Original Research Article

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

Arezoo Amanatfard, Payam Khazaeli, Abbas Pardakhty, Moslem Lari Najafi, Amin Mohammadi

Pharmaceutics Research Center,Insitute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

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) ($C_{12}H_{17}NO$) 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.[2] DEET is a colorless and odorless liquid which is almost insoluble in water but soluble in glycerine, ethanol, and isopropyl alcohol.[7-9] 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

Address for correspondence:
Dr. Moslem Lari Najafi,
Pharmaceutical Research Center, Institute of Neuropharmacology and School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
E-mail: drlarinajafi@gmail.com

Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.167691

Alpha Keto receptors in the insect antenna, that is, sensitive to 1-octen-3-ol.[2]

Because of applying alcohol-based topical formulations due to their water-insolubility; increasing systematic DEET absorption and some toxic effects are investigated. On the other hand, volatility of these compounds is considered to be a limited factor in applying simple alcohol bases since it reduces their stability. Accordingly, designing a new medicinal formulation in this field seems to be necessary.[10]

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*.[11] 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.[10] 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), Brij52 (polyoxyethylene-2-cetyl ether), Brij58 (polyoxyethylene-2-stearyl ether), Brij92 (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

Niosomal formulations were prepared by three different methods.

Dehydration rehydration vesicle (DRV) method, direct mixing method, and homogenizing method.

The first one, DRV method. To make DRVs, one initially makes water-containing, small unilamellar vesicles (SUV), adds contrast media, and lyophilizes the mixture. Upon rehydration, the DRVs re-form, passively entrapping DEET (1%). The sorbitan surfactants (Fluka, Germany), cholesterol (Fluka, Germany) and a charge inducing agent such as dihexadecyl phosphate (Sigma, America) were used in order to induce negative charge in the final membrane structure. Materials were dissolved in chloroform (Fluka, Germany),the solvent was removed by rotary evaporator (Heidolph VV2000, Germany) under vacuum condition for about 2 h 10 ml phosphate buffer; pH 7.4 (Fluka, Germany) at 50-55°Cwas used for hydrating the niosomal film layer. Initial niosomes are obtained then transformed to dry and porous particles using freeze drying method, the dehydrated phase, contains drug added to the noisome suspension and DEET (Merk, Germany) loaded.

The second one, direct mixing method, polyoxyethylene cetyl ether (Fluka, Germany), cholesterol (Fluka, Germany), dihexadecyl phosphate (Sigma, America) and DEET (Merk, Germany), melted and entered in a 10 ml syringe. Another 10 ml syringe at 40°C was filled by Phosphate buffer; pH 7.4 (Fluka, Germany). Mixing between two syringes from a 1 mm pore for 2 min was done. Niosomes were separated with centrifuging (Ependorf 5415-D, Germany) at 1500 rpm during 15 min.

The third one, Homogenizing method, a certain amount of aqueous components (equal amount used in direct mixing method) heated at (40°C) in order to melt and mix. 49 ml phosphate buffer; pH = 7.4 (Fluka, Germany), was added through using homogenizer (IKA T10-Ultra-Turrax, Germany) with 500 rpm for15 min.

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.[12] 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 octadecylsilane-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.[13] 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 noisome 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

