Journal of Pharmaceutical and Health Sciences 2013;1(4), 215-227.

Original Article

JPHS

Preparation and Skin Permeation Study of N, N- Diethyl- meta-Toluamide Semi Solid Formulations

Solmaz Ghaffari[1,2,3,4*], Shirzad Azarmi[5,6], Farzad Kobarfard[7], Amir Mehdizadeh[8], Mohammad Reza Avadi[1], Sanaz Ghaffari[9], Navid Nateghian[8], Fatemeh Ghaffari[5] , Nasim Sadri Alamdari[1]

[1] *Department of Pharmaceutics, Islamic Azad University, Parmaceutical Sciences Branch, Tehran, Iran.*
[2] *Department of Pharmaceutics, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.*
[3] *Quality Control and R&D Department, Iranian Parenteral and Pharmaceutical Co. Tehran, Iran.*
[4] *Young Researchers Club, Pharmacy Faculty, Islamic Azad University of Medical Sciences, Tehran, Iran.*
[5] *Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[6] *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alta, Canada T6G 2N8.*
[7] *Department of Medical Chemistry, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[8] *Quality Control and R&D Departments, Hakim Pharmaceutical Co. Tehran, Iran.*
[9] *Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*

Abstract

N,N-Diethyl meta Toluamide (DEET) is an insect repellent agent that contrary to its benefits, if is used in formulations with high skin permeation, will produce side effects of different severity. This study attempted to achieve a semi-solid DEET containing formulation with good appearance, sufficient spreadity, suitable viscosity for tube and jar filling, compatible pH with skin, reasonable stability, longer release time, and the less skin permeation. To obtain such a formulation, three types of DEET containing semi solids including gels (hydrophile), creams (emulsion) and ointments (lipophile), and their characteristics were compared with each other and with Off! Brand. Results showed that one of the prepared creams with the proper viscosity, stability, appearance and spreadity, had the least drug release in six hours and less skin permeation of DEET as compared with Off!. Hence the preparation was introduced as the optimal formulation.

Keywords: DEET dispersions, skin permeation, franz cell, rheology, stability study

***Corresponding author**: Solmaz Ghaffari, Pharm. D., PhD, Department of Pharmaceutics, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran And Department of Pharmaceutics, Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran
Tel: +98 2188275524 & +98 9125272146 Fax: +98 2155543301
E-mail address: ghafari@pharm.mui.ac.ir

1. Introduction

N,N-Diethyl meta Toluamide (DEET) is an insect repellent agent that used in many different preparations such as semi-solids and aerosols for more than 50 years (Reuvin et al., 1982. Gilbert et al., 1957. Ambrose et al., 1992 and Gilbert et al., 1966). The use of DEET in different forms can prevent spread of many disease, such as lyme, malaria that can transported by insects (Ambrose et al., 1992 and Gilbert et al., 1966. Blomquist et al., 1977 and Koren et al., 2003). Therefore, using DEET can reduce hospitalization costs improved public health.

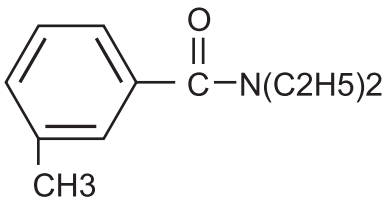

Figure 1. Chemical structure of DEET

Figure 1 illustrates the chemical structure of DEET. DEET has a chemical formula N,N-diethyl- m- toluamide or N, N-Diethyl- 3-methyl- benzamide (C 12 H 17 NO), with molecular weight of 191.27 and density of 0.996-1.002 (5). Despite the benefits of DEET, however, the use of the agent in inappropriate amounts or in formulations that can highly permeate to the skin, several side effects of different intensity may arise (Ambrose et al.,1992 and Koren et al., 2003). Therefore, the most important limitation of using DEET containing products is skin permeation (Koren et al., 2003. Sudakin et al., 2003 and Qui et al.,1997). Studies for finding suitable insect repellants products began from 1942 in USA. These studies investigated over 20000 compounds with repellency character, however, only a compounds were found to be safe for human (Ambrose et al.,1992). Insect repellents are volatile compounds that after spreading on skin, can volatize and repelled insects. These compounds have imids, alcohols, sters, ethers or 1,3 diols, amidsters, hydroxysters, diethers chemical groups. Among them, DEET which is an amid was used more than other compounds in the USA and other countries (McGready et al., 2001. Stinecipher et al., 1997. Mittal et al., 2011. Fei et al.,2011. Barry 1983 and Bell et al., 2002). DEET is non an allergic agent and does not cause irritation and inflammation nor does it damage clothes or produce bad smell. The compound is also stable against sunlight and sweating and is effective against many kind of insects (Blomquist et al., 1977. Koren et al., 2003. McGready et al., 2001 and Qiu et al., 1998). DEET can be in liquids, lotions, creams, ointments and sprays forms in the concentration of 4-100%, however, its children-specific formulations should be of less than 15% concentration. The effectiveness time depends on the percentage of DEET in the formulation (Qiu et al., 1998). Some studies reports that DEET creams provide 100 percent protection when applied at 10 mg/cm2 dose against An. stephensi and at 12 mg/ cm2 against Ae. aegypti. In field tests on human, creams provided complete protection of up to 11 hours against Anopheles mosquitoes and about 6 h against Ae. aegypti (Mittal et al., 2011). Additionally some oral compounds such as garlic and vitamin B1 can act as repellent agents (Reuvin et al., 1982).

