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Original Article

FORMULATION AND EVALUATION OF DISPERSED PERMETHRIN PRONIOSOMES IN POWDER AND MICROEMULSION-BASED HYDROGEL BASES FOR THE TREATMENT OF SCABIES

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ABSTRACT

Objective: This study is aimed to encapsulation of permethrin in proniosomes and formulation and evaluation of dispersed permethrin in powder and micro emulsion-based hydrogel bases.

Methods: Permethrin proniosomes were prepared by modified slurry method using Brij 97, cholesterol, aerosil 200 and drug in different weight ratios, and using two different solvents. The prepared proniosomes were characterized for particle size, shape, flow characteristics, entrapment efficiency.

Results: The studies demonstrated successful preparation of permethrin proniosomes. The effect of using different weight ratios of Drug: Brij 97: Cholesterol and different solvents on entrapment efficiency were studied. The best proniosomes showed entrapment efficiency percent of 55.58%±1.451 for permethrin and zero residual solvents. Such formula was incorporated in a topical powder and micro emulsion-based hydrogel basis and evaluated through particle size, drug content, stability, and clinical trials for efficacy on sarcoptic mite infestation in sheep and rabbits.

Conclusion: Permethrin 5 % micro emulsion-based hydrogel proved to be homogenous, stable, and clinically effective, compared with the topical powder that was unstable under accelerated stability conditions.

Keywords: Proniosomes, Permethrin, Powder, Micro emulsion-based hydrogel, Clinical trial.

INTRODUCTION

Niosomes are vesicles composed of non-ionic surfactants such as polyoxyethylene alkyl ethers, and may be prepared as single or multilamellar vesicles. Surfactants of this type are known to enhance skin permeation, and this is likely to play a role in any modification of permeation using these vehicles [1]. These synthetic nonionic lipid systems vary in size, shape, and structure. Their structure varies from approximately 15 to 3500 nm. The first patent dealing with these lipids came out in 1972 and 1975[2].

Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage. The proniosome approach minimizes these problems as it is a dry and free flowing product which allows for ease of transfer, distribution, measuring and storage. Thus making it a versatile delivery system [3].

Proniosome is a dry free flowing granular product that could be hydrated immediately before use, and would avoid many of the problems associated with aqueous niosome dispersions and problem of physical stability (aggregation, fusion, leaking) could be minimized. The additional convenience of the transportation, distribution, storage and dosing would make 'dry niosome' a promising industrial product [4]. In many references; Proniosomes are dry formulations of water-soluble carrier particles that are coated with a surfactant and hydrated by agitation in hot water for a short period of time [4, 5], yet in the following study the carrier used was aerosil 200, a water-insoluble carrier.

Proniosomes were a successful formulation for transdermal delivery systems [6-13], nebulisable delivery [14], Oral use [5] and topical preparations [15].

Powders for cutaneous application are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, coloring matter authorized by the competent authority [16].

Emulgel is a formulation with an emulsion/hydrogel combination [1]. Emulgels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining; water soluble, longer shelf life, bio-friendly, and pleasing appearance [17]. Micro emulsion-based hydrogel gives two, three, or four-fold more action than normally marketed products [18].

Scabies is a contagious disease of humans and other mammals. It is caused by the mite *Sarcoptes scabiei*, which burrows in the lower stratum corneum of the skin. Mites are tiny arthropods related to spiders and ticks [19]. Treatment is with permethrin, Malathion, or possibly to a lesser extent with lindane which are applied, preferably as an aqueous lotion, to clean, cool, dry skin over the entire body and left on for 8 to 24 h depending upon the preparation [20].

Thereby, a topical insecticide as a proniosome form may have a potential benefit for the treatment of scabies according to its advantages as a drug delivery system.

Permethrin is a pyrethroid insecticide. It's mode of action is reacting with the voltage-gated sodium channels causing paralysis of the insect [21].

In this study permethrin, proniosomes will be prepared and evaluated for entrapment efficiency, flow ability, and particle size. Then the optimum proniosome formulation will be dispersed in topical powder and micro emulsion-based hydrogel basis and evaluated through particle size, drug content and clinical trials for efficacy on *Sarcoptic* mite infestation in sheep and rabbits.