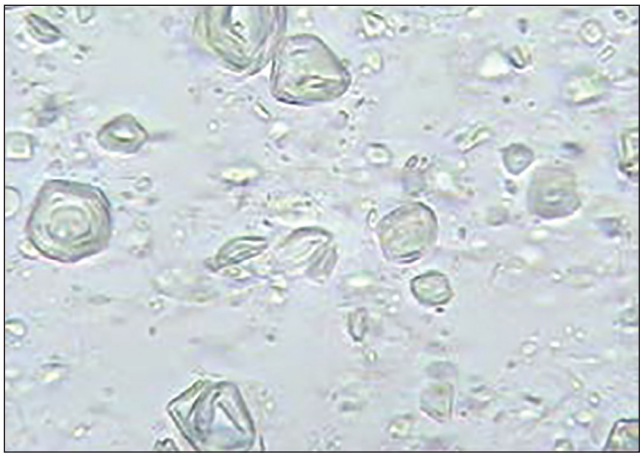

Figure 1: Microscopic photograph (×1000) of formulation number 14

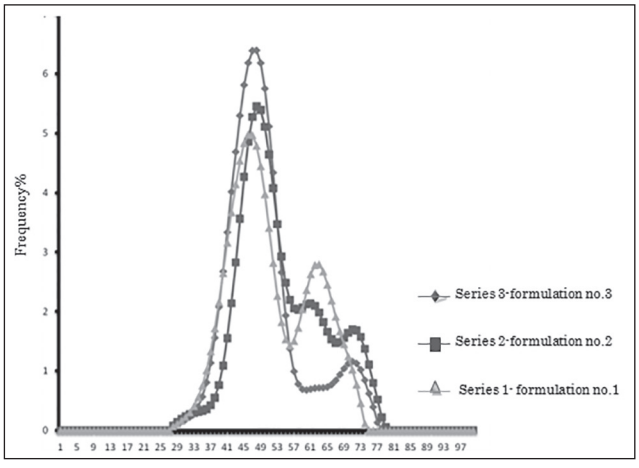

Figure 3: The effect of N,N-diethyl-meta-toluamide amount on the particle size distribution. Particle diameter (μm)

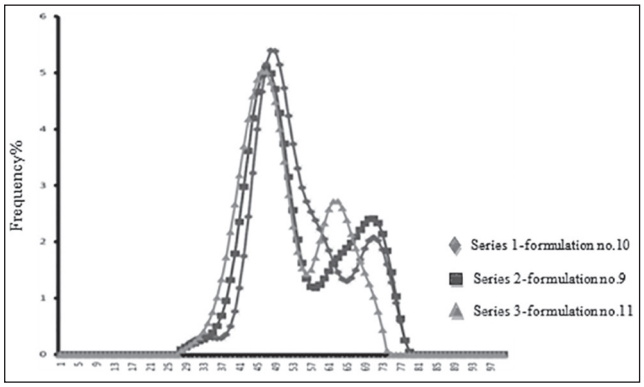

Figure 2: The effect of Brij amount on the particle size distribution. Particle diameter (μm)

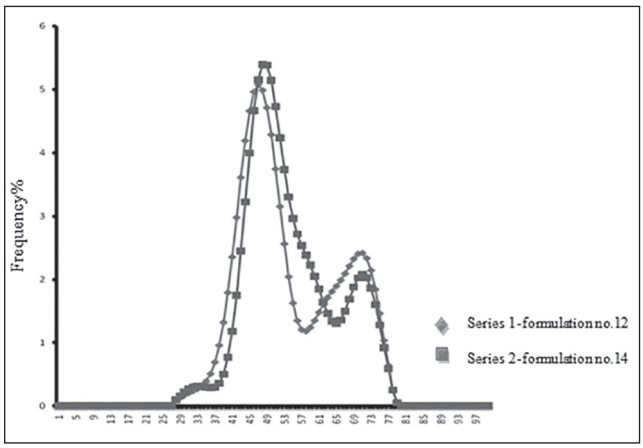

Figure 4: The effect of preparation method on the particle size distribution. Formulation number 12 and number 14 was prepared by direct mixing method

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 nisomes			Cholesterol crystal forming		
									1-week	1-month	3 months	1-week	1-month	3 months
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 nisomes in microscopic focus, Moderate: A sample that is observed 30-50 nisomes in microscopic focus, Few: A sample that is observed <30 nisomes 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 nisomes			Cholesterol crystal forming			Preamble
									1-week	1-month	3 months	1-week	1-month	3 months	
4	217	16	0	21	10	120 times/ 2 min	LUV	Few	—	—	+	—	—	—	Gel
5	217	16	24	21	10	120 times/ 2 min	MLV	Moderate	—	—	+	—	—	—	Asymmetric vesicles' with variable size
6	217	16	30	21	10	120 times/ 2 min	MLV, SUV, LUV	Moderate	—	—	+	—	—	—	Asymmetric vesicles' with variable size
7	217	16	38	21	10	120 times/ 2 min	MLV	Many	—	—	+	—	—	+	Asymmetric vesicles' with variable size (small)
8	217	16	55	21	10	120 times/ 2 min	MLV	Many	—	—	+	—	+	+	Small nisomes