The aim of this study was to prepare a semi-solid DEET containing formulation with good appearance, sufficient spreadity and viscosity and low skin permeation.

2. Materials and Methods

Materials

N, N-Diethyl meta Toluamide (DEET), poly ethylene glycol (PEG) 600, glycerin, cetostearyl alcohol, dimeticon, mineral oil, stearyl alcohol, cetyl alcohol, tween 80 and span 60, pemulen TR-2, methyl and propyl paraben (MPB and PPB), butylated hydroxytoluene (BHT), butylated hydroxyanisole(BHA), ethylene diamine tetra acetic acid (EDTA), sodium lauryl sulfate (SLS) and Triethanolamine (TEA) were purchased from Merck, Germany, ethanol 96° (Bidestan, Iran). Petrolatom and lanolin (Sepidaj, Iran). Carbomer 940 NF and Millipore synthetic membrane 1000 D were purchased from Sigma Aldrich, Germany and Millipore, U.S.A, respectively.

Methods

Formulations containing DEET were designed in three groups of gels, creams and ointments to compare different semi solid preparations and then their physicochemical characteris-

tics including active ingredient, rheological properties, active ingredient release profile, skin permeation, spreadity and formulations stability.
The formulations of gels, creams and ointments ingredients are described in tables 1-3 respectively.

Preparation of gel base formulations

Ingredients were mixed in three different parts named phase A, B and C. Phase A was prepared by mixing appropriate amounts of DEET, pemulen as a polymeric emulsifier and tween 80. Phase B was prepared by adding PEG 600 to distilled water (D/W). Phase C was prepared by mixing carbomer with MPB, EDTA and BHT as preservative and chelator and antioxidant respectively. First, phase A was added to phase B under mixing condition of 800 rpm. The mixture was then to phase C and the solution was mixed under similar condition for 15 minutes to achieve a soft gel. Five different formulations were prepared using the abovementioned method with some differences in ingredients and their used percentages. In F2, half of the water in the formulation was replaced by ethanol in order to provide a more clear gel, however, this change did not result in considerable improvement in the appearance. The viscosity of the formulation were studied, because the predicted filling condition was tubing and the viscosity was effective factor in filling process of semi solids in tubes. Addition of a higher quantity of TEA resulted in better homogenicity in emulgels and higher viscosity (F5).
Comparison of the viscosity of the presented emulgels in led to the following result: V F5>V F3>V F4>V F1>V F2 (Table 1)

Table 1. Gel formulations components

Components	Amount (g)				
	F_1	F_2	F_3	F_4	F_5
DEET	7.5	7.5	7.5	7.5	7.5
PEG 600	20	20	20	20	20
Pemulen TR-2	0.5	0.5	0.5	0.5	0.5
Carbomer 940 NF	1%	1%	1%	0.7%	0.7%
Tween 80	1	1	1	1	1
MPB	0.03	0.03	0.03	0.03	0.03
EDTA	0.05	0.05	0.05	0.05	0.05
BHT	0.05	0.05	0.05	0.05	0.05
Ethanol	----------	20	----------	----------	----------
D/W	40	20	70	69.3	69.3
TEA	q.s	q.s	q.s	q.s	q.s' *

Table 2. Creams formulations components

Components	Amount (g)		
	F6	F7	F8
DEET	7.5	7.5	7.5
D/W	59.5	68	60
Vaseline	17.5	12	------
Mineral oil	605	5	6
Cetostearyl Alcohol	8.35	6.5	------
Nipagin	0.135	0.135	0.135
Nipazol	0.015	0.015	0.015
SLS	1	1	------
Cetyl Alcohol	------	------	4
Stearyl Alcohol	------	------	4
Span 60	------	------	2
Tween 80	------	------	2
P.G	------	------	14.215

