy metal (1 ppm, 2 ppm and 5 ppm) were tested to disperse α–cyclodextrin/1-MCP complex and results are shown below.

Table 22: 1-MCP degradation as function of heavy metal concentration in glycerol at 55°C

| | 1 ppm | | 2 ppm | | 5 ppm | |
|---|---|---|---|---|---|---|
| Retention time* | 4.5 min | 4.7 min | 4.5 min | 4.7 min | 4.5 min | 4.7 min |
| Peak area | | | | | | |
| 5 hrs | 1.4 | 1488.8 | 2.4 | 565.2 | 2.4 | 267.3 |
| Day 1 | 2.2 | 45.7 | 2.6 | 29.6 | 2.3 | 52.0 |
| Day 2 | 1.5 | 22.8 | 2.6 | 13.5 | 2.2 | 10.8 |

*4.5 min: 1-MCP degradation product; 4.7 min: 1-MCP

Results show that the degradation occurred rapidly in all the treatments. As shown, as concentration of heavy metal increased, the peak area of 1-MCP decreased dramatically, and the peak area of 1-MCP degradation products increased correspondingly. The degree

of reduction of 1-MCP in terms of peak area and the increase of peak area of 1-MCP degradation did not increase as much, probably because there were also other degradation compounds formed or the response of the GC to the degradation products were not as sensitive as 1-MCP.

### 5.6.2. Effect of adding different antioxidants in glycerol

Different antioxidants (methylhydroquinone, BHT and triethylamine) were added into glycerol to improve the stability of 1-MCP. Concentrations used were 0.0001%, 0.001%, 0.01% and 0.1%. The peak areas of 1-MCP degradation products, 1-MCP, and 1-MCP isomer were measured to track the change of 1-MCP under these controls. All the samples were stored at 55°C to conduct accelerated shelf life study.

Results show that for all the antioxidants with all the concentrations, 1-MCP isomerization was prevented but not degradation. The prevention of degradation can be at different degrees with different concentrations. For methylhydroquinone, low concentrations are more effective, as the table shown, at concentrations of 0.0001% and 0.001%, there was more preventions, however, as the concentration of methylhydroquinone increased, the degradation occurred more dramatically. For BHT, different concentrations did not make any difference in its effectiveness in preventing 1-MCP degradation. Compared to methylhydroquinone, the effectiveness of BHT is similar. For triethylamine, the effectiveness is similar to BHT and methylhydroquinone, and there is no big difference between different concentrations.

Table 23: Effect of antioxidant on stability of 1-MCP at 55°C

| | Antioxidant concentration | Weight of formulation (g) | Peak area | | |
|---|---|---|---|---|---|
| | | | 1-MCP degradation product | 1-MCP | 1-MCP isomer |
| Methylhydroquinone | | | | | |
| Day 0 | 0.0001% | 0.5854 | | 104.741 | 1.83595 |
| | 0.001% | 0.5464 | | 91.89153 | 1.60043 |
| | 0.01% | 0.5506 | | 86.5881 | 1.64458 |
| | 0.1% | 0.701 | | 149.252 | 2.5779 |
| Day 1 | 0.0001% | | 0.5031 | 60.06351 | 1.19216 |
| | 0.001% | | 0.6726 | 60.8942 | 1.1113 |
| | 0.01% | | 2.25634 | 53.583 | 1.5248 |
| | 0.1% | | 6.2393 | 38.6699 | 1.4114 |
| BHT | | | | | |
| Day 0 | 0.0001% | 0.6189 | | 131.2836 | 2.29032 |
| | 0.001% | 0.5643 | | 102.5078 | 1.72422 |
| | 0.01% | 0.6461 | | 161.6419 | 2.82771 |
| | 0.1% | 0.6114 | | 125.7955 | 2.25033 |
| Day 1 | 0.0001% | | 0.9837 | 59.2748 | 1.5041 |
| | 0.001% | | 0.5726 | 37.131 | 0.949 |
| | 0.01% | | 1.6012 | 53.7888 | 1.5095 |

