Effect of Polysorbate 60 on Physicochemical Properties of Melatonin Niosomes

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ABSTRACT

This study intended to incorporate and evaluate the effect of various concentrations of polysorbate 60 in the development of melatonin niosomes (MN) which contained 5:5:1 molar ratio of span 60: cholesterol: melatonin. Polysorbate 60 at molar ratios of 0, 1.25, 2.5, 5, and 10 were added. Some of vesicles of blank niosomes (BN) and MN started to form some oval shapes, observed by scanning electron microscope (SEM), from $BN_{0.5}$ to $BN_{2.0}$and $MN_{0.25}$ to $MN_{2.0}$, respectively. Mean particle size of melatonin niosomes slightly increased from 2.4 µm of MN_0 to 3.0 µm of $MN_{0.5}$. For BN, particle size tended to reduce with increasing of polysorbate 60. However, size distributions of all niosomes were in a broad. The zeta potential of all formulations tended to increase from -41.3 to -36.6 mV for MN and increased from -43.3 to -39.5 mV for BN. The pH of all niosomes decreased with an increase in the compositions of polysorbate 60. Near infrared spectra revealed the wavelength changed from 974 to 970 nm indicated the hydrogen bonding between polysorbate 60 and other components. The interaction could predict that melatonin niosomes could retard its release, and permeation through skin.

Keywords: Melatonin, Niosomes, Polysorbate 60

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Introduction

Niosomes, bilayer vesicles usually composed of nonionic surfactants and cholesterol, are able to encapsulate both hydrophilic and hydrophobic drugs useful as drug carriers (Shatalebi et al., 2010). It is interested for use in topical applications as niosomes provide advantages in retaining drugs in the stratum corneum and epidermis (Junyaprasert et al., 2012) or promoting cellular uptake (Priprem et al., 2012).

Melatonin (N-acetyl-5-methoxytryptamine), an endogenous neuroendocrine hormone synthesized and secreted from pineal gland, is known as sleep inducer in jet lag as well as antioxidant (Bonnefont-Rousselot, Collin, 2010), immunomodulator (Srinivasan et al., 2008) and anti-inflammatory (Mayo et al., 2005). It is possible to use melatonin for topical anti-inflammatory due to its inhibitory effects of inflammatory mediators and cytokines (Reiter et al., 2000). However, to confine its local effect in a topical application, its physicochemical properties, particularly high partition coefficient (log P = 1.2), are required to be manipulated so as to control its rapid permeation and absorption into the circulation system. This is highly important because systemic effects from rapid permeation and absorption of melatonin include sleep induction which restricts day-time use of the topical melatonin preparations.

Melatonin niosomes (MN), composed of 1:1 sorbitan monostearate 60 (span 60) and cholesterol were able to retard its release at a certain level (Nukulkit et al., 2014). To further retain melatonin at the topical site, surfactants with higher hydrophilic-lipophilic balance (HLB) were hypothesized and used in this preliminary investigation. However, an increase in the HLB values also increased particle sizes of niosomes (Khazaeli et al., 2007) which affect various physicochemical properties as well as permeation and absorption. Polysorbate 60 (tween 60), a non-ionic surfactant widely used in pharmaceutical preparations with a HLB of 14.9, was proposed to increase hydrophilicity (Vaziri Hassas et al., 2014). It is expected that polysorbate 60 will prolong melatonin retention in the tissue to maximize local effect rather than rapid permeation and distribution to cause systemic effects. Incorporation of polysorbate 60 may alter particle size and surface charges which affect permeation (Ruckmani, Sankar, 2010; Akbari et al., 2015).

Near infrared spectroscopy (NIR) is a fast and nondestructive analytical method widely used in pharmaceutical sciences and also industry. This technique provides chemical and physical information. It was used to determination of quality and quantity of pharmaceutical compound including identification, physicochemical and also interaction (Reich, 2005; Jamrogiewicz, 2012) and thus, interested for assessment of interactions that may occur in the complex formation of the modified niosomes. Therefore, this phase of study focuses on pH, particle size, surface potential, NIR and morphology which should provide some concrete physicochemical characterizations for substantial formulations for local retention of melatonin.

Objective of the study

The objectives of this study were to develop melatonin niosomes modified by polysorbate 60 and evaluate the physicochemical properties.

