Preparation and characterization of nonionic nano-vesicles prepared from various surfactant types

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*Bancha Yingngam[1], Nuttapun Supaka[2], Wandee Rungseevijitprapa[1]**

Abstract

The purposes of this study were to prepare and characterize nonionic nano-vesicles prepared from various surfactant types (span 20, span 60, span 80, brij 72, glyceryl monostearate and polyethylene glycol-6 stearate). The vesicles were prepared by film hydration followed by high pressure homogenization technique. Physicochemical properties were investigated by varying molar ratios, total lipids, negative charge inducers, membrane modifiers, electrolytes, and pH of aqueous phases. Results showed that nonionic vesicles approximately 100 to 200 nm depending on surfactant types could be prepared with narrow size distribution (PDI<0.3). The vesicle sizes were increased with increasing cholesterol molar ratios and total lipid concentrations. Mixing nonionic nano-vesicles containing 0, 5, 10 mol% oleic acid as negative charge inducer with osmotic agent, KCl solution at 60, 40, and 80 mM caused significantly increase in vesicle size and aggregation. Rigid nano-vesicles were observed when using precirol ATO5 concentrations $\leq$ 25 mol%. Vesicles were formed independent of pH of aqueous solutions but aggregations were observed at pH 3 and 5. Differential scanning calorimetry thermograms revealed that excipients used in the production of nano-vesicles were presented in amorphous form. These results suggested that a proper selection of excipient can lead to successful encapsulation of active ingredients for drug and cosmetic applications.

Keywords: Vesicles; Molar ratios; Total lipids; Negative charge inducers, Membrane modifiers, Electrolytes, pH of aqueous phases

[1]*Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubonratchathani University, Ubonratchathani 34190, Thailand*

[2]*National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathumthani 12120, Thailand*

**Corresponding author, E-mail: wandeeim@yahoo.com*

1. Introduction

Nonionic nano-vesicles (niosomes) are one of drug and bioactive agent carrier/delivery systems which formed by self assembly of non-ionic surfactants and other additives into bilayers in aqueous phase (Paolino et al., 2008). Encapsulation into this system is feasible for both hydrophilic and lipophilic compounds and thus controls the active release over times. Compared to liposomes, nonionic nano-vesicles are more stable, have less variable in purity and less expensive. Since liposomes are addressed a major problem in degradation of phospholipids in an aqueous system by hydrolysis (Vora et al., 1998). Based on above reasons, they were introduced as alternative carrier systems by using non-ionic surfactants instead phospholipids. In this study, vesicles were prepared with various surfactant types and formulation parameters affected physicochemical properties were investigated. Interaction of vesicle containing various surfactant types and membrane additives was also examined with differential scanning calorimetry.

2. Experimental

2.1. Materials

Span 20, span 60, span 80 were purchased from Sigma-Aldrich (MO, USA). Brij 72 and glyceryl monostearate (GMS) was from Pharma (Bangkok, Thailand). Cholesterol and oleic acid were from Carlo Erba Reagenti SpA (Italy). Precirol ATO5 and Polyethylene glycol-6 stearate (PEG-6 stearate) was from Guttfossé (France). All reagents were of analytical grade.

2.2. Preparation of nonionic nano-vesicles

Nonionic nano-vescicles were prepared as described previously (Yingngam et al., 2007). Briefly, One hundred milliliters of nonionic nano-vesicles were prepared at 20 mM of various surfactant types (span 20, span 60, span 80, brij 72, GMS and PEG-6 stearate) and cholesterol by film hydration method. The obtained vesicles were observed under an optical microscopy attached with a camera (Nikon Eclipse E200, Japan). After that, vesicle size was reduced by high pressure homogenizer (APV 1000, Invensys APV Products, Denmark) at pressure of 500 bars for 10 min. The reproducibility of vesicle preparation and vesicle size reduction technique was performed from 6 batches using 20 mM span 20/ cholesterol at 50:50 mol% as model system.

2.3. Measurement of vesicle size and zeta potential

The vesicles were diluted approximately 40 times with ultra-pure water and investigated vesicle sizes, size distribution or zeta potential using Zetasizer nanoZS (Malvern Instruments, UK). The measurement was performed at 25 ^{o}C for all samples.

2.4. Differential scanning calorimetry (DSC)

Interaction of non-ionic surfactant and cholesterol or other membrane additives was performed using a Mettler Toledo STARe System (DSC822e Module, Switzerland). Thirty microliters of vesicle dispersion or 4-7 mg sample was placed in an aluminium crucible pan and was then carefully sealed with aluminium cap using empty pan as reference. The measurement condition was operated at temperature rate of 10^{o}C/min, and scanning range was from 0 to 130 ^{o}C, following a quench cooling to 0 at 50^{o}C/min, finally the pan was heated from 0 to 180^{o}C at 10^{o}C/min.

3. Results and discussion

3.1. Characterization of vesicle sizes

The mean vesicle size of various vesicles is illustrated in Figure 1. Vesicles displayed vesicle size smaller than 200 nm with size uniformity (Polydispersity index (PDI) < 0.3), except brij 72 exhibited sizes approximately 469 30 nm. The different sizes of various vesicles could be affected from individual properties of surfactants. However, the vesicle size between 200-100 nm was sufficient for the delivery of active ingredient into deeper skin layer (Shim et al., 2004).