Table 3. Ointment formulations components

Components	Amount (g)	
	F_9	F_{10}
DEET	7.5	**7.5**
Vaseline	78.35	92.45
Lanolin	7	———
Mineral oil	7	———
Methyl paraben	0.15	0.05

Preparation method for cream base formulations

Phase A was prepared by adding preservatives and SLS to D/W according to the following procedure: preservatives were solved in warm water and after cooling to 60˚C, SLS was added to the solution. Phase B was prepared by mixing melted vaseline and cetostearyl alcohol and mineral oil with DEET. Then oil phase was added to water phase and mixed for 15 minutes to obtain a soft cream (F6 and F7). Formulation F8, was developed according to following procedure: phase A was prepared by mixing cetyl alcohol, stearyl alcohol, Tween, Span, Mineral oil and DEET. Phase B was made by dissolving of preservatives and PG in D/W. Phase B was then added to phase A and mixed to obtain a soft cream. The stability and spreadity of three different creams was compared.

Preparation of ointment base formulations

Ointment base formulations were prepared by fusion method. To this aim, all ingredients except DEET were mixed and heated to get molten. The mixture was then cooled, to 50˚C. DEET was then added to the vessel and the materials were mixed until reaching the room temperature. Both the F9 and F10 showed good appearance were used to study the effect of lanolin on skin permeation.

Calibration curve

DEET scanning by UV showed a maximum absorbance in 240 and 265 nm, which is consistent with previous studies (Qiu et al., 1998. Shokri et al., 2001 and Nokhodchi et al., 2003). The wave length 240 was selected. Then serial dilutions of DEET including 0.5, 1, 5, 10, 20, 40 ppm in Ethanol and phosphate

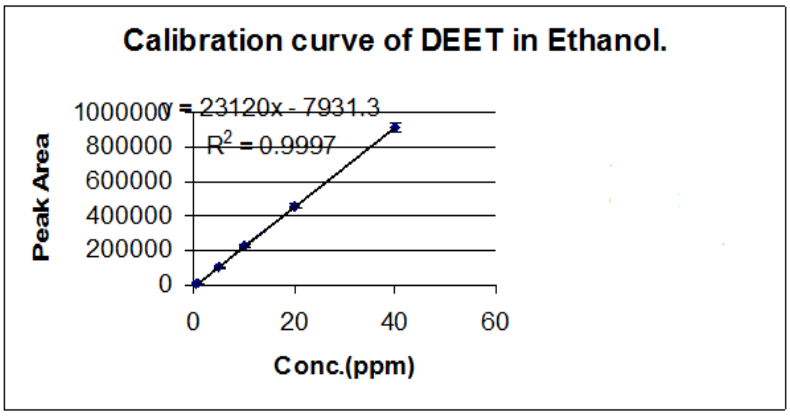

Figure 2. Calibration curve of DEET in ethanol

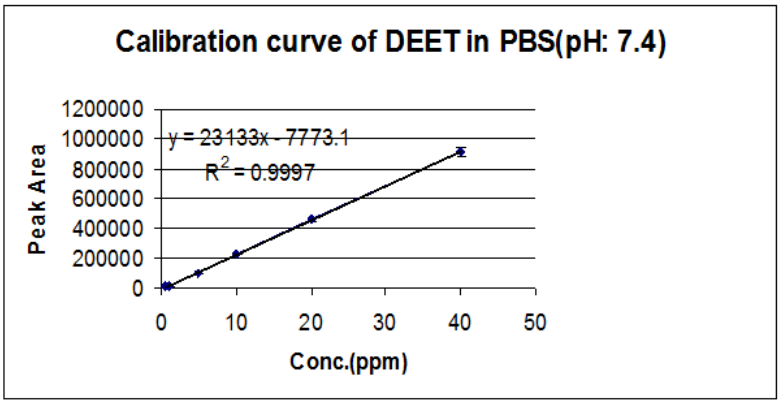

Figure 3. Calibration curve of DEET in PBS

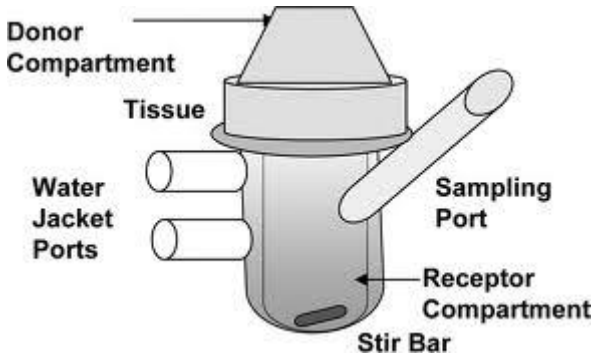

Figure 4. Schematic image of franz cell

buffer solution, PBS, (pH: 7.4) were prepared and injected to HPLC and calibration curve of each medium was analyzed (Figure 2, 3).