MATERIALS AND METHODS

Materials used

Permethrin was obtained from Misr for pharmaceutical preparations (Changzhou Kangmei Chem. Ind. Co., Ltd. China), Cholesterol (Sigma, molecular weight 386.65 g/mol, USA), Brij 97 (Sigma, molecular weight 710, USA), Aerosil 200 (New AWA, India), Carbomer 934, B. F., (USA), Methylparaben sodium (New AWA, India), Propylparaben sodium (New AWA, India), Labrasol (LAS,

PEG-8 caprylic/capric glycerides, Gattefossé, France), Plurol Isostearique (Polyglyceryl-6-isostearate, Gattefossé, France), Filter paper (Whattman, 125 mm), Gas chromatography with flame ionization detector (GC, Shimadzo, Japan), GC with mass detector (Shimadzo, Japan), Zetasizer (Malvern, UK), UV spectrophotometer (Shimadzo, Model 1650-PC, Japan), Scanning Electron Microscope (Electron probe microanalyzer, JEOL, JXA-840A, USA), Sonicator (Crest, USA), pH meter (Jenway UK), Filtration Kit (Consists of a vacuum pump, filtration glass apparatus, 10 mm bochner), All the other chemicals, reagents and solvents used like cyclohexane, acetone, ethyl alcohol, palmetic acid, n-hexane, isopropyl myristate, oleic acid, talc, sodium hydroxide pellets, methylene blue, and methanol were of analytical reagent grade.

Methods

Preparation of permethrin proniosomes

Formulation of permethrin proniosomes with different solvents and cholesterol/Brij ratios

In this study twelve proniosomes (granular powder) were formulated using the slurry method, without rotary evaporator. A mixed factorial design ($2^2X\ 3^1$) was conducted so that two methods of preparation (Method A and B) and two different solvents were used at three different levels of cholesterol/Brij ratios.

An aliquot of 100 mg of Permethrin and 1 g Brij was dissolved at first in the solvent. Cholesterol was then added, portion wise, with stirring using glass rod till complete dissolution.

Table 1: The independent variables and levels for mixed factorial design 2^2 X 3^1

Variable	Levels
Solvent	Cyclohexane
	Acetone
Method of preparation	Method A
	Method B
Cholesterol/Brij molar ratio	0.77
	2.31
	3.86

According to method A an aliquot of 1.16 g of aerosil 200 was then triturated with the solution using a mortar and pestle. The mixture was left to dryness in a 9 cm glass Petri dish at room temperature. After complete dryness, the powder was transferred to a mortar and lightly triturated (To guard against destruction of the formed proniosomes). While method B is as method A except aerosil 200 was added after dryness, with light trituration using a mortar and pestle.

Formulation of permethrin proniosomes with different permethrin concentrations and cholesterol/brij ratios

The effect of permethrin concentration and cholesterol/Brij ratio on entrapment efficiency was studied as well on eight formulations using the same method of preparation. Using method A of preparation, and cyclohexane as a solvent, the following $4^1\ X2^1$ mixed factorial design was used, so that two independent variables were studied, permethrin concentration and cholesterol/Brij molar ratio. Permethrin concentration had four levels, and cholesterol/Brij had two levels according to the following table:

Table 2: The independent variables and levels for 2^1 X4^1 mixed factorial design

Variable	Levels
Permethrin concentration	4.86 %
	13.22 %
	18.51 %
	22.77 %
Cholesterol/Brij molar ratio	0.77
	3.86

Eight preparations were prepared using the previous factorial design. Brij amount used was 62 mg for all the preparations while cholesterol amount was 26.7, and 134.3 mg for cholesterol/Brij molar ratio 0.77, and 3.86 respectively.

Evaluation of the resulting proniosomes

The resulting proniosomes were evaluated for visual appearance, bulk density, Carr's index, microscopical examination, permethrin entrapment efficiency, and residual solvent.

Bulk density (ρ_B) and tapped density (ρ_T): Each powder was separately passed through a sieve with aperture equals to1 mm. Bulk and tapped density was determined according to the USP 32.

Carr's index (C) was calculated for preparations no. 1-12 using the following formula:

$$C = 100 \times (1-\rho_B/\rho_T)$$

Microscopical examination: The best formula was selected and examined under the electron microscope.

Table 3: Permethrin preparations with its preparation details

Preparation No.	Cholesterol/Brij molar ratio	Method of preparation	Solvent used	Permethrin concentration %
1	3.86	A	Cyclohexane	2.29
2	3.86	A	Acetone	2.29
3	2.31	A	Cyclohexane	2.84
4	2.31	A	Acetone	2.84
5	0.77	A	Cyclohexane	3.73
6	0.77	A	Acetone	3.73
7	3.86	B	Cyclohexane	2.29
8	3.86	B	Acetone	2.29
9	2.31	B	Cyclohexane	2.84
10	2.31	B	Acetone	2.84
11	0.77	B	Cyclohexane	3.73
12	0.77	B	Acetone	3.73
13	0.77	A	Cyclohexane	4.86
14	0.77	A	Cyclohexane	13.22
15	0.77	A	Cyclohexane	18.5
16	0.77	A	Cyclohexane	22.77
17	3.86	A	Cyclohexane	4.86
18	3.86	A	Cyclohexane	13.22
19	3.86	A	Cyclohexane	18.5
20	3.86	A	Cyclohexane	22.77