3.2. DSC characterization

DSC is general used to investigate gel to liquid phase transition (Tc) of vesicles. DSC thermograms showed crystallinity peak of cholesterol. The Tc of all the tested composition was almost negligible due to decrease in crystallinity of cholesterol and precirol. Consequently, the stability of the system was increased (Figure 2).

3.3. Effect of process parameters on physicochemical properties of vesicles

In this study, the size reduction condition was optimized and the inter-day reproducibility was studied. The high pressure homogenization pressures and times gave no different between batch to batch with good reproducibility (n=6).

3.4. Effect of formulation parameters on physico-chemical properties of vesicles

Cholesterol concentration was varied from 0 mol% to 50 mol%. The size increased with increasing concentration of cholesterol. The mean diameter of vesicles increased with increasing cholesterol concentration at 106 4 110 3, 173 3 and 189 12 nm for 0, 10, 30 and 50 mol%. Besides, increasing total lipid concentration also led to increasing in vesicle sizes. Adding oleic acid as negative charge inducer to the vesicle resulted in the changes of the zeta potential and reduction of vesicle size.

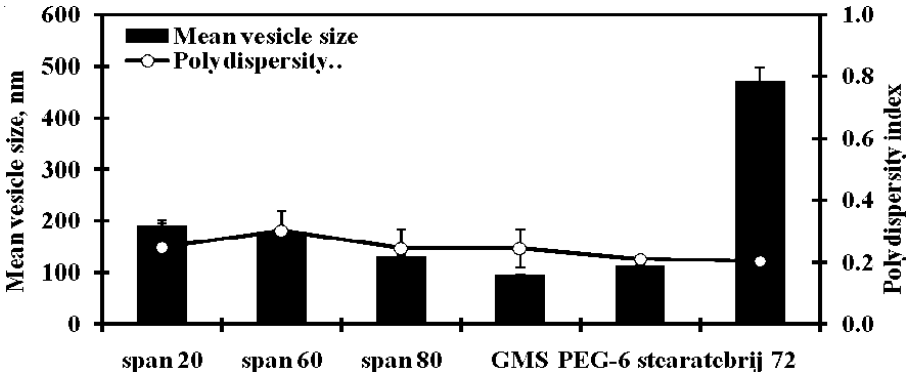
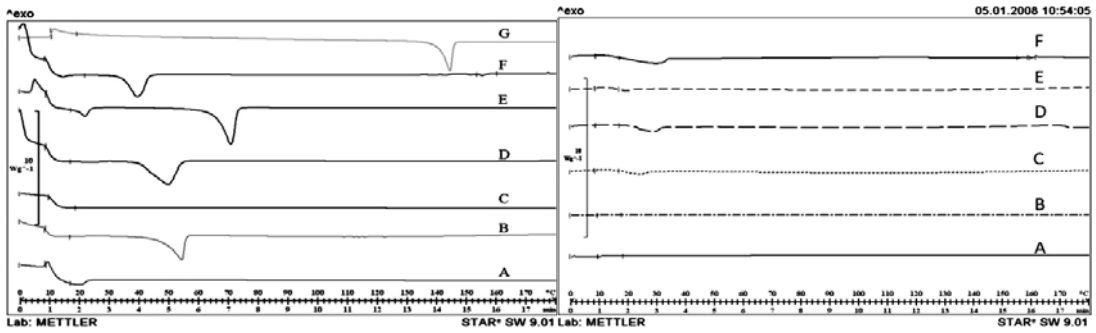


Figure 1. Mean vesicle size of various vesicles composing of 20 mM surfactant/cholesterol at 50:50 mol%; span 20/cholesterol, span 60/cholesterol, span 80/cholesterol, brij 72/cholesterol, GMS/cholesterol and PEG-6 stearate/cholesterol. Each value represents the mean S.D. (n=3)



A. B.

Figure 2. DSC thermograms of vesicles composing of 20 mM surfactant/cholesterol at 50:50 mol%; (**2A**) (A) span 20, (B) span 60, (C) span 80, (D) brij 72, (E) GMS (F) PEG-6 stearate and (G) cholesterol, and (**2B**) (A) span 20/cholesterol, (B) span 60/cholesterol, (C) span 80/cholesterol, (D) brij 72/cholesterol, (E) GMS/cholesterol and (F) PEG-6 stearate/cholesterol.

To improve rigidity of vesicles, precirol ATO5 was employed by varied from 0 to 25 mol%. The precirol ATO5 concentration lower 25 mol% resulted in rigid vesicle formation and higher 25 mol%, rigid vesicles were transformed to rigid particles. The difference in vesicle size was not detected for all formulations. Furthermore, the integrity of vesicles adding rigidity modifier was carried out using DSC. The obtained thermograms were compared to bulk surfactant, cholesterol and precirol ATO5. The presence of surfactant and cholesterol and precirol ATO5 in the vesicles could reduce crystallinity of cholesterol and precirol ATO5.

