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

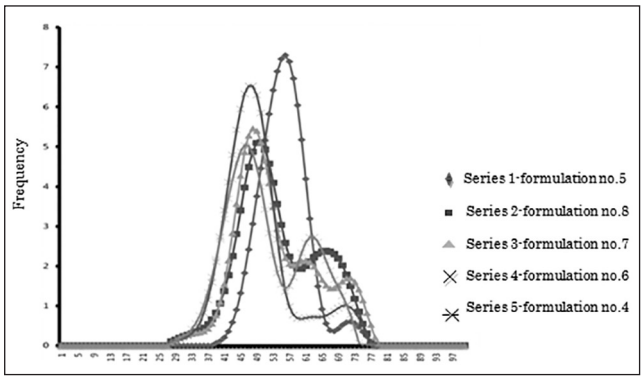

Figure 5: The effect of cholesterol amount on the particle size distribution. Particle size (μm)

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)	Un loaded DEET (mg)	Un loaded DEET (mg)	Average (mg)	Percent of entrapment
		1st measurement	2nd measurement	At 3rd measurement		
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.

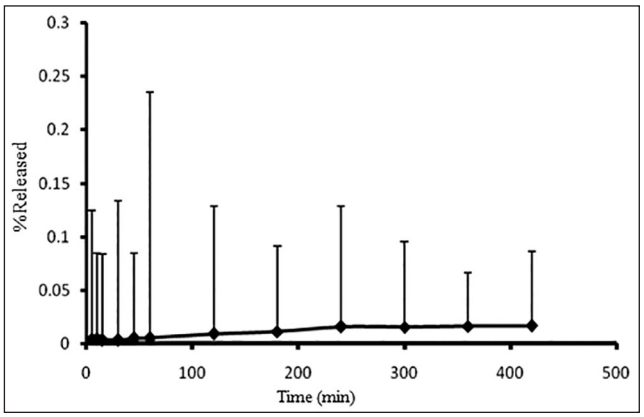

Figure 6: Release of N,N-diethyl-meta-toluamide

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