Assay for free permethrin [22]

An aliquot of each preparation was separately transferred into Büchner attached to the filtration kit, and using 125 mm filter paper. An aliquot of 20 ml methanol was poured into the Büchner during the operation of the vacuum pump. The vacuum pump was stopped when no methanol was retained on the filter paper. The filtrate was transferred to 25 ml volumetric flask and volume was completed using methanol. The solution was measured using UV spectrophotometer at λ_{max} 272 nm.

The residual solvent determination in the prepared proniosomes using GC-mass [23]: The residual of cyclohexane in the best formula was determined. The concentration limit of cyclohexane was 3880 ppm in the proniosomes. Cyclohexane is considered class two residual solvent [23]. The procedure of analysis was done according to the USP 32.

The effects of different factors and their interaction were evaluated statistically. ANOVA test was performed to test the significance of the difference between the tested factors using computer software statveiw 4.57.

Preparation of permethrin proniosomes 26.67 % (PP)

Permethrin, Brij 97 was dissolved at first in cyclohexane. Cholesterol was then added, portion wise, with stirring using glass rod till complete dissolution. Aerosil 200 was then triturated with the later solution using a mortar and pestle. The mixture was left to dryness at room temperature. After complete dryness, the powder was transferred to a mortar, and triturated in a delicate manner and passed through 280 µm sieve.

Preparation of permethrin 5 % topical powder (PTP)

Palmitic acid was dissolved in ethyl alcohol at room temperature. Palmitic acid solution was sprinkled on talc powder, and the mixture was triturated using mortar and pestle. The mixture was left to dry at room temperature; then it was triturated again using the mortar and pestle. The mixture was passed through 280 µm sieve.

Preparation of permethrin 5 % micro emulsion-base hydrogel (PMG)

Determination of range of water to prepare micro emulsion

The following formula was mixed together to get a homogenous oily mixture.

Preparation of micro emulsion

The oily phase was mixed together as follows: 28.42 g LAS, 14.27 g plurol isostearique, 4.58 oleic acid, and 7.64 g isopropyl myristate. The aqueous phase was prepared by dissolving methylparaben sodium and propyl paraben sodium in purified water as follows: 0.2 g methylparaben, 0.05 g propylparaben, and 45 ml purified water**.** The aqueous phase was added, portion wise, to the oily phase at room temperature with continuous stirring using a glass rod to prepare the micro emulsion.

Table 4: The formula was as follows

S. No.	Ingredient	Quantity	Function
1.	Permethrin	5.00 g	Insecticide
2.	Cyclohexane	90 ml	Solvent
3.	Cholesterol	5.42 g	Basic proniosomal component
4.	Brij 97	2.58 g	Nonionic surfactant
5.	Aerosil 200	5.75 g	Carrier

Table 5: The formula of powder vehicle was as follows

S. No.	Ingredient	Quantity	Function
1.	Palmitic acid	8.84 g	Absorption enhancer
2.	Talc	43.16 g	Diluent
3.	Ethyl alcohol	Quantity sufficient	Solvent

The above mixture was mixed with pp in a closed suitable plastic container; Quantities were 9.38 g PP and 40.62 powder vehicle.

Table 6: The formula used to prepare the micro emulsion

S. No.	Ingredient	Quantity (g)	Function
1	LAS	9.3	Surfactant
2	Plurol Isostearique	4.67	Surfactant
3	Oleic acid	1.5	Absorption enhancer
4	Isopropyl myristate	2.5	Absorption enhancer

Purified water was added to the above mixture by titration, and range of water added that led to transparency of the mixture was determined.

Evaluation of micro emulsion

The micro emulsion prepared was evaluated for transparency (by the naked eye).

Polarizing microscope: a sample of 1 g was examined under a polarizing microscope to detect any liquid crystals.

Globule size and zeta potential was measured using zeta sizer.

pH was measured directly using pH meter.

Conductivity was measured directly using conductivity meter.

Viscosity was measured using rotational viscometer at different shear rates and stresses.

Preparation of gel

An aliquot of one g of carbomer 934 was dispersed, portion wise, in 80 ml purified water using a magnetic stirrer. Adjusting pH of the gel was done using 5 N sodium hydroxide (3 ml) to 5.5-6.0. The weight was completed to 100 g using purified water.

Preparation of micro emulsion-based hydrogel

The prepared micro emulsion and gel were mixed together at room temperature using mortar and pestle. The mixing ratio was 1: 1.