| | | | | | |
|---|---|---|---|---|---|
| | 0.1% | | 0.7693 | 63.62245 | 1.5385 |
| Triethylamine | | | | | |
| Day 0 | 0.0001% | 0.5902 | | 107.3504 | 1.8427 |
| | 0.001% | 0.5864 | | 154.7576 | 2.6966 |
| | 0.01% | 0.585 | | 110.2093 | 1.835 |
| | 0.1% | 0.6317 | | 131.5661 | 2.2049 |
| Day 1 | 0.0001% | | 0.8879 | 57.6307 | 1.6708 |
| | 0.001% | | 0.6879 | 58.6 | 0.1046 |
| | 0.01% | | 1.5667 | 55.7665 | 1.8097 |
| | 0.1% | | 1.8997 | 43.5779 | 1.5385 |

5.6.3. Effect of chelating agents

EDTA and citric acid were tested as chelating agent to prevent 1-MCP degradation. Similarly to antioxidant, chelating agent at certain concentrations was able to prevent 1-MCP degradation only to certain extend. At some concentrations, the color of the formulation changed to red after 1 day of storage at 55°C, indicating the occurrence of certain chemical reactions. The results are summarized in the table below.

Table 24: Effect of chelating agent on stability of 1-MCP at 55°C

| | Antioxidant | Weight of | Peak area |
|---|---|---|---|

| | concentration | formulation (g) | 1-MCP degradation product | 1-MCP | 1-MCP isomer |
|---|---|---|---|---|---|
| EDTA | | | | | |
| Day 0 | 1% | 0.5919 | | 103.8933 | 1.66812 |
| | 0.5% | 0.6011 | | 108.4599 | 1.79404 |
| | 0.1% | 0.6023 | | 108.4791 | 1.82064 |
| | 0.05% | 0.5987 | | 98.54951 | 1.96394 |
| | 0.01% | 0.5957 | | 110.8286 | 1.71719 |
| Day 1 | 1% | | 1.90714 | 9.56936 | 1.09231 |
| | 0.5% | | 1.24425 | 69.19106 | 2.23799 |
| | 0.1% | | 1.26588 | 67.5887 | 1.50081 |
| | 0.05% | | 0.98766 | 77.16534 | 1.69642 |
| | 0.01% | | 0.9677 | 78.29698 | 1.791 |
| Citric acid | | | | | |
| Day 0 | 5% | 0.3476 | | 49.01 | 0.58033 |
| | 1% | 0.6115 | | 97.708 | 0.92458 |
| | 0.5% | 0.5204 | | 90.38618 | 1.72932 |
| | 0.05% | 0.586 | | 115.1563 | 3.61806 |
| Day 1 | 5% | | 0.9889 | 46.78 | 0.5631 |
| | 1% | | 1.3588 | 16.98676 | 0.5333 |

| | | | | | |
|---|---|---|---|---|---|
| | 0.5% | | 1.3799 | 13.48166 | 0.5683 |
| | 0.05% | | 1.5668 | 13.1335 | 0.60798 |

5.6.4. Replacing glycerol with mineral oil

As shown in the previous section that compared the retention effect of glycerol and mineral oil for 1-MCP, mineral oil also had equivalent effect as glycerol, therefore, mineral oil was tested for the same test. Results show that 1-MCP did not degrade in mineral oil. However, another challenge with using mineral oil is that cyclodextrin powder cannot be dispersed uniformly and a dispersion solution needs to be developed for application.

