

# HPMC Inhibit Mannitol Re-crystallization in Air-dried Liposome Formulations

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**Abstract:** Liposome defined as a spherical vesicle, which is formed when phospholipids are being hydrated with an aqueous environment. This study investigated the role of HPMC to inhibit mannitol crystallization in the air-dried liposome formulation. HPMC has been used in solid dispersion to prevent crystallization drugs. The mannitol and HPMC were included in the developed formulations with four different ratios and air-dried at 40°C for 120 h. Liposome was composed of SPC:DDA: Chol (9:3:1 in molar ratio). XRD data showed crystalline-forming properties as a function of mannitol and HPMC ratio. The formula with the highest mannitol: HPMC ratio (4:1) showed the most apparent crystallinity, while the lowest proportion (2:3) indicated amorphous solid. DTA analysis found that the following formula showed a broad endothermic peak at 150-170°C, indicating amorphous solid. SEM data supported these results where no crystalline structure was observed in the latter formulation. The rest formula showed partially amorphous and partially crystalline. It can be concluded that the incorporation of HPMC as a dispersion matrix potentially inhibits crystal formation in the developed formulation

## 1 INTRODUCTION

Liposome was defined as a spherical vesicle, which is formed when phospholipids are being hydrated with an aqueous environment (Kaur, 2011). Liposome, which is widely used in drug delivery, is well known for its versatility for delivering both hydrophilic and lipophilic agents (Çağdaş, Sezer, & Bucak, 2014; Chen, Han, Cai, & Tang, 2010). Research on producing liposomes into solid materials has been conducted extensively since solid liposome has more advantages in terms of stability compared to the liposome in water suspension. Decreasing molecular mobility in a solid-state may decrease chemical degradation, which leads to physical instability (Mohammed, Bramwell, Coombes, & Perrie, 2006).

However, dehydration could also be detrimental for the liposome since removing water from the system will cause the vesicle structure to collapse. To prevent this, compounds that are rich in hydroxyl group are employed to interact with the phosphate head, thus replacing the water molecules during drying (Franks, 2007; Ingvarsson, Yang, Nielsen,

Rantanen, & Foged, 2011). Mannitol is an alcoholic sugar that is widely used in the drying process of the liposome. However, mannitol is easily re-crystallized and potentially damaging bi-layer the membrane (Li et al., 2016).

We tried to address the problem by employing hydroxypropylmethylcellulose (HPMC) as a dispersing matrix, which will help to suppress the re-crystallization of mannitol (Kiew, Cheow, & Hadinoto, 2015). HPMC is a cellulose-derived polymer that methylated and hydroxy-propylated (Rowe, 2009). It is widely used as bioadhesive material, a controlled release agent, a dispersion agent, and an increasing viscosity agent. To produce a stable liposome vesicle, we used a combination of soy phosphatidylcholine (SPC) as principal lipid constituent, bromide salt of dimethyl dioctadecyl ammonium (DDAB) which is cationic, and cholesterol (Patent No. US20150079156 A1, 2015).

We observed the effects of mannitol in combination with HPMC as a dispersing matrix in different ratios on the physical characteristics of the solid dried products. Powder X-Ray Diffraction

(PXRD), and Differential Scanning Calorimetry (DSC) were used to investigate the properties of the products. The procedures of physical characterization were conducted according to our previous work (Yusuf, 2013).

## 2 METHODS

### Materials

The lipid phase for liposome formulation was Dimethyl-Dioctadecylammonium (Sigma Aldrich, Singapore) and Soy Phosphatidylcholine (Lipoid GmbH, Germany), and cholesterol (Sigma-Aldrich, Singapore). The use of cholesterol was to enhance liposomal membrane stability. The protectant used in this research was Mannitol (Sigma-Aldrich, Singapore), a poly-alcohol compound. Hydroxypropyl-methyl-cellulose (Shin-Etsu, Japan) was selected as a dispersion matrix to increase physical stability of the products in terms of increasing the total mass of the products. Methanol (E. Merck) was chosen to dissolve all of the lipid phases in the liposomal ingredients. All materials used were of analytical grade.

### Research Procedure

The technique for liposome formula preparation was thin-film hydration methods in which the lipid phase solution was evaporated and hydrated using water medium. The lipid phase was dissolved in methanol proportionally SPC:DDA: Cholesterol = 9:3:1.