Preparation of PMG

An aliquot of 9.38 g of pp was mixed with 40.62 g emulgel using mortar and pestle at room temperature.

Evaluation of PTP and PMG

The prepared PTP was evaluated for physical appearance, assay, fineness, scanning electron microscopy, and accelerated stability. While PMG was evaluated as powder except for fineness, and viscosity were evaluated instead.

Physical examination

The PTP and PMG were examined visually for homogeneity.

Assay for permethrin using GC (using flame ionization detector)

Standard preparation: An aliquot of 100 mg working standard of permethrin was transferred to 50 ml volumetric flask, dissolved in n-hexane. And volume was completed using the same solvent.

Test preparation: An aliquot of 500 mg of PTP/PMG was transferred to 100 ml volumetric flask. It was dissolved in n-hexane and volume was completed using the same solvent.

Fineness (Sieve test)

The topical powder was examined for fineness using sieve 280 µm.

PMG rheological properties

PMG was evaluated for rheological properties as follows: One gram of the PMG was transferred to the plate of the cone and plate viscometer (Brookfield viscometer, Model III Brookfield, DV-I, USA). The water bath was adjusted at 25 ˚C. The viscometer was run from 0.5 to 100 RPM. The upward and down-ward curve was plotted with lag time of 1 minute.

Rheological parameters used for evaluation:

a. Viscosity values at each RPM.

b. Yield value: This is the point at which the extrapolation of the straight part of the up-curve of the rheogram intersects the shearing stress axis [24].

c. Hysteresis loop area formed by the up-and down curves of the rheograms, which has been proposed as a measure of the thixotropic breakdown [24].

d. Thixotropy is an isothermal and comparatively slow recovery, on standing of a material, of a consistency lost through shearing [24].

e. Farrow's number: the power law is given as $\sigma = K\gamma^n$ or $\log \sigma = K + n \log \gamma$, where σ is shear stress, γ is the shear rate, K and n are constants. Values of $n>1$ corresponds to dilatants or shear thickening behavior and values of $n<1$ to shear-thinning behavior. Newtonian liquids have n= 1 [25].

Scanning electron microscopy

A sample of PMG was prepared and examined under scanning electron microscope for proniosomes.

Accelerated stability study

According to the international conference on harmonization (ICH) guidelines for stability testing, the accelerated stability conditions are 40±2 ˚C for temperature and 75±5 % for the relative humidity. The intervals of testing were 0, 3, 6 mo. PTP & PMG were packaged into a glass container with a plastic closure. The products were tested every interval for the physical appearance, and permethrin assay.

Clinical evaluation

The clinical study was conducted to evaluate the efficacy of topically applied PTP, PMG and compare it with commercially available Permethrin 5 % Lotion for the treatment of naturally infested sarcoptic mange in rabbits and sheep [26].

RESULTS AND DISCUSSION

Permethrin proniosomes preparations

Permethrin proniosomes are all powders and having a white color. According to fig. 1, Preparation No1, 5 & 6 show better flow ability than other preparations and also shows the distribution of Carr's index among the different preparations.

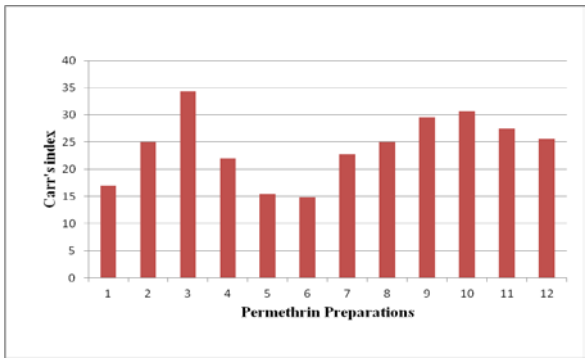

Fig. 1: Carr's index for different permethrin preparations

Fig. 2 shows the proniosomes under the electron microscope for the preparation No 19. Proniosomes vesicles size range is from 8-30 µm. This size range may be attributed to surfactants with longer alkyl chains generally give larger vesicles (Brij 97 Molecular weight 710) [27]. And increasing hydrophilicity of the surfactant monomer led to a larger vesicle (Brij 97 HLB 12), a result that is expected since surface energy decreases with increasing hydrophobicity [28].