3. Release study

Franz cell (Ashke Shishe, Tehran, Iran) was used for release study. The characteristics of used franz cells was as follow (Figure 4): The volume of each cell was 36 ml, the effective surface diameter was 2 cm and water can be circulated between two layers of glasses that were designed to control of temperature. 300 mg of each selected formulations was used as donor phase. The receptor phase could be phosphate buffer or ethanol both of which were studied. However, buffer system was more similar to living condition of animals or human. Ethanol was used as the receptor phase to observe its effect on dissolution and skin permeation of DEET. Some other studies showed that using ethanol may lead to increased skin permeation because ethanol evaporates rapidly, leaving an increased DEET concentration that can lead to a more skin permeation (Koren et al., 2003). In this study ethanol was used as one of receptor phases to observe its effect on DEET permeation through the membrane or skin. The separator of the donor and receptor phases was synthetic membrane that was soaked in PBS 24 hours before usage. The temperature of circulating water was set at the temperature of human skin, 32.1˚C. Samples were gathered from receptor phase in intervals of 1, 2, 4, 5, 6 hours by replacing fresh receptor phase. Each sample was then injected to HPLC using Hitachi system as described below:

The column was C18, the UV Detector (L-7420 Merck- Hitachi, Germany) which was set at λ max 240 nm was used and system was equipped to pump (L-7100 Merck- Hitachi, Germany) and loop (20μl). The mobile phase was methanol and water with a ratio of 80/20 and the flow rate was 0.7 ml/min. The retention time of DEET was 5 minutes. One sample peak of HPLC was shown in Figure 5.

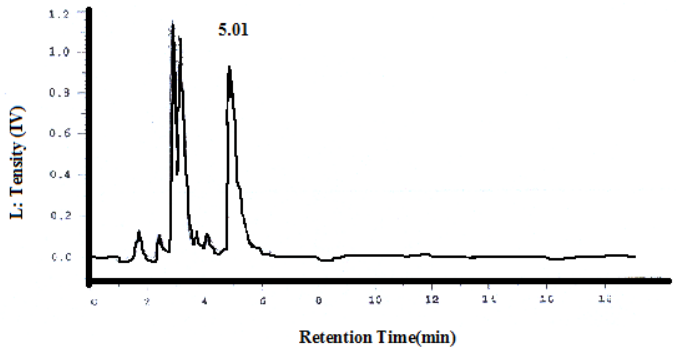

Figure 5. DEET HPLC peak sample

All steps described above were carried out for the four selected prepared formulations and DEET (Off! Brand, Johnson & Johnson, USA) one of which being special for children and the other for adults. Both formulations contained 7.5% DEET.

Skin permeation study

Abdominal area of skin of 280-340 g male rats were used after removing the hair using special Moser hair remover (Germany). Each abdominal skin area was treated by chloroform. All steps of this part of the study were similar to the release study and the skin was used as separator between donor and receptor phases (Mittal et al., 2011. Fei et al., 2007., Nokhodchi et al., 200. Puglia et al., 2001. Cooper et al., 1984. Hagedron et al., 1995 and Domb et al., 1995).

Physicochemical characteristics of the formulations

Spreadity:

Appropriate amounts of formulations and standards (Off!) were used on the human skin and the formulation smell and human sense was observed.

Measurement of pH:

The pH of all formulations was controlled to be compatible with human skin.

Determination of creams type:

To find that each cream was O/W or W/O, the removability of formulations was studied by diluting them with water.

Rheology study:

Viscosity and torque of the selected formulations of each group (F4, F8, F10) were studied with Bob and Cup rheometer (Brookfield-programmable- DV3, U.S.A) which could give data such as:

Stability studies:

F8 underwent physical and chemical stability study. Because it was the selected formulation, the study continued for three months at 4˚, 25˚ and 40˚ C and humidity of 65% and for 9 months at 30˚ C.

