The Preservation of Liposomes During Air Drying Using a Matrix Containing Maltodextrin and HPMC

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Abstract: This study used maltodextrin as a protectant to stabilize liposomes during air drying and Hydroxypropyl Methyl Cellulose (HPMC) as solid dispersion matrix that could provide a barrier to the coalescence of liposomes. The purpose was to optimize the composition of the matrix to protect liposomes. The liposome suspension was prepared with the thin-film hydration method using three lipid components with the molar ratio of SPC:DDA:Chol = 9:3:1. The maltodextrin was dissolved in water and used in the experiment as the hydration liquid. The formulations included maltodextrin and HPMC with 4 (four) different ratios. Then, they were air-dried at the same condition (40ºC for 120 hours). The solid products were characterized using Powder X-Ray Diffraction (PXRD), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM). The PXRD analysis showed that all of the formulations developed in this study had an amorphous structure. However, the formulations showed peak splitting in the DSC analysis. The differences in the crystalline lamellar thickness of maltodextrin might be the cause of these results during the air drying. The successful preservation of liposomes was analyzed using SEM photomicrography. Compared with the other formulations, F2MO3 created the best protection for liposomes. The inclusion of HPMC as a dispersion matrix into the liposome formulation potentially inhibits crystal formation during the drying process and, therefore, provides better protection for the lipid bilayer.

1 INTRODUCTION

Liposome has particular advantages in vaccine delivery, especially when cationic lipid components are included in its formulation as a lipid bilayer (Agger *et al.*, 2008). One of the most potential cationic lipids is DDA (dimethyl dioctadecyl ammonium bromide) (Kaur *et al.*, 2014). The use of DDA in liposome formulations is still complicated because it involves stability issues. First, due to its physical instability, DDA is not suitably used as a single lipid constituent (Kett *et al.*, 2015). Second, the electrostatic repulsion caused by its positive charge is not sufficient to prevent the physical aggregations of liposomes (Kallerup *et al.*, 2015).

This research offers strategies to enhance the stability of liposomes by adding sugars and dispersing matrix, i.e., hydroxypropyl methylcellulose (HPMC), into the developed formulation. The role of sugar, e.g., maltodextrin, and HPMC is to provide the glassy amorphous matrix to inhibit the recrystallization of the components in the formulations (Corveleyn & Remon, 1996; Ingvarsson *et al.*, 2011; Yu, 2001). To prove the hypothesis, this research evaluated the physical behavior of these components during the drying process. Air drying is preferable because it is simpler (i.e., it does not need special equipment) and cheaper compared with the alternatives, namely freeze-drying and spray-drying process.

2 MATERIALS AND METHOD

2.1 Materials

For the components of the liposomes, this research used Dimethyl-dioctadecyl ammonium (Sigma Aldrich, Singapore) and soy phosphatidylcholine S-

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Table 1: The formulations of liposomes

Formulation Codes	Maltodextrin Concentrations	HPMC Concentrations	Maltodextrin:HPMC (Weight ratio)
F2MO1	5%	2.5%	2:1
F2MO2	10%	2.5%	4:1
F2MO3	5%	7.5%	2:3
F2MO4	10%	7.5%	4:3

100 (Lipoid GmBh, Germany). The addition of cholesterol (Sigma-Aldrich, Singapore) to the formulations was expected to provide a membrane stabilizer. Maltodextrin (Sigma-Aldrich, E:13-17) was used as a lyoprotectant to stabilize the liposomes during the air-drying process. HPMC 15000 (Metolose 90SH-15000SR, Shin-Etsu, Japan) functioned as a dispersion matrix to increase the mass of the end product. This research chose methanol (analytical grade, Merck) as a solvent to facilitate the mixing of liposomal ingredients.

2.2 Methods

The liposomes were prepared with the thin-film hydration methods. The membranes consisted of SPC, DDA, and cholesterol dissolved in methanol. The molar ratio of the constitutive elements of the lipid bilayer was SPC:DDA:Cholesterol = 9:3:1. The lipid phase was then evaporated in a vacuum condition at 45°C for 60 minutes using a rotary evaporator (Büchi, Germany). The process left a thin film on the evaporator wall. Afterward, the hydration procedure started using the pre-warmed solution of maltodextrin (5 mL) in various concentrations (Table 1). The hydration process was carried out at a temperature of 50°C for 10 minutes. The appearance of white-milky suspension indicated a successful formation of liposomes. Then, the liposome suspensions were sonicated for 5 minut es. The HPMC powder was weighed according to the details in Table 1 and dispersed in 5 mL of purified water to form HPMC gel. The liposome suspensions were added to the HPMC gel, mixed until homogeneous, and divided into the vials for drying.