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

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 nisomes			Cholesterol crystal forming			Preamble
									1-week	1-month	3 months	1-week	1-month	3 months	
9	217	16	24	21	10	120 times/ 2 min	MLV	Few	—	—	+	—	—	—	Asymmetric and big particles
10	240	16	24	21	10	120 times/ 2 min	MLV	Few	—	—	+	—	—	—	Asymmetric and big particles
11	250	16	24	21	10	120 times/ 2 min	MLV	Few	—	+	+	—	—	—	Asymmetric and big particles

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

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 nisomes		Cholesterol crystal forming		Preamble
									1-week	1-month 3 months	1-week	1-month 3 months	
12	240	16	24	42	10	30 min	MLV	Many	—	—	+	—	—	Small particles
13	240	16	24	42	10	120 times/ 2 min	MLV	Many	—	—	—	—	—	Small and medium particles
14	240	16	24	42	10	500 times/ 7 min	MLV	Many	—	—	—	—	—	Medium particles

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

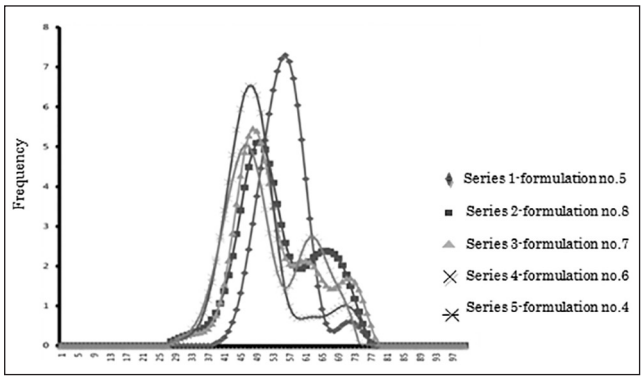

Figure 5: The effect of cholesterol amount on the particle size distribution. Particle size (μm)

have sufficient stability, sufficient loading, with low skin irritation, and toxic effects probably.

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 noisome 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 incopatability 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

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	Tubular and big with crystals
16		92.4	46.4	0.2	—	—	—	Surfactant droplets
17	Brij58	314.7	46.4	—	+	MLV	Few	Big niosomes
18		314.7	46.4	0.2	—	—	—	Complete dissolution of niosomes and surfactants
19	Brij92	99.84	46.4	—	+	MLV	Many	Similar shape, tubular and separated nisomes
20		99.84	46.4	0.2	—	—	—	Surfactant droplets
21	Tween 20	171.9 48.5	46.4	—	+	MLV	Few	With crystal
22	Span 20	171.9 48.5	46.4	0.2	—	—	—	Without noisome and surfactant droplets
23	Tween 40	205.4 46.48	46.4	—	+	MLV	Many	Big niosomes
24	Span 40	205.4 46.48	46.4	0.2	+	MLV	Very few	Surfactant droplets

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

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	200	30	65	1.5	30	+	Many	Small and useable niosomes
26	Brij52	200	30	45	1.5	30	+	Many	Small and useable niosomes
27	Brij52	200	60	45	1.5	30	+	Many	Small and useable niosomes
28	Brij52	200	60	45	1.5	60	+	Many	Small and useable niosomes
29	Brij52	200	60	45	1.5	20	+	Many	Small and useable niosomes
30	Brij52	353	60	45	2	30	+	Many	Small and useable niosomes
31	Brij52	353	65	45	2	30	+	Many	Small and useable niosomes
32	Brij52	353	70	45	2	30	+	Many	Small and useable niosomes

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

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

Sample name	DEET (mg)	Un loaded DEET (mg) 1st measurement	Un loaded DEET (mg) 2nd measurement	Un loaded DEET (mg) At 3rd measurement	Average (mg)	Percent of entrapment
1st sample	42	34.6	35	34.7	34.8	17.2
2nd sample	42	32.5	33.8	33.4	33.2	20.9
3rd sample	42	35.4	36.6	36.1	36	14.2

DEET: N,N-diethyl-meta-toluamide

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

Measurement	Time (min)											
	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	0.08	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.

