Thai Journal of Pharmaceutical Sciences (TJPS)

5th International Conference on Pharmaceuticals, Nutraceuticals and Cosmetic Science (IPNaCS 2017)

Investigation of Polyoxyethylene Alkyl Ether (POAE) Vesicles Formation by Proniosome Gel Method

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Keywords: Proniosome, Niosome, Brij, Polyoxyethylene Alkyl Ether

Introduction

Niosomes are non-ionic surfactant based vesicles that have been developed alternative to liposomes to overcome the problems using phospholipids. Niosomes were first developed and reported by L'Oreal in 1975. Niosomes can be used to carry of both hydrophilic and lipophilic drugs. These liposomes like vesicles are formed from the hydrate mixture of non-ionic surfactant, cholesterol and charge inducing substance. The stable vesicles form only with the presence of proper mixture of the lipids and do not form spontaneously.[1] Niosomes have been studied as drug delivery system to carry drugs, bioactive substances for various expected uses. Recent niosomal preparation method which has been in the interest of many researchers is proniosome gel method.[2-6] Proniosome gel is basically mixture of many phases of liquid crystal which on hydration tends to form unilamellar or multilamellar vesicles. Stable niosomes can be prepared by adding membrane modifiers along with surfactant and drugs. Nonionic surfactants have received the most attention for topical and percutaneous absorption since they have low irritancy potential and provide skin permeation enhancing ability by disrupting the stratum corneum lamellae structure. Park *et al.* studied various types of polyoxyethylene alkyl ethers as enhancers of ibuprofen through rat skin. They found that polyoxyethylene alkyl ether showed high ability for enhancing the drug absorption. The surfactant containing ethylene oxide chain length of 2-5, hydrophlie-lipophile balance (HLB) value of 7-9 and alkyl chain length of C16-C18 are the very effective enhancing promoters.[7]

The objective of this study was to investigate the formation of quercetin niosome using proniosome gel method and comparing six kinds of polyoxyethylene alkyl ethers (POAE) with different HLB values and some important physical properties as shown in Table 1. This method avoids the use of unacceptable inorganic solvent and energy-expensive procedures such as ultrasonication or extrusion. The method is nowadays called coacervation-phase separation technique. It is based on the initial formation of a proniosome mixture containing nonionic surfactant, isopropyl alcohol and water which is converted to niosome by a simple dilution step.

Table 1 The physical properties of polyoxyethylene alkyl ethers used in the study.[8]

Trade name	Chemical information	HLB	MW	Melting point (°C)
Brij 30	Polyoxyethylene (4) lauryl ether	9.7	362	14
Brij 52	Polyoxyethylene (2) cetyl ether	5.3	330	32.8
Brij 58	Polyoxyethylene (20) cetyl ether	15.7	1124	38
Brij 93	Polyoxyethylene (2) oleyl ether	4.9	356	10
Brij 97	Polyoxyethylene (10) oleyl ether	12.4	709	10
Brij 98	Polyoxyethylene (20) oleyl ether	15.3	1149	30-40

Methods

Preparation method

The composition of the formulations in this research followed the previous described study.[9] A 0.02 g of quercetin with 1 g of non-ionic surfactant:cholesterol mixture and 0.005 g of dihexadecyl phosphate were mixed with 1.5 ml of isopropyl alcohol in a beaker and warmed in a water bath up to 60±5ºC for 5 min. A 1.5 ml of phosphate buffer (pH 6.0) was added and still warmed on the water bath for further 2 min till the clear gel was observed. The mixture was allowed to cool down and converted to proniosome gel. Niosome gel was diluted by adding 10 ml of phosphate buffer (pH 6.0) previously warmed at 60±5°C and gently mixed. The mixture was sonicated for 3 rounds of 3 min interval.

The resulted niosomal dispersions were kept in well closed glass tubes in dark place at 4°C for further characterization. Six types of polyoxyethylene alkyl ethers were formulated by proniosome gel method as indicated in Table 1. The obtained niosomes were observed for their shape under microscope.

Optical microscopic examination and surface morphology

An optical microscope with a camera attachment was used to observe the shape and the completion of vesicle formation after diluting and storing for 24 h. The morphology of a selected formula was confirmed using cryo-scanning electron microscope (JEOL, JSM-6010L). The sample was immersed in liquid nitrogen and the image was captured at a desired magnification.