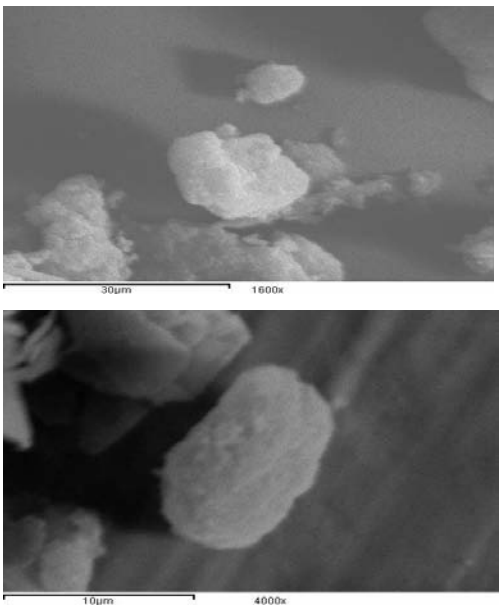

Fig. 2: The Proniosomes for permethrin preparation No. 19 under electron microscope

Preparations No 1-3, 17-20 have permethrin retention in proniosomes of 65.41, 42.38, 42.24, 65.4, 47.14, 55.58 & 49.75 % respectively. While preparations No 4 to 16 have permethrin retention of 26.05, 35.11, 24.86, 31.16, 31.96, 29.83, 18.45, 5.01, 25.19, 24.86, 13.78, 11.81& 19.49 % respectively, showing the least permethrin retention.

Cyclohexane as a solvent in the preparations shows more or less better entrapment efficiency than acetone. Acetone and cyclohexane vapor pressures are 240, 104 m bar respectively [29, 30]. Accordingly cyclohexane is less volatile than acetone. It is reported that elevated vapor pressures trigger crystallization of disordered materials [1], accordingly slow evaporation of cyclohexane may allow molecules of cholesterol, Brij, and permethrin to be more organized resulting in more entrapment of permethrin within proniosomes.

The entrapment efficiency of permethrin in the proniosomes increases with higher cholesterol/Brij molar ratio. And using method A in the preparation leads to the same result. Such result

may be attributed to high cholesterol content (~ 50 % total lipid) and larger vesicle size that are reported to increase entrapment efficiency. Also, cholesterol has the property to prevent leakage from the niosomal formulation that may explain such results [5].

According to Prabagar Balakrishnan *et al.* [27] as the HLB of the surfactant increases above 10, the minimum amount of cholesterol necessary to form vesicles increases, because more cholesterol is necessary to compensate for the larger head group. Such explanation may be valid as well for the above results, especially because the HLB value of Brij 97 is 12.

Method A has an advantage over method B in that no physical stress is performed on the prepared proniosomes. This may explain better entrapment efficiency of permethrin in the proniosomes by using such method. Also, addition of aerosil 200 before drying of the preparation shows better results especially in the flow ability of the powder, and entrapment efficiency.

Table 7: Percentage of permethrin entrapment efficiency in different preparations

Preparation	% of retained permethrin in niosomes *
1	65.41±2.517
2	42.38±2.50
3	42.24±2.521
4	26.05±2.511
5	35.11±2.508
6	24.86±2.49
7	31.16±2.501
8	31.96±2.499
9	29.83±2.510
10	18.45±2.508
11	5.01±2.503
12	25.19±2.502
13	24.86±1.408
14	13.78±1.715
15	11.81±0.721
16	19.49±2.001
17	65.40±2.051
18	47.14±0.357
19	55.58±1.451
20	49.75±1.087

*All values are expressed as mean±SD (n=3)

According to table 7, entrapment efficiency into proniosomes increases as well with cholesterol/permethrin ratio. The improvements in drug entrapment with increased cholesterol may be due to: (1) with increased cholesterol, the bilayer hydrophobicity and stability increased (Bernsdorff *et al.*, 1997) [31] and permeability decreased (Kirby *et al.*, 1980)[32], which may lead to efficiently trapping the permethrin drug into bilayers as vesicles formed. (2) In contrast, higher amounts of permethrin may compete with cholesterol for packing space within the bilayer, hence excluding cholesterol during assembly into the vesicles, leading to more leakage, permeability and less stability [27].

The entrapment efficiency of the prepared permethrin proniosomes is comparable to that of norfloxacin proniosomes as studied by Debey Akhelish *et al.* Where entrapment efficiency was 72.69 % using span 60: cholesterol molar ratio170: 80, and maltodextrin as a carrier [5]. But beclomethasone dipropionate entrapment efficiency reported by Elhissi A. *et al.* was 34.6 % using span 60: cholesterol ratio 1: 1 and sucrose as a carrier [14].