4. Results and Discussion

Selection of the best formulation of each type of semi solids

Among gel formulations after replacing 50% of water with ethanol in formulation F2, the clearance of gel did not show significant improvement in comparison with F1. In addition, due to addition of ethanol, more skin permeation was predicted. Among five gel formulations, F4 was selected for additional studies because of good appearance, sufficient viscosity and it pleasantness for user. Among cream formulations the cases 6 and 7 did not show good stability in the first days after preparation. However, F8 showed good appearance and stability, therefore, it was selected for more further investigation. Among ointments formulations, F9 had a bad smell because of presences of lanolin in formulation F10 was more preferred by the users. Hence, F10 was selected as ointment formulation.

Selection of suitable wave length for HPLC analysis

The result DEET UV scanning is shown in Figure 6a, and its second derivatives, is displayed in Figure 6b. As seen, the maximum absorbance of DEET was detected at 240 and 265 nm.

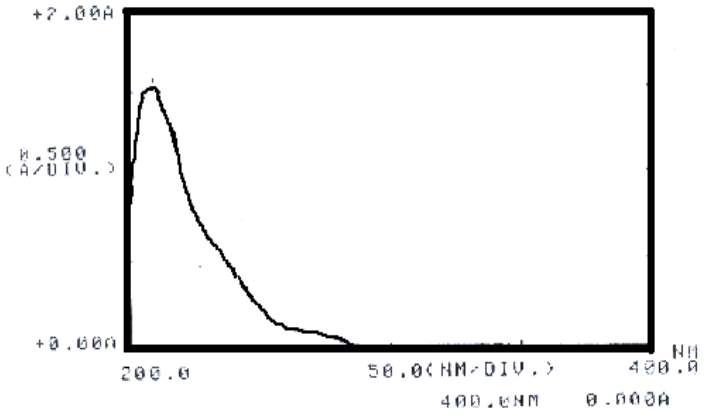

Figure 6a. DEET UV scan, in ethanol (6a), DEET UV scan, Second derivatization in ethanol (6b)

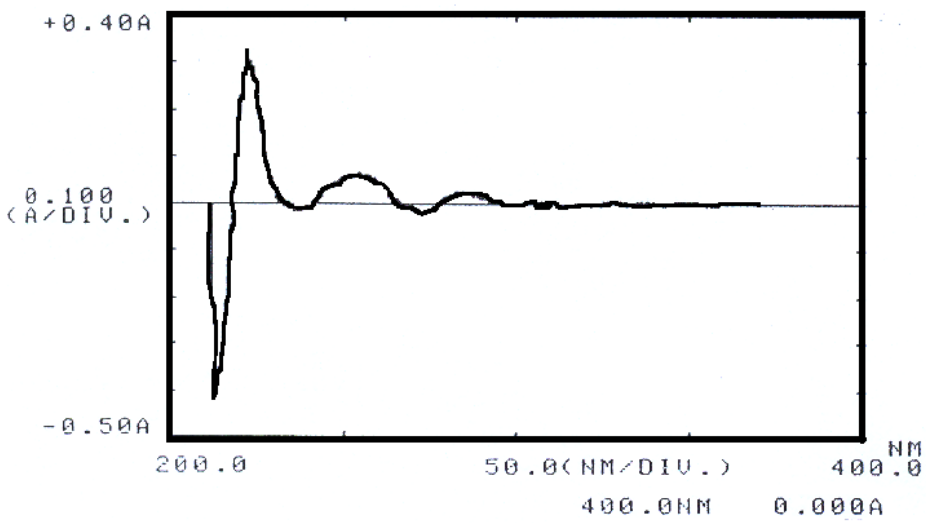

Figure 6b. DEET UV scan, in ethanol (6a), DEET UV scan, Second derivatization in ethanol (6b)

HPLC analysis

One sample of HPLC peaks is shown in Figure 5. The peak was sharp and without significant tailing.

pH results

All the formulations were in good range of pH falling within the range of human skin compatibility (pH: 5-5.6).

Selection of the best formulation

All of the selected formulations had good spreadity but the greasy after use of ointments and dryness of skin after use of gels did not produce a pleasant sense. therefore, F8 was selected as a cream based formulation to compare with the famous brand (Off!).

Determination of cream type

F8 was removable by water and O/W creams could be remove easily with water if needed.

