Preparation and characterization of physicochemical properties of N, N-diethyl-meta-toluamide niosomes

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Abstract

Introduction: The insect repellent compounds are used to protect humans, animals and plants against insect bites. Aromatic amides have insect repellent effects. N,N-diethyl-meta-toluamide (DEET) \((\text{C}_{12}\text{H}_{17}\text{NO})\) is one of the best insect repellents has been used for many years. DEET is a colorless, odorless liquid that is approximately insoluble in water and soluble in glycerin, ethanol, and isopropyl alcohol. Due to the solubility problem of DEET, its topical formulations usually have alcoholic bases, but these kind of formulations increase skin permeation and also systemic absorption of DEET, which leads to some toxic effects. The main goal of this study was to prepare the formulation of DEET niosomes in a topical dosage form with suitable stability properties. Materials and Methods: Three different methods were used to prepare niosome formulations: Dehydration rehydration vesicle method, direct mixing method, homogenizer method. Sorbitan surfactants, cholesterol, polyoxyethylenecetyl, phosphate buffer (pH 7.4), and charge inductive compounds like cetyltrimethylammonium bromide were used to provide a net negative charge to the final membrane structure. A high-performance liquid chromatography method was then used for the determination of the loaded DEET. Results: A large number of niosomes were multi-layered and have a spherical shape. In comparison, syringe method against direct mixing is more appropriate because of creation MLV and uniform niosomes but the best method is homogenizer method. Drug entrapment was between 14% and 21% in selected formulation. Conclusion: According to this study, homogenizing method can be used for formulation of DEET in niosome form in topical formulations.

Key words: Insect repellents, N,N-diethyl-meta-toluamide, niosome

INTRODUCTION

Insect-transmitted diseases remain a major source of disease and death worldwide. Mosquitoes alone transmit diseases to more than 700 million persons annually.

They have a main role in leishmaniasis and several kinds of bacterial infections, like Rocky Mountains spot fever, Tularemia, etc.\(^1\) Carbon dioxide is a powerful and conservative attraction and activator for most blood-sucking insects around 350 substances of diverse chemical composition have been identified in the human skin, including l-lactic acid, short- and long-chain fatty acids, aldehydes, alcohols, aromatic compounds, amines, acetates, and ketones. In addition, 1-octen-3-ol which is secreted in human’s sweat and breath, is also a significant factor in attracting insects to human beings.\(^2\)

Insect repellent products are substances that protect humans, animals, and plants against insects by changing the host’s odor or restraining the olfactory receptors in insects.\(^3\) These compounds are often volatile and operate neither kill insects nor destroy the attraction factors such as: Heat, moisture, body sweat, CO\(_2\), and body perspirations, but prevent finding the individual’s location by insects.\(^4\) Insect repellents are part of the organic matters group of straight chain unsaturated fatty acids with 2-decanoic acid as the impressive.\(^5\)

Amides and especially aromatic amides, generally remove mosquitoes, other insects\(^6\) and leeches.\(^7\) N,N-diethyl-meta-toluamide (DEET) has been used since 1957 in public as an insect repellent compounds standard.\(^8\) DEET is a colorless and odorless liquid which is almost insoluble in water but soluble in glycerine, ethanol, and isopropyl alcohol.\(^9\) Besides, is an effective insect repellent compound on many reptiles, especially against black mosquitoes, malaria mosquitoes, ticks, and fleas.\(^1\) According to the recent researches, DEET restrains
Vesicles prepared from nonionic surfactants or niosomes are consisted of one or more two-layered structures of nonionic surfactants, creating a surrounded space. These vesicles were introduced by Handjani-vila et al. However, the niosome forms, due to their slow released profile and long-term effect, can always create a low concentration of DEET on the skin and reduce skin absorption which is quite depending on the structure and concentration of substance. This study provides a topical niosomal DEET formulation in order to.

MATERIALS AND METHODS

The nonionic surfactants used as vesicle-forming materials were Span 20 (sorbitan monolaurete), Span 40 (sorbitan monopalmitate), Brij 52 (polyoxyethylene-2-cetyl ether), Brij 58 (polyoxyethylene-2-stearoyl ether), Brij 92 (polyoxyethylene-2-oleanether), Tween 20, Tween 40, Tween 60 and Tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO, USA), sodium chloride 0.9% injection solution, cholesterol (Chol) was bought from Fluka, Switzerland.

All organic solvents and the other chemicals were of analytical grade and were obtained from Merck, Germany, DEET, dihexadecyl phosphate.