Entrapment efficiencies for proniosomal gels are higher than that of proniosomes powders. Clotrimazole proniosomal gel has 91.92 % entrapment efficiency using lecithin: cholesterol 18:2 ratio as reported by Samita Singla *et al.* [33]. The encapsulation efficiency of ornidazole Proniosomal gel formulations are in the range of 38% to 78% as shown by G. V. Radha *et al.* [34]. While Anindita De *et al.* showed the proniosome gel vesicles prepared with span 60, 40 and cholesterol had maximum entrapment efficiency (76.77 %±1.54) [28].

The permethrin proniosomes dry powders were prepared so that no aqueous phase was used. And so the proposed structure for them has compacted spheres with no aqueous core nor compartments. While entrapped permethrin was embedded within the hydrophobic bilayer of such spheres.

Preparation No 19 was selected as it had comparatively good entrapment efficiency (55.57 %) and can be used for preparing topical dosage forms due to the high concentration of permethrin. Residual solvent on such preparation and was found to be zero. This result indicates that such method of preparation and the proniosomes have an acceptable safety to the patient according to USP 32 acceptance criteria [23].

To evaluate the effect of organic solvent, cholesterol/Brij ratio, and preparation method on entrapment efficiency and Carr's index, statistical analysis was done. ANOVA test was performed to test the significance of the difference between the tested factors using computer software stat view 4.57, and using fisher's PLSD test between the different pairs. Tables 8 & 9 show that organic solvent, cholesterol/Brij ratio and preparation method had a significant effect on entrapment efficiency ($P<0.05$). While tables 10 & 11 show that both cholesterol/Brij ratio and preparation method had a significant effect on Carr's index (flow ability) ($P<0.05$). However, type of organic solvent had no significant effect on Carr's index.

Table 8: The statistical analysis for the entrapment efficiency of permethrin according to the factorial design (ANOVA table)

Source of variation	DF	Sum of squares	Mean squares	F-value
Organic solvent	1	390.458	390.458	61.651
Cholesterol/Brij ratio	2	2530.626	1265.313	199.786
Organic solvent X Cholesterol/Brij ratio	2	604.265	302.132	47.705
Preparation method	1	2246.760	2246.760	354.752
Organic solvent X Preparation method	1	856.148	856.148	135.181
Cholesterol/Brij ratio X Preparation method	2	223.987	111.994	17.683
Organic solvent X Cholesterol/Brij ratio X Preparation method	2	273.943	136.971	21.627
Residual	24	152.000	6.333	------

DF: Degrees of freedom

Table 9: Fisher's PLSD test for the main effects of factors used in the factorial design

Source of variation		Fisher's PLSD test for entrapment efficiency		
		Different pairs	Mean difference	P-value
Organic solvent	Cyclohexane acetone	Cyclohexane, acetone	6.587	0.001 S*
Cholesterol/Brij ratio	0.77	0.77, 2.31	13.473	0.001 S*
	2.31	0.77, 3.86	20.160	0.001 S*
	3.86	2.31, 3.86	6.688	0.001 S*
Preparation method	Method A Method B	Method A, Method B	15.8	0.001 S*

S*: Significant at $P<0.05$, PLSD: Pairwise least significant difference

Table 10: The statistical analysis for the Carr's index of permethrin preparations according to the factorial design (ANOVA table)

Source of variation	DF	Sum of squares	Mean squares	F-value
Organic solvent	1	2.789	2.789	1.235
Cholesterol/Brij ratio	2	473.866	236.933	104.906
Organic solvent X Cholesterol/Brij ratio	2	168.114	84.057	37.217
Preparation method	1	267.322	267.322	118.361
Organic solvent X Preparation method	1	12.461	12.461	5.517
Cholesterol/Brij ratio X Preparation method	2	163.996	81.998	36.306
Organic solvent X Cholesterol/Brij ratio X Preparation method	2	156.214	78. 107	34.583
Residual	24	54.205	2.259	------

DF: Degrees of freedom

Table 11: Fisher's PLSD test for the main effects of factors used in the factorial design

Source of variation		Fisher's PLSD test for Carr's index		
		Different pairs	Mean difference	P-value
Organic solvent	Cyclohexane Acetone	Cyclohexane, Acetone	0.557	0.2775
Cholesterol/Brij ratio	0.77	0.77, 2.31	-6.738	<0.001 S*
	2.31	0.77, 3.86	1.650	0.0128 S*
	3.86	2.31, 3.86	8.388	<0.001 S*
Preparation method	Method A Method B	Method A, Method B	-5.450	<0.001 S*

S*: Significant at P<0.05, PLSD: Pairwise least significant difference

The different interactions under study between the different factors used were shown to be not significant at (P<0.05). Tables 9 &11 show that there was no interaction between different factors used. This means that the different factors do not affect each other.