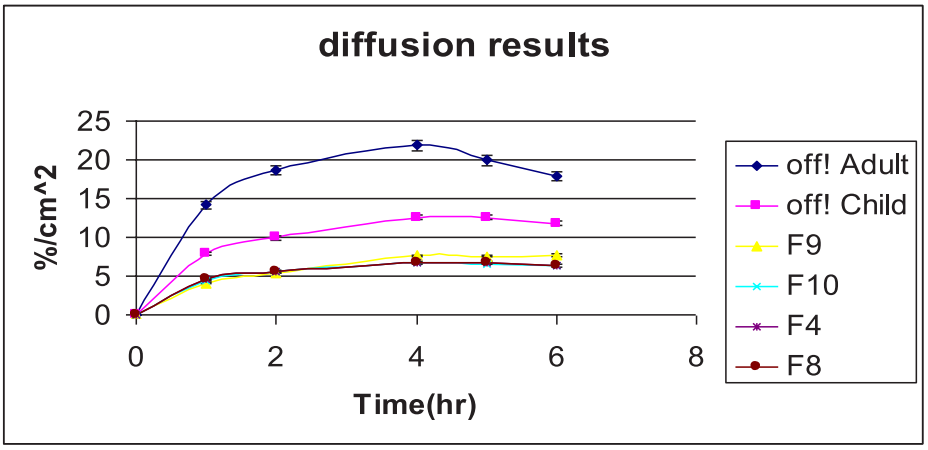

Figure 7. Diffusion of standard products (Off!) and selected prepared formulations

Release studies

Figure 7 illustrates the release algorithm for all type of the selected formulations (gel: F4, creams: F8, and two standards: adult Off! and Off! for child and ointments: F10&9 are the same but the amount of release versus time (rate of release) are different in various dosage forms. The release of the Off! formulation faster than the designed formulations and between prepared formulations. Moreover, no significant difference in the release rates of the prepared formulation was observed. However, DEET showed faster release rate from the gel compared with other formulations due to its lipophilic structure of and lack of affinity with water phase. Lower release rate of DEET from creams compared to gels, may be due to the affinity of DEET to lipid phase. But as it is clear the release pattern of creams and ointments were similar. Perhaps, the presence of oil phase in some particular percentage is sufficient to maintain DEET in the formulation.

Then optimum formulation (F8) was for skin permeation was compared with that of the standard (Off!).

Skin permeation test

Figure 8 compares the skin permeation of F8 with the standards. As seen, the lowest cumulative skin permeation of F8 occurred at the end time (6 hs).

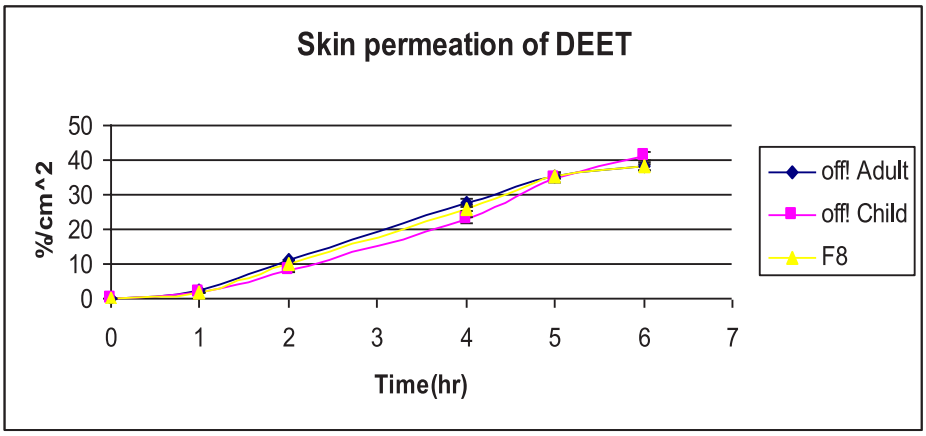

Figure 8. Skin permeation of standard products(Off!) and the best formulation(F8)

Rheology study

Figure 9-11 show the rheological behavior of the selected formulations in different RPMs. As already predicted, RPM increase leads to decrease of the viscosity. At the first stage of experiments the viscosity of cream was more than emulgel and that was more than ointment. It can be assumed that the presence of stearyl and cetyl alcohol in cream enhance the viscosity. All selected formulation showed plastic behavior.

Stability study

In tables 4-9 the results of stability study are shown for F8. All results of stability study were reasonable. Some other studies was done on stability of DEET formulations in liposphere system by Domb et al. Examination of the rabbits during the experiment and after necropsy showed no evidence of toxicity or irritation. The 10% DEET-liposphere formulation was stable at room temperature for at least 1 year (Domb et al., 1995).