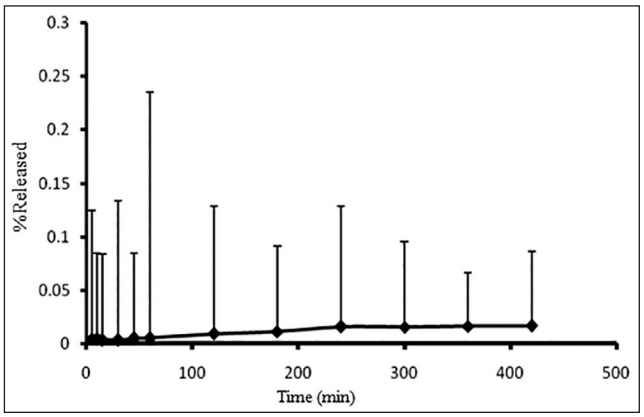

Figure 6: Release of N,N-diethyl-meta-toluamide

REFERENCES

1. Fardin M. Insect Repellents. J Med. 2002;204:1838-1840.
2. Ditzen M, Pellegrino M, Vosshall LB. Insect odorant receptors are molecular targets of the insect repellent DEET. Science 2008;319:1838-42. doi:10.1126/science.1153121.
3. David B. Troy PB. Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott Williams and Wilkins; 2006:1733. Available at: http://www.amazon.com/Remington-The-Science-Practice-Pharmacy/dp/0781746736. Accessed August 19, 2014.
4. Feldman EG D DE. Hand Book of Nonprescription Drugs. 18th ed. Washington; 2006:185.
5. Ralph Gordon Harry JBW. Harrys Cosmeticology. Chcago. (Technical LS&, ed.).; 1996:206-211.
6. Suryanarayana M V, Pandey KS, Prakash S, *et al*. Structure-activity relationship studies with mosquito repellent amides. J. Pharm. Sci. 1991;80:1055-7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1687691. [Last Accessed on 2014 Aug 19].
7. Sweetman S. *Martindale:* The Compelet Drug Referenced. Pharmaceutical Press; 2007:1857-1858.
8. Maradele J SAPE. The Merck Index. New York: Merck research laboratories; 2001:483.
9. Torner P, Ganderton D LW. The Pharmaceutical Codex. London: The pharmaceutical press; 1986:540.
10. United States Pharmacopoeia (USP) — National Organization for Rare Disorders. Available at: https://www.rarediseases.org/advocacy/alliances/usp. [Last Accessed on 2014 Aug 19].
11. Handjani-Vila RM, Ribier A, Rondot B, Vanlerberghie G. Dispersions of lamellar phases of non-ionic lipids in cosmetic products. Int. J. Cosmet. Sci. 1979;1:303-14.
12. Walash D. Paucilamellar lipid vesicles. 1988.
13. Van den Bergh BA, Vroom J, Gerritsen H, Junginger HE, Bouwstra JA. Interactions of elastic and rigid vesicles with human skin *in vitro*: Electron microscopy and two-photon excitation microscopy. Biochim. Biophys. Acta 1999;1461:155-73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10556497. [Last Accessed on 2014 Aug 22].
14. Khazaeli P, Pardakhty A, Shoorabi H. Caffeine-loaded niosomes: Characterization and *in vitro* release studies. Drug Deliv. 2007;14:447-52.
15. Biswal S, Murthy PN SP. Vesicles of Non-ionic Surfactants (Niosomes) and Drug Delivery Potential. Int J Pharm Nanotech. 2008;1:1-8.
16. Ciotti SN WN. Follicular liposomal delivery systems. J. Liposome Res. 12:143-8. doi:10.1081/LPR-120004787.
17. Naresh Ahuja, Vipin Saini, Vijay Kumar Bishnoi, Atul Garg M, Hisoria JS and KN. Formulation and evaluation of llansoprazole noisome. J Chem. 2008;1:561-563.

How to cite this article: Amanatfard A, Khazaeli P, Pardakhty A, Najafi ML, Mohammadi A. Preparation and characterization of physicochemical properties of N, N-diethyl-meta-toluamide niosomes. Int J Pharma Investig 2015;5:259-65..

Source of Support: Nil. **Conflict of Interest:** None declared.