Methods

Niosome morphology was evaluated by an optical microscope (Olympus, Japan). By this method, kind of the niosomes multilamellar vesicle (MLV, SUV), their shapes (spherical, tubular, polyhedron), wall thickness, crystal formation, separation of surfactants and cholesterol particles, and niosomal aggregation can be studied.

Particle size distribution

In order to study the particle size distribution, Malvern zeta-sizer with laser ray scattering technique was used. The particle size of each formulation was determined 3 times.

Drug loading

In order to revise the drug loading, niosomes were separated from niosome suspensions by centrifuging. An amount of 1 ml of niosome suspensions was poured in a centrifuge tube and centrifuged at 15,000 for 30 min. The upper phase was separated from the lower phase containing buffer phosphate and extra amount of nonentrapped DEET, then analyzed.

Release study

In order to revise the drug release, the Franz diffusion cell model was used. The diffusion cell has a receptor part with a volume of about 37 ml. One milliliter of niosomic suspension was placed on cellophane separating membrane.

Method of analysis

High-performance liquid chromatography method was used for measuring the DEET in different cases of loading and releasing studies. To adjust the conditions, a C18 250 mm × 4.6 mm column filled by octadecysilane-coated silica gel was used. Mobile phase contains water and methanol. Procedure was done by a gradient method with the speed of 1.3 ml/min. UV-detector, with 254 nm wavelength, was used. In this method, percent of methanol phase reaches from 5% to 95% during 30 min and remains in this ratio (water/methanol: 5/95) for 5 min.
RESULTS

Vesicle-forming ability
The ability of surfactants in forming niosome by use of different methods is summarized in Tables 1-6 and the morphology of selected formulation (number 14) was shown in Figure 1.

Physicochemical properties
A large number of niosomes were multi-layered and have a spherical shape.

According to the results, the amount of Brij 52 had no effect on number and size of niosomes [Figure 2] with the amount of DEET had direct effect [Figure 3]. In comparison, syringe method against direct mixing is more appropriate because of creation MLV and uniform niosomes but the best method is homogenizer method [Tables 1-6 and Figure 4].

Physical stability
Physical properties include niosome aggregation and lack of cholesterol crystals, were investigated. Cholesterol crystals were formed when cholesterol molar ratio was more than surfactant [Figure 5]. There was no phase separation in any formulations even after three methods, shown in Tables 1-4.

Drug entrapment
The results show that drug entrapment was between 14% and 21% in selected formulation (number 14), shown in Table 7.

Release profile
In order to release a study, cellophane membrane was dipped in buffer phosphate (pH = 7.4) as a receptor phase for 24 h. The receptor temperature was fixed at 37 ± 1 by water circulation.

Using magnet stirrer was led to have uniform distribution of temperature and drug in the receptor phase. Sampling was done at specified intervals time at sync condition. After every sampling, 1 ml of fresh receptor phase was replaced.

Result of release study is shown in Table 8 and Figure 6.

DISCUSSION

Niosomal formulation of DEET would lead to a decrease in skin penetration and an increase in duration of action. Niosomes may
### Table 1: Niosome formulation by syringe method, Brij52 and effect of DEET amount on niosome's morphology

| Formulation | Brij52 (mg) | Dihexadecyl phosphate (mg) | Cholesterol (mg) | DEET (mg) | Phosphate buffer (ml) | Mixing speed | Appearance | Relative number of niosomes | Aggregation of niosomes | Cholesterol crystal forming |
|-------------|-------------|-----------------|-----------------|----------|-------------------|-------------|-----------|-----------------------------|------------------------|--------------------------|
| 1           | 217         | 16              | 38              | 8        | 10                | 120 times/min | SUV       | Moderate                    | —                      | +                        |
| 2           | 217         | 16              | 38              | 21       | 10                | 120 times/min | MLV       | Moderate                    | +                      | —                        |
| 3           | 217         | 16              | 38              | 42       | 10                | 120 times/min | MLV       | Many                        | —                      | +                        |

Classification of niosomes by number of them – Many: A sample that is observed more than 50 niosomes in microscopic focus, Moderate: A sample that is observed 30-50 niosomes in microscopic focus, Few: A sample that is observed <30 niosomes in microscopic focus, DEET: N,N-diethyl-meta-toluamide, SUV: Small unilamellar vesicles, MLV: Multi lamellar vesicle