Results

Table 2 summarizes the vesicles formation of the noisome gel containing quercetin at 0.02 g/g of lipid mixture. It was interesting to note that non-ionic surfactants with very high HLB values, i.e., Brij 58, Brij 97 and Brij 98 which their HLB values are 16, 12.4 and 15, respectively could not form vesicles. In contrast, non-ionic surfactants with low HLB values, i.e., Brij 30, Brij 52 and Brij 93 could provide the vesicles spontaneously formed. The lack of homogeneity of size might be due to the fixed sonication time in the process of preparation.

Table 2 Summaries the effect of cholesterol proportion on the vesicles formation

Surfactant	Appearance under microscope		
	1:1*	**2:1***	**3:1***
Brij 30	Incomplete formation of vesicles with the unentrapped crystals were observed	Vesicles were formed with the unentraped crystals were observed	Homogeneous vesicles were formed without the quercetin crystals were observed
Brij 52	Vesicles were formed	Vesicles were formed with the unentrapped crystals were observed	Vesicles were formed with the unentrapped crystals were observed
Brij 58	No formation of vesicles	No formation of vesicles	No formation of vesicles
Brij 93	Incomplete formation of vesicles	Small vesicles were formed with the unentrapped crystals were observed	Small vesicles were formed with the unentrapped crystals were observed
Brij 97	No formation of vesicles	No formation of vesicles	No formation of vesicles
Brij 98	No formation of vesicles	No formation of vesicles	Small and homogenous vesicles were formed

*Nonionic surfactant to cholesterol molar ratio

Figure 1 demonstrates the morphological shape of the formed vesicles. Quercetin niosomes form Brij 30 at 1:1 and 2:1 molar ratios revealed the unentrapped crystals and the incomplete assembling of vesicles. At 3:1 molar ratio, the vesicles were completely formed and the quercetin was well incorporated. Brij 52 showed high ability to assemble into niosomes at all ratios with large size and well incorporation of quercetin

while Brij 58 which has high HLB value of 15.7 did not exhibit a complete vesicle but small pieces of lipid agglomerate were observed. In case of Brij 93 which the HLB value is 4.9, at all molar ratios, vesicles were formed in spherical shape and rather homogeneous. The population of small size was dominant. However, this kind of surfactant was not selected because of the incomplete incorporation of quercetin.