Materials and Methods

Melatonin (Shanghai Chemical, PR China), cholesterol (Sigma, USA), sorbitan monostearate 60 (Span 60), (Sigma, USA), polysorbate 60 (tween 60), (Namsiang, Thailand) and hexane (Labscan, Thailand) were used as received.

Preparation of niosomes

Niosomes were formed by hand shaking method (Moghassemi, Hadjizadeh, 2014). Span 60 and cholesterol (1:1 molar ratio) were dissolved in hexane, mixed with water or melatonin solution and rotary evaporated at 60°C (BUCHI Rotaevap R-3, Switzerland). All of the melatonin niosomes (MN) of 0.1 mg/ml (0.2 molar ratios) were freshly-prepared. Polysorbate 60 used and the codes of each formula are shown in Table 1.

Table 1 The compositions of blank niosomes (BN) and melatonin niosomes (MN) composed of sorbitan monostearate 60 (Span), cholesterol (Chol) and polysorbate 60 (PS).

Code	Composition (Molar)			
	Melatonin	Span	Chol	PS
BN_0	-	1	1	0
MN_0	0.2	1	1	0
$BN_{0.25}$	-	1	1	0.25
$MN_{0.25}$	0.2	1	1	0.25
$BN_{0.5}$	-	1	1	0.5
$MN_{0.5}$	0.2	1	1	0.5
$BN_{1.0}$	-	1	1	1
$MN_{1.0}$	0.2	1	1	1
$BN_{2.0}$	-	1	1	2
$MN_{2.0}$	0.2	1	1	2

Characterization of niosomes

The pH of niosomes were evaluated by pH meter (Mettler Toledo, Germany).

Particle sizes and size distributions of niosomes were measured by laser-light scattering analysis (Mastersizer 2000, Malvern, U.K).

The electrostatics or charges repulsion between particles of niosomes were measured by Zeta potential (Nano-z, Malvern Zetasizer, U.K).

Near infrared spectroscopy (NIR, XDS Near-infrared, FOSS, Denmark) was used to determine the interaction between components in niosomes by changing of NIR spectrum.

The morphology of niosomes were observed by a scanning electron microscope (SEM) (SNE4500M, SEC, Korea). Samples were diluted with water and dropped on the stub, allowed to dry in room temperature, and coated with gold. Representative photographs of sample were taken after scanning observations.

Statistical analysis

Data represents in mean ± SD. Statistical analysis of difference between formulations was performed by using independent *t*-test. The level of significance was taken at $p < 0.05$.

Results

The representative morphologies of niosomes observed by SEM are shown in Figure 1. The niosomes appeared as spherical vesicles, there were some vesicles which appeared to be oval or deviated from spherical shape. For BN, it was found the oval vesicles started from $BN_{0.5}$, followed by $BN_{1.0}$ and also $BN_{2.0}$. For MN, the oval vesicles started on the $MN_{0.25}$, $MN_{0.5}$, $MN_{1.0}$, and $MN_{2.0}$. There were some aggregations appeared in the $BN_{1.0}$ and

$BN_{2.0}$ and $MN_{0.25}$, $MN_{0.5}$, $MN_{1.0}$, and $MN_{2.0}$. Increasing amount of polysorbate 60 influenced shape and morphology of niosomes. Mean particle sizes of niosomes measured by laser-light scattering are illustrated in Table 2. The BN_0 showed mean particle size of 2.47 µm and 0.39, 0.25, 1.04, and 0.20 µm for $BN_{0.25}$, $BN_{0.5}$, $BN_{1.0}$, and $BN_{2.0}$, respectively. While the size of MN_0 slightly increased by increasing amount of polysorbate 60 from 2.44 of MN_0 to 2.61 of $MN_{0.25}$, and to 2.95 µm of $MN_{0.5}$. Polydispersity indices (PDI), which indicated the size distributions, varied in wide range. PDI of blank niosomes were 5.30 of BN_0, 16.98 of $BN_{0.25}$, 1.83 of $BN_{0.5}$, 25.57 of $BN_{1.0}$, and 0.65 of $BN_{2.0}$. For melatonin niosomes, the PDI were 5.72 of MN_0, 2.69 of $MN_{0.25}$, 2.69 of $MN_{0.5}$, 1.17 of $MN_{1.0}$, and 7.15 of $MN_{2.0}$.