The thin-film, which was formed after the evaporation using a rotary evaporator (Büchi, Germany) for 60 minutes hydrated with a solution of mannitol in various concentrations that had previously warmed to facilitate miscibility. Had been done at 50°C for 10 minutes, the appearance of white liquid suspension indicate the formation of liposomal suspension. Liposome suspension sonicated for 5 minutes to produce a smaller vesicle size. HPMC powder was weighed according to Table 1, and dispersed in 5 mL purified water to form HPMC gel.

Liposome suspension was combined into HPMC gel and stirred until a homogeneous mixture achieved and portioned into vials for air-drying. The temperature and time of the air-drying was 40°C for 72 hours.

Table 1: The composition of Mannitol and HPMC in the Formula

Formula	Mannitol Weight (g)	HPMC Weight (g)	Mannitol: HPMC
FMO1	0,250	0,125	2:1
FMO2	0,500	0,125	4:1
FMO3	0,250	0,375	2:3
FMO4	0,500	0,375	3:4

### Differential Thermal Analysis (DTA)

The thermal profiles of the solid samples were analyzed using DTA instrument (Mettler Toledo, Switzerland). The sample is put into aluminum crucibles and scanned from 30°-300°C at a heating rate of 10°C/min.

### X-Ray Diffraction Analysis (XRD)

The crystallinity of the dried products analyzed using Powder X-Ray Diffraction Instrument (Phillips X'Pert PRO PANalytical, Netherlands). The samples inserted into the sample holder and flatten. The condition of analysis was using Cu metal target and K $\alpha$  filter. The voltage and current were 40 kV and 40 mA, performed at room temperature, in a range of 2 $\theta$  of 5-40°.

### Scanning Electron Microscopy (SEM)

A small amount of the samples was dispersed and glued onto 25 mm diameter sample holder. The samples were sputter-coated with 5 nm layer of Au-Pd (Gold-Palladium). The observation was completed using 500 and 1000 magnitude.

## 3 RESULTS AND DISCUSSION

The formulations profile was partially amorphous. The results could be seen from the presence of broad peaks in the temperature range 96-116 for FMO1, FMO2, and FMO3, which correspond to endothermic transition temperatures for HPMC. The results show that some of the material has been incorporated to form amorphous solid dispersions and is the expected profile of the product because it indicated the success of the vitrification mechanism in maintaining the physical stability of the dry liposome (Ingvarsson et al., 2011).

Table 2: Endothermic peak list in DTA thermogram

	T1 (°C)	ΔH1 (J/g)	T2 (°C)	ΔH2 (J/g)	T3 (°C)	ΔH3 (J/g)
SPC	99.54	284.49				
DDAB	90.18	145.50				
Cholesterol	149.31	79.8				
Mannitol					168.3	190
HPMC	88.37	169.95				
FMO1	116.3	17.4			166.5	72.2
FMO2	96.7	10.7	157.4	9.50	172.5	113.0
FMO3	100.3	32.1	157.5	11.4	171.8	48.7
FMO4			160.3	62.3		

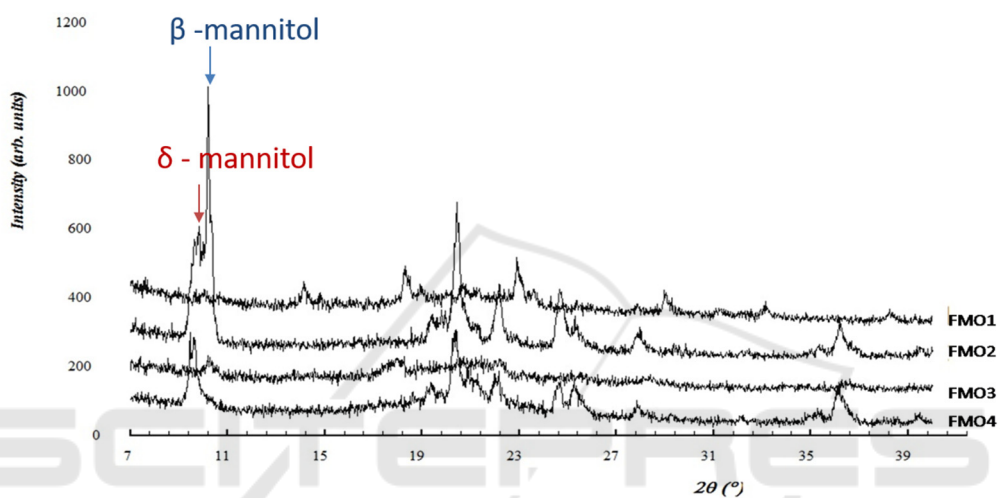


Figure 2: The X-ray diffractogram profile of air-dried liposome formulation with mannitol in combination with HPMC in a different ratio, according to table 1.