### Table 2: Niosome formulation by syringe method Brij52 and effect of cholesterol amount on niosome's morphology

| Formulation name | Brij52 (mg) | Dihexadecyl phosphate (mg) | Cholesterol (mg) | DEET (mg) | Phosphate buffer (ml) | Mixing time and period | Appearance | Relative number of niosomes | Aggregation of niosomes | Cholesterol crystal forming |
|------------------|-------------|-----------------|-----------------|----------|-------------------|------------------------|-----------|-----------------------------|------------------------|--------------------------|
| 4                | 217         | 16              | 0               | 21       | 10                | 120 times/2 min        | LUV       | Few                         | —                      | +                        |
| 5                | 217         | 16              | 24              | 21       | 10                | 120 times/2 min        | MLV       | Moderate                    | —                      | +                        |
| 6                | 217         | 16              | 30              | 21       | 10                | 120 times/2 min        | MLV, SUV, LUV | Moderate                   | —                      | +                        |
| 7                | 217         | 16              | 38              | 21       | 10                | 120 times/2 min        | MLV       | Many                        | —                      | +                        |
| 8                | 217         | 16              | 55              | 21       | 10                | 120 times/2 min        | MLV       | Many                        | —                      | +                        |

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### Table 3: Niosome formulation by direct mixing method, Brij52, and effect of Brij52 amount on niosome's morphology

| Formulation name | Brij52 (mg) | Dihexadecyl phosphate (mg) | Cholesterol (mg) | DEET (mg) | Phosphate buffer (ml) | Mixing time and period | Appearance | Relative number of niosomes | Aggregation of niosomes | Cholesterol crystal forming |
|------------------|-------------|-----------------|-----------------|----------|-------------------|------------------------|-----------|-----------------------------|------------------------|--------------------------|
| 9                | 217         | 16              | 24              | 21       | 10                | 120 times/2 min        | MLV       | Few                         | —                      | +                        |
| 10               | 240         | 16              | 24              | 21       | 10                | 120 times/2 min        | MLV       | Few                         | —                      | +                        |
| 11               | 250         | 16              | 24              | 21       | 10                | 120 times/2 min        | MLV       | Few                         | +                      | —                        |

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Table 4: Niosome formulation by direct mixing method, Brij52 and effect of different methods on niosome’s morphology

| Formulation name | Brij52 (mg) | Dihexadecyl phosphate (mg) | Cholesterol (mg) | DEET (mg) | Phosphate buffer (ml) | Mixing time and period | Appearance | Relative number of niosomes | Aggregation of niosomes | Cholesterol crystal forming |
|------------------|------------|---------------------------|------------------|-----------|----------------------|------------------------|-----------|---------------------------|------------------------|--------------------------|
| 12               | 240        | 16                        | 24               | 24        | 42                   | 10 min                 | Many      | Many                      | +                      | --                       |
| 13               | 240        | 16                        | 24               | 24        | 42                   | 10 min                 | Many      | Many                      | +                      | --                       |
| 14               | 240        | 16                        | 24               | 24        | 42                   | 10 min                 | Many      | Many                      | +                      | --                       |

Figure 5: The effect of cholesterol amount on the particle size distribution. Particle size (µm)

The amount of Brij is not efficient on numbers and size of niosomes. The presence of DEET leads to niosomal destruction and appearance of surfactant drops. Niosome preparation in the absence of cholesterol yield jelly and single lamellar products that could not entrap DEET so more and bigger niosomes obtained by adding cholesterol. However, excess increasing of cholesterol produces cholesterol crystals and decreases the size of niosomes. Finally, decreasing niosome size by increasing cholesterol amount is observed.

In this study, several methods were used in the formulation of DEET niosomes. First, DRV method was used that is the simplest and most common one and has been used because of some drugs like insulin.[14,15] But because of lipophilicity of DEET, and some incompatibility with surfactant, this method was not suitable.

Syringe method creates MLV niosomes. This method is a mixing procedure for two immiscible phase systems so aqueous and nonaqueous phases were entered into two separate syringes, then mixed together through a connection. Doxorubicin and minoxidil niosomes were prepared by this way.[16,17]

This method was not suitable because of different sizes and polyhedral niosomes with low loading were prepared.

By two above methods, the best molar ratio of components were determined. Hence, the final method was done using a homogenizer that especially has been used for two phase systems such as preparation of lansoprazole niosomes.