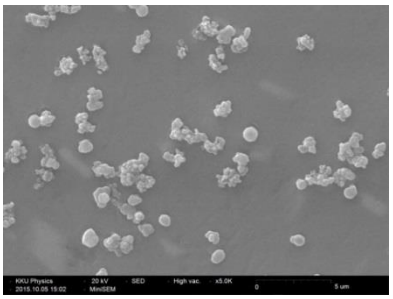
Table 2 Mean particle size and polydispersity index (PDI) of niosomes. Data represents in mean ± SD (n =3)

Formulation	Particle size (µm)	PDI
BN_0	2.47 ± 0.01	5.30
MN_0	2.44 ± 0.03	5.72
$BN_{0.25}$	0.39 ± 0.01	16.98
$MN_{0.25}$	2.61 ± 0.02*	2.69
$BN_{0.5}$	0.25 ± 0.00	1.83
$MN_{0.5}$	2.95 ± 0.00*	2.69
$BN_{1.0}$	1.04 ± 0.03	25.57
$MN_{1.0}$	0.21 ± 0.01*	1.17
$BN_{2.0}$	0.20 ± 0.01	0.65
$MN_{2.0}$	0.21 ± 0.01	7.15

*$p < 0.05$ when compared to BN with same ratio

Results from the measurements of pH and zeta potential of the niosomes are presented in Table 3. The pH of niosomes decreased with an increase in the compositions of polysorbate 60, from 6.14 of BN_0 to 4.96 of $BN_{2.0}$ and from 6.36 of MN_0 to 5.13 of $MN_{2.0}$. Zeta potential measurements showed that increasing polysorbate 60 in the niosomes compositions causes a tendency towards positive zeta potential. The zeta potential increased from -43.30 mV for BN_0 to -39.47 mV for $BN_{2.0}$ and the melatonin niosomes increased from -41.27 mV for MN_0 to -36.63 mV for $MN_{2.0}$.

(a)



(b)

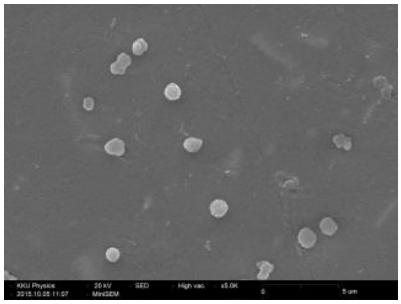


Figure 1 Scanning electron microscope photographs of (a) $MN_{0.25}$ and (b) $MN_{0.5}$ (Magnification 5000×)

NIR spectra (Figure 2) suggested potential interactions between polysorbated 60 and the other components used to form the niosomes. Hydrogen bonding was likely to interact between hydroxyl groups of polysorbate 60 with the others as the wavelength changed from 974 to 970 nm. The second derivative computation was processed to enhance the

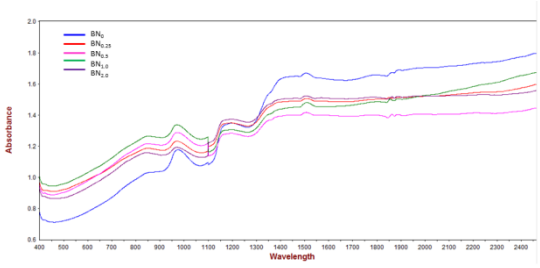
spectral resolution and compensate the baseline between samples (Figure 3).

Table 3 pH and zeta potential (mV) of niosomes.

Data represents in mean ± SD (n =3)

Formulation	pH	Zeta potential (mV)
BN_0	6.14 ± 0.03	-43.30 ± 2.60
MN_0	6.36 ± 0.01	-41.27 ± 0.97
$BN_{0.25}$	6.00 ± 0.13	-41.13 ± 1.01
$MN_{0.25}$	6.26 ± 0.07	-41.23 ± 1.38
$BN_{0.5}$	5.96 ± 0.02	-40.60 ± 0.78
$MN_{0.5}$	6.05 ± 0.02	-40.37 ± 0.25
$BN_{1.0}$	5.74 ± 0.02	-36.57 ± 0.35
$MN_{1.0}$	5.83 ± 0.01	-39.80 ± 0.87
$BN_{2.0}$	4.96 ± 0.02	-39.47 ± 0.72
$MN_{2.0}$	5.13 ± 0.04	-36.63 ± 1.37

(a)



(b)