Table 25: Effect of mineral oil on 1-MCP degradation

| | Weight of formulation (g) | Peak area | | |
|---|---|---|---|---|
| | | 1-MCP degradation product | 1-MCP | 1-MCP isomer |
| Day 0 | 0.3555 | | 307.6462 | 6.13592 |
| Day 1 | 0.3477 | | 255.041 | 11.6753 |
| Day 2 | 0.3674 | | 356.8571 | 11.1855 |
| Day 4 | 0.2434 | | 318.6776 | 6.13592 |

5.6.5. Development of mineral oil based formulation

The same formulation used before was adapted and glycerol was replaced using mineral oil. Results show that it can disperse α–cyclodextrin powder uniformly and 1-MCP is

stable in the formulation for an extended period of time.

Table 26 shows that 1-MCP is distributed uniformly in the formulation. Among 8 samples tested, the 1-MCP level ranges from 0019% to 0.021%.

Table 26: Uniformity of 1-MCP in mineral oil based formulation

| Sample weight (g) | 1-MCP peak area | 1-MCP level in sample (%) |
|---|---|---|
| 1.5275 | 466.28012 | 0.020 |
| 1.5160 | 441.73026 | 0.020 |
| 1.5317 | 492.64673 | 0.021 |
| 1.5119 | 454.05414 | 0.021 |
| 1.531 | 425.7867 | 0.019 |
| 1.5168 | 479.23288 | 0.020 |
| 1.5166 | 455.07831 | 0.021 |
| 1.5223 | 458.19614 | 0.021 |

Table 27 shows that 1-MCP was stable in mineral oil based formulation at 55°C for over 10 days (0.61%-0.7%). The test extended to 24 days and 0.47% was retained (30% loss compared to initial at 0.67%). The results show that it is stable for almost two weeks which is equivalent to 12 months of room temperature shelf life (24 months in Europe regulation), indicating the successful development of a stable formulation as stated in sub-objective 3.

Table 27: Stability of 1-MCP in mineral oil based formulation at 55°C

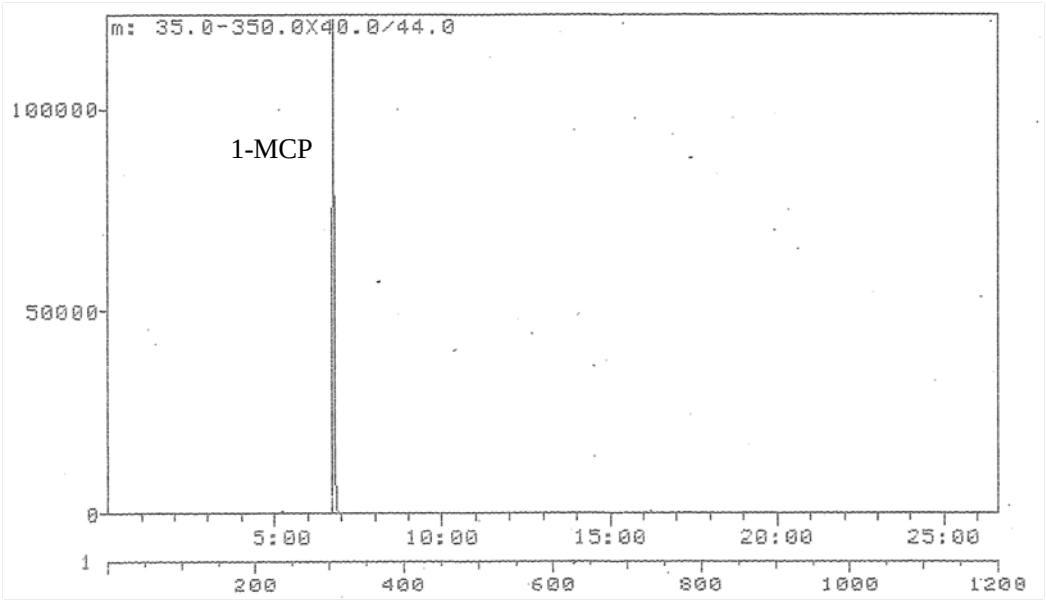
| Day | Sample weight (g) | 1-MCP level (%) |
|---|---|---|
| 0 | 1.17 | 0.67 |
| 1 | 1.18 | 0.66 |
| 2 | 1.18 | 0.63 |
| 3 | 1.17 | 0.62 |
| 4 | 1.17 | 0.68 |
| 5 | 1.18 | 0.62 |
| 6 | 1.16 | 0.66 |
| 7 | 1.17 | 0.69 |
| 8 | 1.14 | 0.67 |
| 9 | 1.1 | 0.61 |
| 10 | 1.18 | 0.7 |
| 12 | 1.17 | 0.64 |
| 13 | 1.15 | 0.60 |
| 14 | 1.17 | 0.59 |
| 15 | 1.1 | 0.57 |
| 19 | 1.1 | 0.47 |
| 24 | 1.1 | 0.47 |