CONCLUSIONS

The selected formulation (number 14) showed desirable properties such as multilamellar and spherical shape, suitable size distribution, and sufficient drug loading. The release kinetic of DEET from niosome formulation shows a
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Table 5: Formulation of noisome by DRV method

| Formulation | Surfactant | Cholesterol | DEET | Existence of niosome | Appearance | Relative number of niosomes | Preamble |
|-------------|------------|-------------|------|-----------------------|------------|-----------------------------|----------|
| 15          | Brij52     | 92.4        | 46.4 | —                     | +          | MLV                         | Many     |
| 16          | Brij52     | 92.4        | 46.4 | 0.2                   | —          | —                           | Surfactant droplets |
| 17          | Brij58     | 314.7       | 46.4 | —                     | +          | MLV                         | Few      |
| 18          | Brij58     | 314.7       | 46.4 | 0.2                   | —          | —                           | Complete dissolution of niosomes and surfactants |
| 19          | Brij92     | 99.84       | 46.4 | —                     | +          | MLV                         | Many     |
| 20          | Brij92     | 99.84       | 46.4 | 0.2                   | —          | —                           | Surfactant droplets |
| 21          | Tween20    | 171.9       | 46.4 | —                     | +          | MLV                         | Few      |
| 22          | Span20     | 171.9       | 46.4 | 0.2                   | —          | —                           | Without noisome and surfactant droplets |
| 23          | Tween40    | 205.4       | 46.4 | —                     | +          | MLV                         | Many     |
| 24          | Span40     | 205.4       | 46.4 | 0.2                   | +          | MLV                         | Very few |

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Table 6: Formulation of noisome by direct mixing method with Tween 80

| Formulation | Surfactant | Cholesterol | Temperature | Percent of tween 80 | Mixing time and period | Existence of niosome | Relative number of niosomes | Preamble |
|-------------|------------|-------------|-------------|---------------------|------------------------|----------------------|---------------------------|----------|
| 25          | Brij52     | 20          | 30          | 65                  | 1.5                    | 30                   | +                         | Many     |
| 26          | Brij52     | 20          | 30          | 45                  | 1.5                    | 30                   | +                         | Many     |
| 27          | Brij52     | 20          | 60          | 45                  | 2                     | 30                   | +                         | Many     |
| 28          | Brij52     | 20          | 60          | 45                  | 2                     | 30                   | +                         | Many     |
| 29          | Brij52     | 20          | 60          | 45                  | 2                     | 30                   | +                         | Many     |
| 30          | Brij52     | 35          | 60          | 45                  | 2                     | 30                   | +                         | Many     |
| 31          | Brij52     | 35          | 65          | 45                  | 2                     | 30                   | +                         | Many     |
| 32          | Brij52     | 35          | 70          | 45                  | 2                     | 30                   | +                         | Many     |

Classification of niosomes by number of them – Many: A sample that is observed more than 50 niosomes in microscopic focus, Moderate: A sample that is observed 30-50 niosomes in microscopic focus, Few: A sample that is observed <30 niosomes in microscopic focus

Table 7: Drug entrapment in selected formulation (n = 14)

| Sample name | DEET (mg) | Un loaded DEET (mg) | Un loaded DEET (mg) | Un loaded DEET (mg) | Un loaded DEET (mg) | Average (mg) | Percent of entrapment |
|-------------|-----------|---------------------|---------------------|---------------------|---------------------|--------------|-----------------------|
| 1st sample  | 42        | 34.6                | 35                  | 35                  | 35                  | 34.7         | 34.8                  |
| 2nd sample  | 42        | 32.5                | 33.8                | 33.8                | 33.8                | 33.4         | 33.2                  |
| 3rd sample  | 42        | 35.4                | 36.6                | 36.6                | 36.6                | 36.1         | 36                    |

DEET: N,N-diethyl-meta-toluamide

Table 8: DEET released (%) from the selected formulation (n = 14)

| Measurement | 5 | 10 | 15 | 30 | 45 | 60 | 120 | 180 | 240 | 300 | 360 | 420 |
|-------------|---|----|----|----|----|----|-----|-----|-----|-----|-----|-----|
| Mean (%)    | 9.58 | 8.64 | 7.29 | 7.04 | 9.82 | 10.66 | 18.16 | 22.41 | 31.85 | 31.38 | 32.75 | 32.92 |
| SD          | 0.12 | 0.08 | 0.08 | 0.13 | 0.08 | 0.23 | 0.12 | 0.08 | 0.113 | 0.06 | 0.05 | 0.07 |

Mean ± SD, n = 3, SD: Standard deviation, DEET: N,N-diethyl-meta-toluamide

first order release, followed by a gradual release for at least 7 h which seems a good profile for long duration of action and low systematic side effects and that may be an ideal formulation for a topical insect repellent.
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