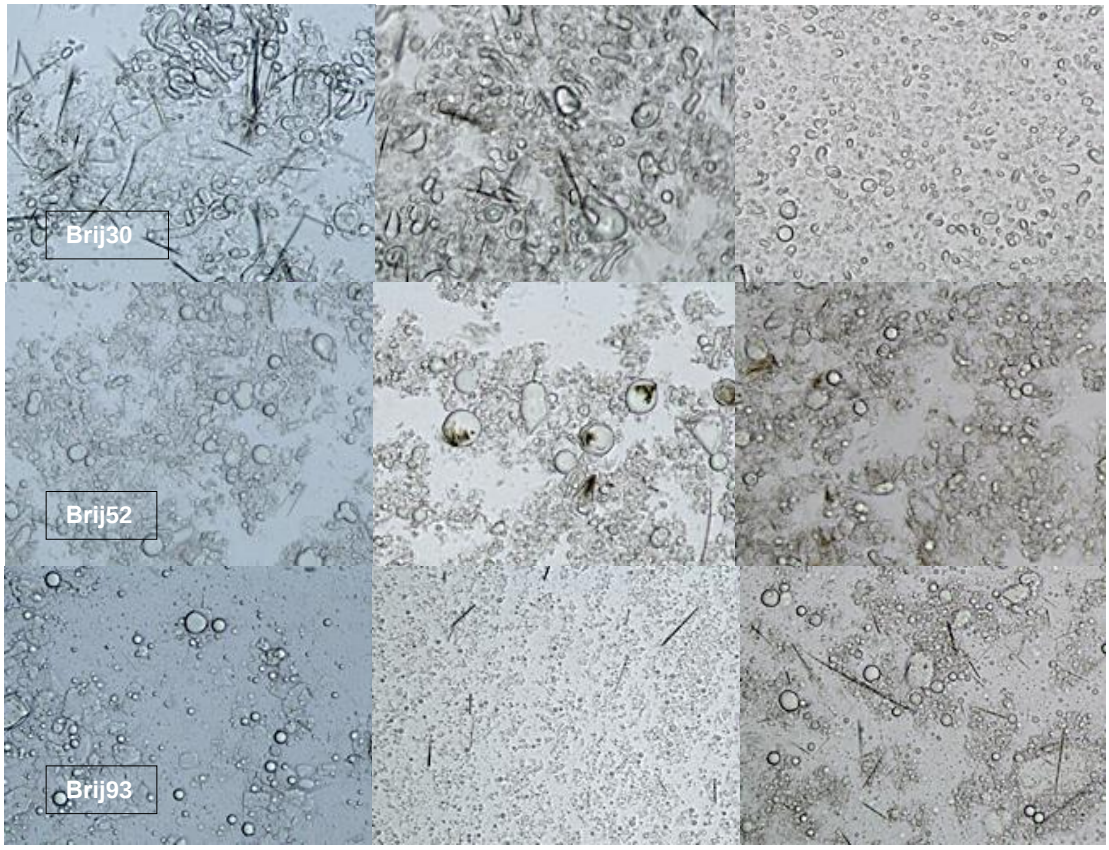

Figure 1 Optical micrographs (400x) of quercetin niosomes prepared from Brij 30, Brij 52 and Brij 93 at the surfactant:cholesterol molar ratios of 1:1 (left), 2:1 (middle) and 3:1 (right).

In case of Brij 97 and Brij 98 at 1:1 and 2:1 molar ratios of nonionic surfactant to cholesterol, vesicle was not formed. Complete spherical vesicles were observed only from Brij 98 at 3:1 molar ratio.

Figure 2 demonstrates the cryo-SEM image of niosome dispersion prepared from Brij 52 which was prior frozen in liquid nitrogen. It was found that the vesicles were in spherical shape, large size and homogeneous.

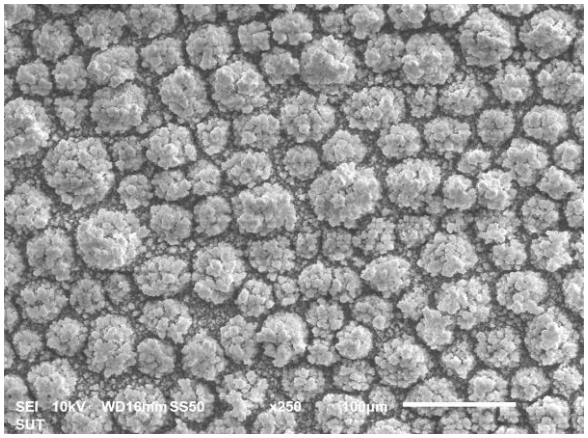

Figure 2 Cryo-SEM image of Brij 52 niosome dispersion prepared from proniosome gel at the 2:1 molar ratio of nonionic surfactant to cholesterol. The scale bar represents 100 micrometers.

Discussion

The six POAE were investigated for the effect of cholesterol proportion on the vesicle formation using proniosome gel method. It was found that the high HLB value nonionic POAE could not form the vesicle by this method. Low HLB value POAE formed the vesicles with occasionally incomplete entrapment of quercetin upon the ratio of cholesterol added in the individual Brij. Cholesterol is well known to be an important substance in the vesicle bilayer to increase the drug entrapment efficiency. Recently, Wilkhu *et al.*[10] studied the organization of surfactants into niosome using thermo gravimetric analysis and found that cholesterol played a key role in assembling of bilayer. Without cholesterol, the vesicle cannot be formed and higher concentration of cholesterol decreased the time required for niosome assembly. The study also found that the heat enthalpy for melting of the lipid mixture decrease upon the increasing of the cholesterol while addition of dicetyl phosphate increased the melting enthalpy. Cholesterol did not melt but dissolved into the molten mixture upon heat. The intercalation of cholesterol within the bilayer reduces the average area per molecule and overall critical packing parameter (CPP). In general, if CPP < 0.5, it indicates that the micelles are formed. If the CPP of the nonionic surfactant is in between 0.5–1, the spherical vesicles will form while CPP > 1 the invert micelles will form.[11] For higher HLB values of POAE, the polyoxyethylene head groups are more likely to dissolve in the buffer medium, thus micelles are expected to occur and they solubilise the added quercetin.

Conclusion

This study showed that POAE with HLB between 5-9 can be used to prepare niosome by proniosome gel method when they are added optimum proportion of cholesterol. However, many other factors might affect the assembling ability of the vesicles such as dilution temperature, ionic strength of the medium and sonication time. These factors should be separately studied for each certain nonionic surfactant.

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