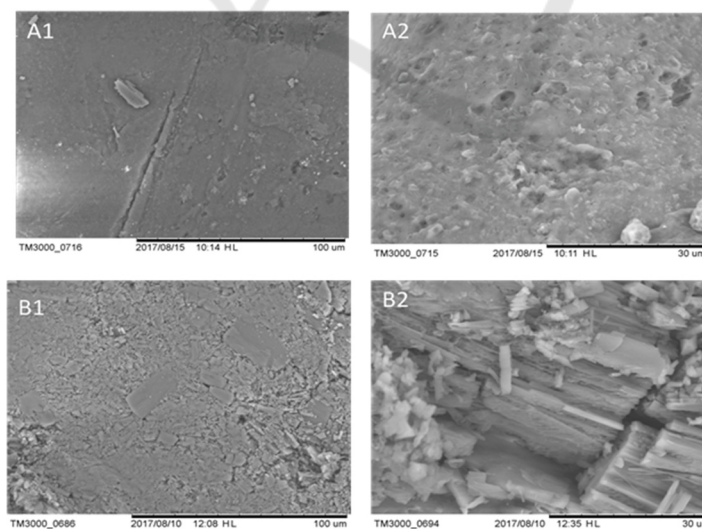


Figure 3: Morphology of liposome formulation characterized by SEM. A1 and A2 were FMO3, while B1 and B2 were FMO4.

However, there are still sharp peaks that may correlate with mannitol transition temperatures. The results of the thermal analysis showed that there were new peaks (listed in Table 2) different from the original substances. Mannitol is a material which has different morphological phases (polymorphism),  $\beta$ - and  $\delta$ -mannitol polymorph had endothermic peaks in 157° and 167° Celsius, respectively (Barreneche, Gil, Sheth, Inés Fernández, & Cabeza, 2013). The peak of pure mannitol was observed in 168.3°C, indicating  $\delta$ -mannitol existence; no other peak was observed. However, observations of thermal analysis of the formulations showed two peaks that existed together in FMO2 and FMO3. From this data, we could conclude that  $\beta$ -mannitol was formed during the formulations processes as there was no raw material which had endothermic peaks at such temperature.

Interestingly, only  $\beta$ -mannitol observed in FMO1, and only  $\delta$ -mannitol was found in FMO4. The difference between the FMO1 and FMO4 formulas is in the ratio of the weight of mannitol: HPMC, which are 2:1 and 3:4, respectively. Mannitol levels in FMO1 are higher than HPMC, so the shape of the delta mannitol, which has existed since the beginning, is relatively unchanged. Whereas in FMO3, the HPMC ratio is higher than that of mannitol; this is what might trigger the shift from delta to beta mannitol. High HPMC ratios also occur in FMO3, but in this formula, the amorphous form dominates, as evidenced by the relatively high endothermic enthalpy at 100.3 ( $\Delta H_1=32,1$  J/g). The differences in endothermic enthalpies of the peaks indicating different energy levels of thermodynamic transitions in the formulations. These results will be confirmed using the crystallinity profile from X-Ray powder diffraction.

The X-Ray diffractogram results showed that FMO3 was an amorphous form, characterized by the absence of intensive crystalline peaks (Kiew et al., 2015). Nevertheless, the sharp peak of mannitol detected from the formulas FMO2 and FMO4. According to the previous investigations, the characteristic peaks of  $\beta$ -mannitol was 10.56° and 14.71°, the  $\alpha$ -mannitol was 13.79°, and  $\delta$ -mannitol was 9.57° (Vanhoorne et al., 2016). In FMO2, it is confirmed that there is a mixture of  $\beta$ - and  $\delta$ -mannitol all at once. The results show that the difference in the ratio of mannitol: HPMC in the formula is very influential on the phase behavior even though although the process is carried out uniformly.

Morphological analysis with SEM instruments also supports the DTA and XRD examination. The FMO3 formula is known to provide an amorphous and porous surface image, while FMO4 on the same

scale shows a high crystallinity (Haque & Roos, 2005). As a carrier for dry liposomes, the FMO3 profile is preferable because liposomes can be incorporated and protected during the drying process (Nugraheni, Setyawan, & Yusuf, 2017).

## 4 CONCLUSIONS

The incorporation of HPMC as a dispersion matrix potentially inhibits crystal formation in the developed formulation, especially in FMO3, which is the most suitable carrier for air-dried liposome compared to the other formulations.

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