Effect of osmotic gradient on vesicle tendency and aggregation were shown in Figure 3A-B. The osmotic gradient of KCl solution was challenged directly into vesicle dispersion in order to predict physical stability of vesicle. For non-charge vesicles, concentration of KCl solution increased up to 60 mM did not affected vesicle sizes. Comparing to 5 and 10 mol% negative charge, the lower charge vesicles were more sensitive to osmotic solution than higher negative charge (Figure 3A). This could be due to the negatively charge of vesicle that was suppressed from the K+ counter ion and salting out effect and vesicle-vesicle interaction thus causing consequently aggregation. To confirm this hypothesis, the zeta potential of vesicle dispersion with 5 and 10 mol% was also investigated (Figure 3B).

Another important parameter for prediction the vesicle formation was changing pH of aqueous system used for film hydration. Figure 4 shows partially aggregated of vesicle at pH 5 of acetate buffer, but the vesicle remained unchanged at pH 7 and 9. This finding revealed that vesicle could be formed without aggregation in the presence of medium to high pH solution.

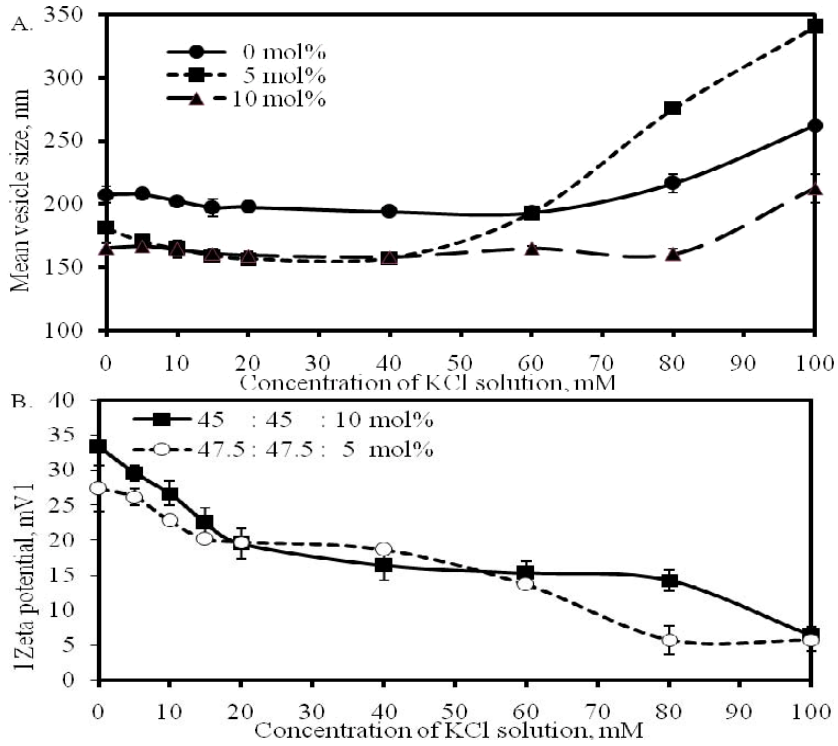


Figure 3. Effect of osmotic gradient of KCl solution mixed with nonionic nano-vesicles on osmotic sensitivity, aggregation tendency and zeta potential value; (**3A**) vesicles tendency and aggregation and (**3B**) changes of zeta potential value. Each value represents the mean S.D. (n=3)

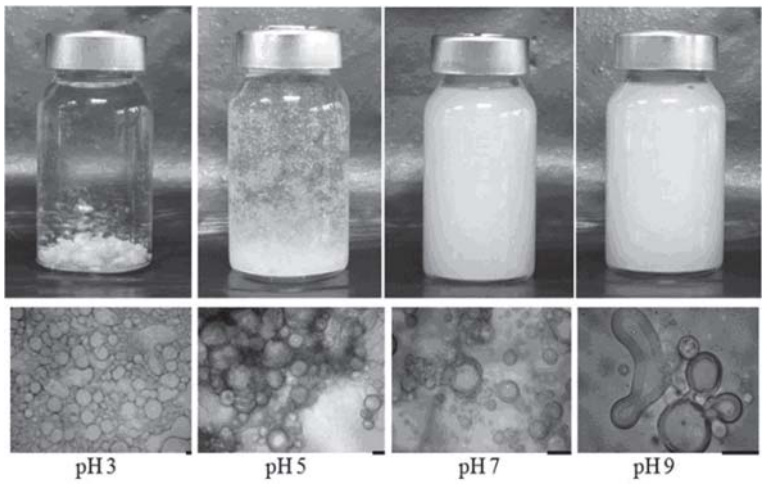


Figure 4. Effect of pH aqueous phase on vesicle formation of span 20/cholesterol which dry film was hydrated with 100 mM acetate buffer; pH 3, pH 5, pH 7 and pH 9 (scale bar was 1 μm).

4. Conclusion

This study demonstrated nonionic nano-vesicle could be prepared from various surfactant types. Several formulation parameters affected on physicochemical properties of vesicles. These formulations will be further investigated for entrapment efficiency, skin permeation and efficacy.

Acknowledgements

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