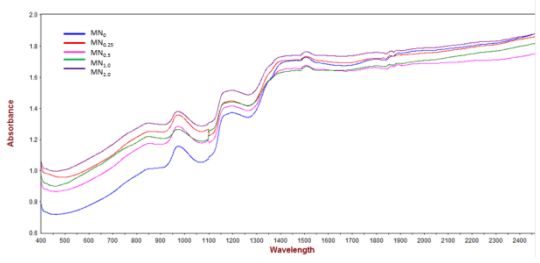
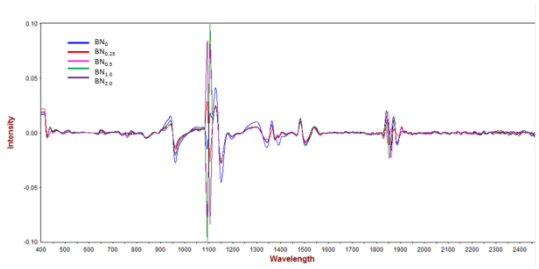


Figure 2 Near infrared spectra of all formulations of (a) blank niosomes, and (b) melatonin niosomes

(a)



(b)

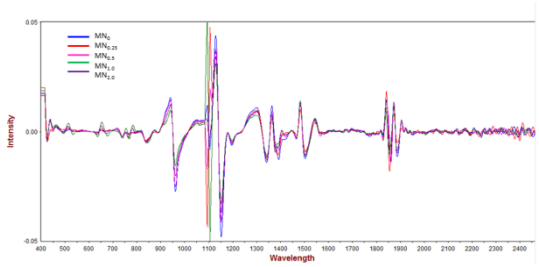


Figure 3 The second derivative near infrared spectra of all formulations of (a) blank niosomes, and (b) melatonin niosomes

Discussion

Incorporation of polysorbate 60 into the MN increased particle size of niosomes to reach its maximum at a molar ratio of polysorbate 60 of 0.5. On the contrary, the mean particle size of BN reduced with increasing of polysorbate 60. The HLB values of Span of 4.7 and polysorbate 60 of 14.9 are very different and could influence the hydrophilicity and surface energy of the system. Moreover, water uptake into vesicle increases with higher hydrophilicity (Kamboj et al., 2014), thus, there is a limitation of the use of polysorbate 60 in the niosomes formula.

The particle sizes of MN were larger than BN as molecules of melatonin could associate between surfactant molecules forming the bilayers of niosomes. However, the size distributions indicated as PDI varied in a broad because all of the niosomes

were formed by self-assembly. The different preparation methods might be considered for further study. Moreover, it might be possible that the niosomes particles started aggregation resulting in the high values of size distributions measured by laser-light scattering.

The surface potentials of the niosomes were increasingly positive with an increase in the non-ionic polysorbate 60. The increasing of charge may lead to aggregation between particles which also showed in SEM. Addition of the electrostatic stabilizer might be considered for further in order to prevent aggregation between particles. Incorporation of polysorbate 60 might change the formation of vesicles affect surface charge of niosomes. Bayindir, Yuksel, (2010) reported that surfactant type might affect the zeta potential values.

The NIR spectra indicated OH interaction between polysorbate 60 and other components. Polysorbate 60 might form hydrogen bond at the positions as illustrated in Figure 4.

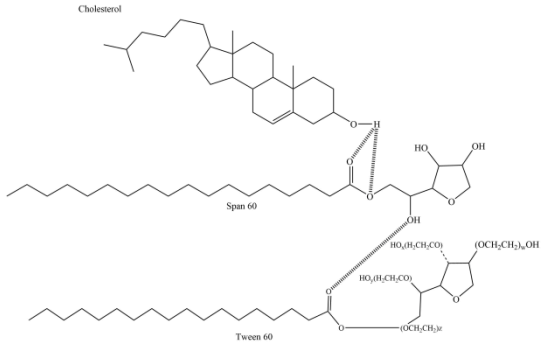


Figure 4 The predicted position of hydrogen bonding between cholesterol, span 60, and polysorbate 60 (tween 60)

Conclusions

Melatonin niosomes increased in particle size with amount of polysorbate 60 increased until the ratio 0.5 M of polysorbate 60. The interaction between polysorbate 60 and other components could predict that melatonin niosomes could retard melatonin release, and permeation through skin.

Acknowledgements

This study was supported by the Melatonin Research Group, Khon Kaen University, Khon Kaen, Thailand.

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