5. Conclusion

This successfully introduced a suitable cream formulation human skin compatibility and acceptable skin permeation to avoiding DEET toxic effects. Future studies can make effort towards the scale up the production process. In addition, further development of the product can involve preparing an ointment with less skin permeation compared our prepara-

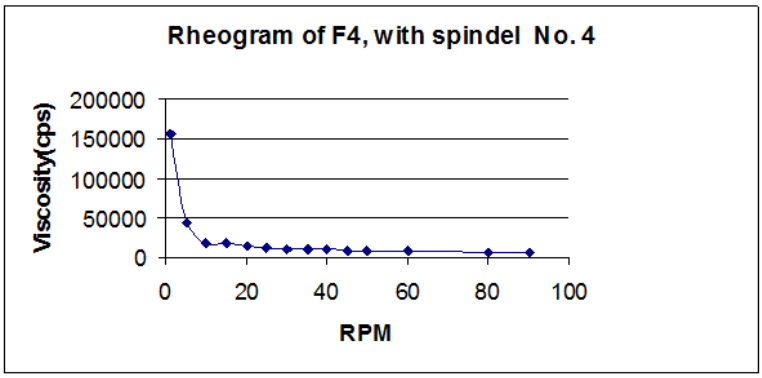

Figure 9. Rheogram of F4 with Brookfield Rheometer, Spindel No. 4 in 24˚C

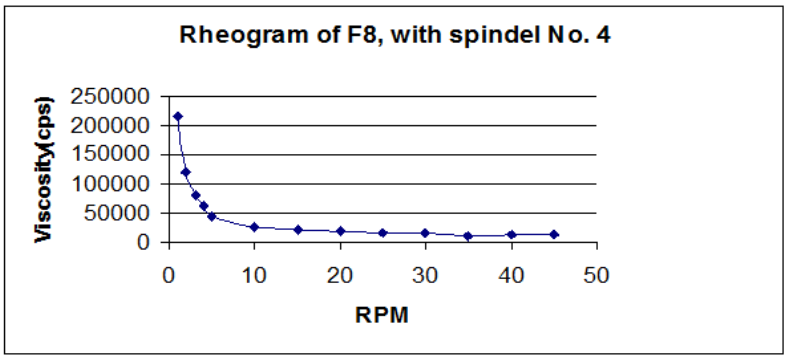

Figure 10. Rheogram of F8 with Brookfield Rheometer, Spindel No. 4 in 24˚C

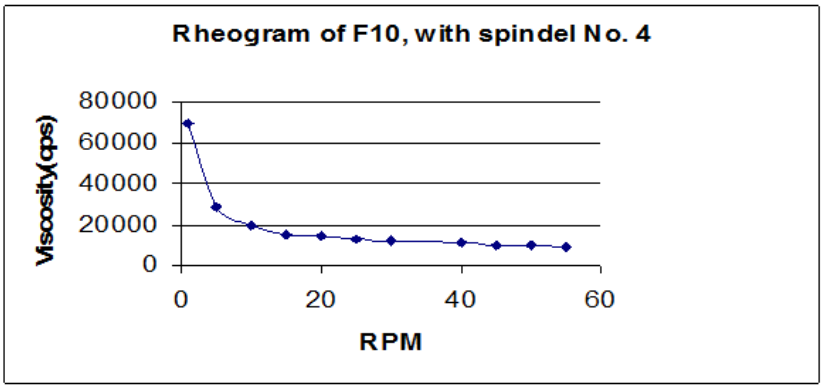

Figure 11. Rheogram of F10 with Brookfield Rheometer, Spindel No. 4 in 24˚C

Table 4. Assay test for standard

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
Standard(off!)® cream	0.067	0.067	0.067	0.067	0

Table5. Assay test for F8 in the production day

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
F_8	0.067	0.066	0.065	0.066	0.0081

Table 6. Assay of F8 after 9 months in 30˚C

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
F_8	0.065	0.065	0.067	0.0656	0.00087

Table 7. Assay of F8 after 3 months in 4˚C

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
F_8	0.066	0.067	0.065	0.066	0.00081

Table 8. Assay of F8 after 3 months in 25˚C

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
F_8	0.064	0.065	0.067	0.0653	0.012

Table 9. Assay of F8 after 3 months in 40˚C and 65% humidity

	Amount of active ingredient (mg/ml)				
	Test 1	**Test 2**	**Test 3**	**Average**	**SD**
F_8	0.056	0.054	0.077	0.0623	0.0102

tion for the users that cannot use the product repeatedly along the day such as soldiers or farmers. Previous studies have shown that that the use of PEG in DEET containing formulations can reduce the skin permeation of DEET by making interaction with this substance (McGready et al., 2001 and Stinecipher et al., 1997). Hence, adding PEG in gels can enable more control of side effects.