The effect of permethrin concentration and cholesterol/Brij ratio on entrapment efficiency was also evaluated using statistical analysis (ANOVA test, stat view 4.57 software). Tables 12 & 13 show that both permethrin concentration and cholesterol/Brij ratio had a significant effect on entrapment efficiency (P<0.05). The different interactions under study between the different factors used were shown to be not significant at (P<0.05).

The selected proniosome formulation represents a product of new preparation approach. Using a modified slurry method without vacuum, and aerosil 200 as an insoluble carrier. Such permethrin proniosome is not applicable to be used as such topically because it is granular in nature, other than proniosomal gels [35]. But it should be loaded on a base material of emulsion, gel, ointment, etc. that can be used topically. It has the advantage of being powder, easy handling, storage, distribution, simple preparation, and no residual solvents.

Table 12: The statistical analysis for the entrapment efficiency of permethrin according to the factorial design (ANOVA table)

Source of variation	DF	Sum of squares	Mean squares	F-value
Permethrin concentration %	3	803.565	267.855	125.558
Cholesterol/Brij ratio	1	8256.234	8256.234	3870.140
Permethrin concentration X Cholesterol/Brij ratio	3	147.892	49.297	23.108
Residual	16	34.133	2.133	------

DF: Degrees of freedom

Table 13: Fisher's PLSD test for the main effects of factors used in the factorial design

Source of variation		Fisher's PLSD test for entrapment efficiency		
		Different pairs	Mean difference	P-value
Permethrin concentration %	4.86 %	4.86, 13.22	15.855	<0.0001 S*
	13.22 %	4.86, 18.50	10.803	<0.0001 S*
	18.50 %	4.86, 22.77	10.792	<0.0001 S*
	22.77 %	13.22, 18.50	-5.052	<0.0001 S*
		13.22, 22.77	-5.063	<0.0001 S*
		18.50, 22.77	-0,12	<0.9891
Cholesterol/Brij ratio	0.773.86	0.77, 3.86	-37.095	<0.0001 S*

S*: Significant at P<0.05, PLSD: Pair wise least significant difference

Permethrin proniosomes 26.67 % (PP)

PP is homogenous and having white color. It shows good flow ability having bulk density, tapped density, and Carr's index 0.40 g/ml, 0.49 g/ml, and 18.36 respectively. The assay of permethrin was 98.2 %, and permethrin retention in the proniosomes was 55.6 %. Such results show that PP were successfully prepared and had the same retention of permethrin as preparation no. 19 (same formula).

Permethrin 5 % topical powder (PTP)

PTP was visually homogenous, and the assay of permethrin was 98.17 %. The powder passed the 280 µm sieve. Such results indicate that the topical powder was successfully prepared.

According to fig. 3 the scanning electron microscope revealed the proniosomes in the topical powder having rounded shape, and its size ranges from 8-30 µm.

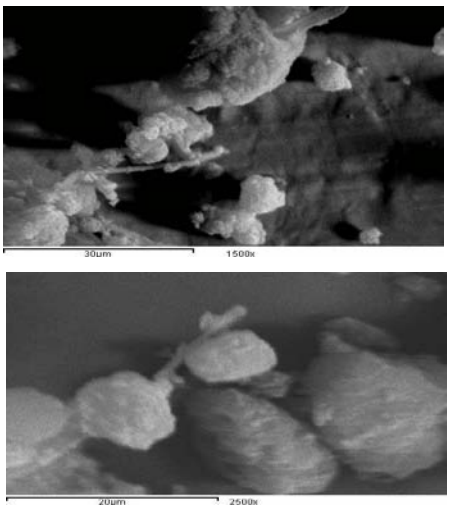

Fig. 3: The Proniosomes for Permethrin 5 % topical powder (PTP) under scanning electron microscope

PTP stability testing showed that the product was stable physically, no change in color, appearance, or homogeneity. Yet, the assay of permethrin was as follows 98.17, 80.12, 66.34 % after zero, three, six months respectively storage at accelerated stability conditions. This result may be attributed to the loss of permethrin that is not retained within the proniosomes (44.4 %) under accelerated stability storage conditions.

Such results indicate that PTP is not stable under such conditions, and may not be stable enough during the shelf life.

Range of water needed to prepare micro emulsion

An aliquot of 32.72 g micro emulsion was prepared using 14.75 ml purified water. The micro emulsion began to be transparent using 11.4 ml water and changed to translucent at 18.1 ml, the midpoint was 14.75 ml.

Permethrin 5 % micro emulsion-based hydrogel

The micro emulsion prepared was a clear light yellow micro emulsion, Examination under a polarizing microscope indicates that there are neither crystals nor liquid crystals and that it is isotropic. The micro emulsion under a light microscope, as indicated by fig. 4, shows sponge-like structure of bi continuous structure micro emulsions.