The characterizations of the solid products by XRD, DSC, and SEM were carried out according to Nugraheni *et al.* (2017). The condition of the XRD analysis was as follows: Cu as an anode, Kα filter, a generator set to 40 kV/30 mA, and room temperature. The study was carried out at a 2theta

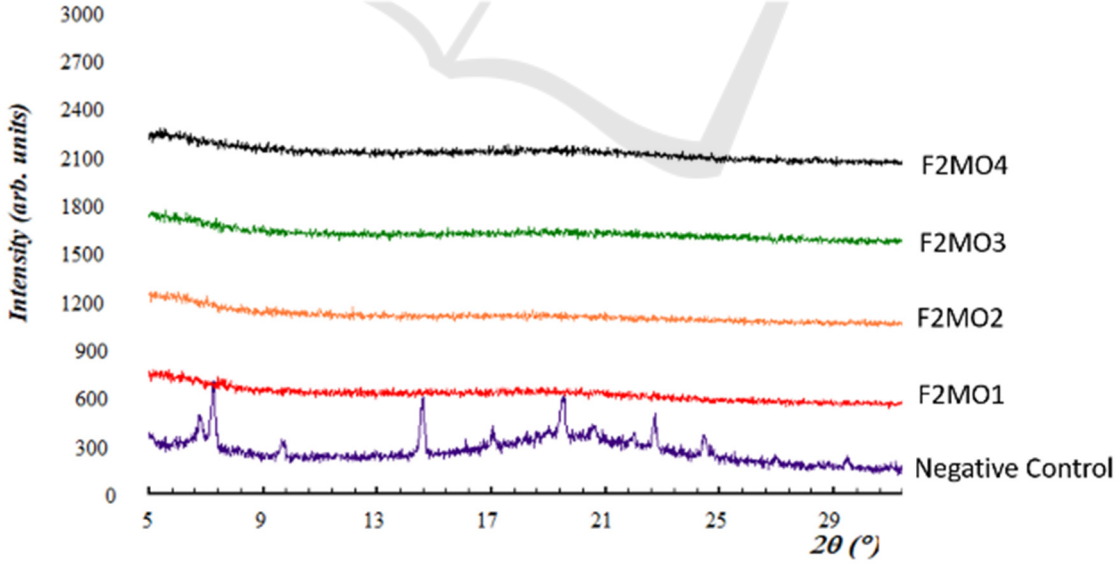

Figure 1: The X-Ray Diffraction pattern of samples containing different concentrations of maltodextrin and HPMC (as seen in Table 1). The negative control (purple) was dried lipid components without maltodextrin and HPMC.