6. Acknowledgments

The authors would like to acknowledge the research vice chancellor of Islamic Azad University for financial support of this project. The technical assistance of Dr T. Gazori, Dr N.Chitgar and Mr R. Jafarian is appreciated. Also, the authors thank Isfahan University of Medical Sciences and Iranian Parenteral and Pharmaceutical Co (IPPC) for their kind collaboration. for th

7. References

Ambrose An. A comparison of the acute toxicity, neuropathology, and electrophysiology of N,N-diethyl-m-toluamide and N,N-dimethyl-2,2-diphenylacetamide in rats. Fund App Toxicol 1992; 79-88.

Barry BW. Properties that influence percutaneous absorption. Dermatological formulations, Marcel Dekker New York. 1983;127-133.

Bell JW, Veltri JC, Page BC. Human Exposures to N, N-diethyl-m-toluamide Insect Repellents Reported to the American Association of Poison Control Centers 1993-1997, Int J of Toxicol. 2002; 21:341-352.

Blomquist L, Thorsell W. Distribution and fate of the insect repellent 14C-N,N-Diethyl-m-Toluamide in the animal body. II. distribution and excretion after cutaneous application. Acta Pharmacol Toxicol. 1977; 41:235-243.

Cooper E. Increased skin permeability of lipophilic molecules. J Pharm Sci. 1984; 73:1153-1155.

Domb AJ, Marlinsky A, Maniar M, Teomim L. Insect repellent formulations of N,N-diethyl-m-toluamide (deet) in a liposphere system: efficacy and skin uptake. J Am Mosq Control Assoc. 1995;11(1):29-34.

Fei B, Xin JH. N, N-diethyl-m-toluamide–Containing Microcapsules for Bio-Cloth Finishing, Am. J Trop Med Hyg. 2007;77(1):52-57.

Gilbert IH. Evaluation and Use of Mosquito Repellents. JAMA. 1966; 196:253-255.

Gilbert IH, Gouck HK, Smith CN. Field tests on Diethyl-toluamide (DEET), a highly effective repellent against mosquitoes in the nip ah palm-mangrove swamps in Malaya. Soap Chem Special. 1957;33:95-97.

Hagedron U, Lippold BC. Absorption of sunscreens and other compounds through human skin in vivo: Derivation of a method to predict maximum fluxes. Pharm Res. 1995; 12(9):1354-1360.

Koren G, Matsuee D, Bailey B. DEET-based insect repellents: safety implications for children and pregnant and lactating women. CMAJ. 2003; 169(3):209-212.

McGready R, Hamilton KA, Simpson JA, Cho T, Luxemburger C, Edward R, Looareesuwan S et al. Safety of the insect repellent N,N-diethyl-M-toluamide (DEET) in pregnancy. Am J Trop Med Hyg. 2001;65(4):285-289.

Mittal PK, Sreehari U, Razdan RK, Dash AP, Ansari MA. Efficacy of Advanced Odomos repellent cream (N, N-diethyl-benzamide) against mosquito vectors. Indian J Med Res 2011; 133:426-430.

Nokhodchi A, Shokri J, Hassan-Zadeh D, Ghafurian T, Barzegar-Jalali M. The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int J Pharm. 2003; 250: 359-369.

Puglia C, Bonina F. Evaluation of alternative strategies to optimize ketorolac transdermal delivery. Int J Pharmaceutics. 2001; 228:79-87.

Qui H, Jun W. Reduced transdermal absorption of N,N-diethyl-m-toluamide from a new topical insect repellent formulation. Pharm Dev and Tech. 1997; 2(1):33-42.

Qiu H, McCall JW, Jun HW. Formulation of topical insect repellent N,N-diethyl-m-toluamide (DEET): vehicle effects on DEET in vitro skin permeation. Int J Pharm. 1998; 163(1-2):167-176.

Reuvin H, Yagupsky P. Diethyltoluamide-containing insect repellent. Arch Dermatol. 1982; 118:582-583.

Shokri J, Nokhodchi A, Dushbolaghi A, Hassan-Zadeh D, Ghafurian T, Barzegar- Jalali M. The efect of surfactants on the skin penetration of diazepam. Int J Pharm. 2001; 288:99-107.

Stinecipher J, Shah J. Health Risks and Benefits of Insect Repellents: Deet Health Concerns. J Toxicol Env Health. 1997; 52:119-135.

Sudakin LD, Trevathan WR. DEET: A Review and Update of Safety and Risk in the General Population. Clinic Toxicol. 2003; 41(6):831-839.