## 5.7. Biological efficacy of developed formulation

### 5.7.1. Biological efficacy test using GC-MS

GC-MS results show that 1-MCP released from our developed formulation is in its biologically active monomer form and no dimer or isomer (biologically inactive) were identified at 100 ppm which is much higher than a common 1-MCP treatment level at 1 ppm. Dimer and isomer were only identified at higher concentration 10,000 ppm.

A: 100 ppm




B: 10,000 ppm

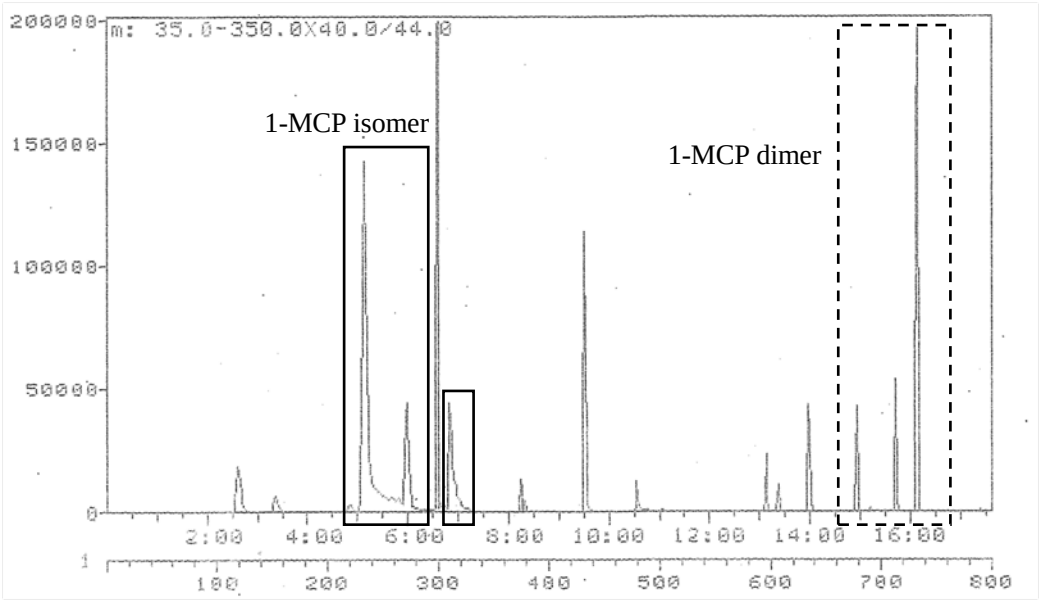



Figure 43: GC results of 1-MCP release from the formulation at two concentrations (A) 100 ppm, and (B) 10,000 ppm

5.7.2. Real food test

The results show that after 4 days, treated banana still retained fresh color (color stage 4.5), whereas control banana advanced to color stage 5.5 and developed sugar spot. Therefore, the 1-MCP released from modified β-cyclodextrin proved its biological efficacy.