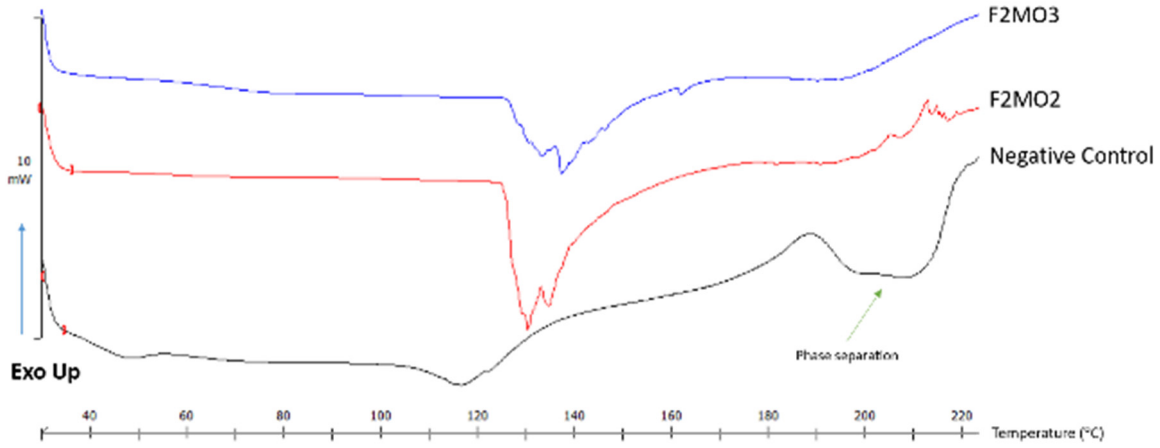

Figure 2: The DSC thermogram pattern of samples containing different concentrations of maltodextrin and HPMC (as seen in Table 1). The negative control (black) was dried lipid components without maltodextrin and HPMC.

range of 5 to 40°. Meanwhile, in the DSC thermal analysis, the samples were placed in aluminum crucibles and scanned from 30°C-300°C with a heating rate of 10°C/min. The morphology of the liposomes in the solid gel was analyzed with SEM. The portions of the dried product were scattered and glued onto 25-mm diameter plates, which were attached to the SEM specimen mounts. The specimens were sputter-coated with a 5-nm layer of Au-Palladium.

3 RESULTS AND DISCUSSION

The solid systems of all formulations were relatively amorphous. Compared with the negative control, there was nearly no high-intensity peak detected in the X-Ray diffractogram pattern analysis (Figure 1). The amorphous system is the ideal condition because it can preserve the liposomes in the formulations. This physical feature is necessary because the crystalline components can damage the integrity of the bilayer membrane and cause ruptures on it (Li *et al.*, 2016). The X-Ray diffractogram of all formulations also strongly confirmed the role of vitrification mechanism to preserve the liposomes during drying (Ingvarsson *et al.*, 2011).

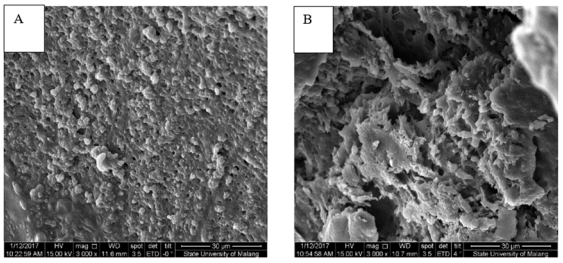

Figure 3: The different SEM microphotographs, indicating that the increase of maltodextrin in formulations containing the same amount of HPMC produces a different surface profile. (A) F2MO3, (B) F2MO4.

Contrary to Nugraheni *et al.* (2017) in which the liposome formulations from maltodextrin were freeze-dried, the DSC profiles in this research exhibited endothermic peak splitting at 120-150^{0}C (Figure 2). These results imply the heterogeneity of the samples. The different melting points of the crystal with a different lamellar thickness in the polymer were probably the cause of the endothermic peak splitting (Montanheiro *et al.*, 2016). The reorganization or recrystallization of amorphous material in thermal treatment, e.g., heating during the air-drying process, might be responsible for this phenomenon (Pereira *et al.*, 2016).

Compared with the other formulations, F2MO3 and F2MO2 were more homogeneous (Figure 2). The phase separation for samples containing neither Maltodextrin nor HPMC occurred at 120-180^{0}C. This finding shows that both components are crucial in the formulations.

The SEM photograph (Figure 3) of the sample's surface changed when the maltodextrin increased. The increase of maltodextrin in the formulations containing the same amount of HPMC produced more porous and rougher surface. The porosity of the matrix is essential to facilitate the rehydration of the samples (Yusuf *et al.*, 2017). F2MO3 produced a

smoother surface than F2MO4, which would accommodate the preservation of liposomes in the matrix.

Yusuf *et al.* (2017) have created several formulations with different disaccharides, but the resultant crystallinity profile was unfavorable because the phase separations still occurred in the results. However, this research developed the formulations using amorphous oligosaccharide. The finding shows that F2MO3 is the formulation that meets the desired physical characteristics. The high HPMC-maltodextrin ratio inhibits molecular re-arrangement and, thereby, provides better protection for the liposome vesicles.

4 CONCLUSIONS

The inclusion of Maltodextrin and HPMC as a dispersion matrix into the liposome formulations potentially inhibits crystal formation during the drying process and, consequently, provides better protection for the lipid bilayer in liposomes.

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