Figure 44: Bananas treated with 1-MCP vs control

In addition to pre-harvest application as a spraying solution, the formulation can also be used as post-harvest. The potential products can be in tablet or capsule form. The schematic diagram below demonstrates the application. The formulation can also be stored in a water soluble pouch which would be placed into water to release 1-MCP.

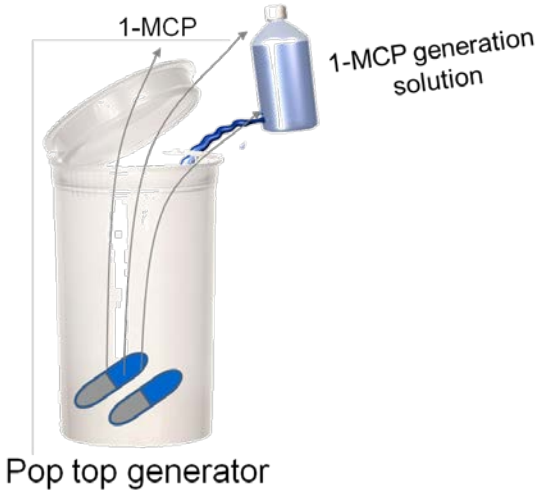



Figure 45: Potential application in post-harvest

# 6. CONCLUSION

**Sub-objective 1:** Modification of β-cyclodextrin was optimized to yield 90-95% powder. The powder has better modified pore size and particle size, which are more favorable for 1-MCP encapsulation than β-cyclodextrin.

**Sub-objective 2:** 1-MCP can be encapsulated under 0.1 atm headspace concentration both using solid-gas method (inclusion level 0.5%) and in solution-gas method (inclusion level 0.3%).

**Sub-objective 3:** A stable formulation was developed consisting of mineral oil, HPC and polysorbate for both α and modified β-cyclodextrins. After 30 days of storage at room temperature, more than 90% of 1-MCP can be retained in the formulation. After 14 days of storage at 55°C (equivalent to 1 year storage at room temperature), more than 80% of 1-MCP can be retained in the formulation.

**Sub-objective 4:** After hydration using different concentrations from 0 to 0.5% xanthan gum, we were able to spray the liquid formulation and control the release of 1-MCP from the sprayed liquid in a controlled manner for 1 to 6 hours to obtain complete release.

**Sub-objective 5:** 1-MCP released from the formulation is biologically effective that no dimer or isomer formation at treatment level (1 ppm). It was able to extend the shelf life of bananas (color stage 2-2.5) from 2 days to 5 days at room temperature.

# 7. FUTURE WORK

**Task 1: Identify an alternative encapsulant for 1-MCP**

In this dissertation, modified β-CD was tested as an encapsulant for 1-MCP, however, its inclusion ratio is not comparable to α–CD, because the pore size after modification is still not suitable to trap 1-MCP. Therefore, identification of an alternative encapsulant that is more cost effective with higher 1-MCP loading is critical for developing 1-MCP technology.

**Task 2: Study the 1-MCP degradation, isomerization, and dimerization**

In this dissertation, we have shown that 1-MCP can degrade with the presence of heavy metal, it is critical to further investigate the mechanism of the degradation. Based on the experience of our research group, isomerization (although was solved in this work), is a common issue for some commercial 1-MCP products and the mechanism also needs to be further understood. We did not investigate much on the dimerization of 1-MCP, but it is known that it occurs at high concentration. Further study on the dimerization of 1-MCP at different concentrations would be helpful to improve the biological efficacy of 1-MCP (1-MCP application usually is at low concentration, dimerization would not occur, but the understanding of the functional relationship between concentration and dimerization is useful for designing the treatment concentration to deliver biological effective 1-MCP where higher concentration is required).

**Task 3: Conduct more real food test**

In this dissertation, we have proved the biological efficacy of our formulation on bananas, real food test on both pre-harvest and post-harvest applications needs to be conducted to determine the release profile and efficacy of 1-MCP for real application.

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