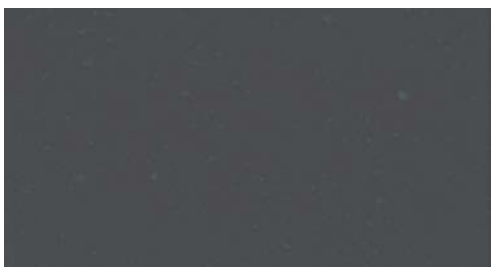

Fig. 4: Micro emulsion under light microscope showing sponge phase like structure

Examination using Zetasizer shows the average globule size of the micro emulsion 57.17 d. nm, while the zeta potential (–106 mV). Zeta potential is a useful tool to estimate the stability of the dispersed system, the high value of zeta potential (-106 mV) would likely prevent particle aggregation to occur owing to electrostatic repulsion between the particles [36]. The pH of the micro emulsion is 5.5 and the conductivity is 23.6 µS/cm. The flow pattern of the micro emulsion is newtonian flow according to fig. 5, and the average viscosity is 227.8 cp according to table 16. These results can be explained by the relatively small drop size (57.17 d. nm) which leads to an increase of viscosity, the lipophilic properties of oleic acid that increase the oil phase viscosity,[37] the oily character of Plurol isosteric,[38] and the bi continuous structure [39, 40]. Also, the percent of water and surfactant/co-surfactant have a more positive effect and negative effect on viscosity, respectively [40].

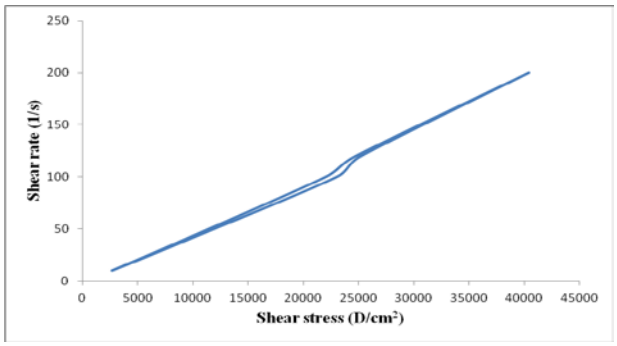

Fig. 5: Rheogram for the micro emulsion using rotational viscometer

The micro emulsion was clear transparent of yellow color. While microemulsion-based hydrogel was homogeneous and translucent. The prepared PMG shows visual homogeneity, and having permethrin assay of 97.5 %, indicating successful preparation.

According to fig. 6 and table 14 PMG shows a yield value of 1000 D/cm^2, and shear-thinning behavior having Farrow's no. 0.221 (less than 1). Also the micro emulsion-based hydrogel is thixotropic having hysteresis loop area 10 D/sec cm^2.

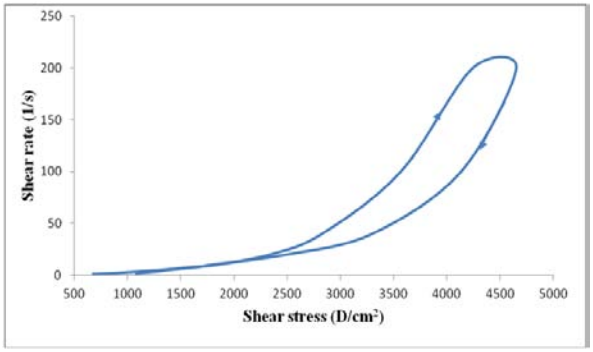

Fig. 6: The Rheogram for permethrin 5 % micro emulsion-based hydrogel (PMG)

The proniosomes of the PMG as shown in fig. 7 are round shaped; vesicles sizes range from 8 to 30 µm.

PMG stability testing showed that the product was stable physically, no change in color, appearance, or homogeneity. And the assay of permethrin was as follows 97.47, 97.10, and 96.50 % after zero, three, six months respectively storage at accelerated stability conditions.

Such results indicate that PMG is stable under such conditions, and may be stable enough during the shelf life.

Table 14: Rheological properties of permethrin 5 % micro emulsion-based hydrogel (PMG)

Condition at 25 ˚C, spindle 52		
Shear Rate (1/s)	**Shear stress (D/cm²) ***	**Viscosity (cp) ***
1	1085±0.33	165100±50.00
2	1116±1.22	55830±60.83
4	1321±1.64	32240±40.00
5	1384±3.24	27990±65.57
8	1683±6.42	20840±79.37
10	1777±2.64	17770±26.46
20	2500±5.26	12580±26.46
40	3303±18.81	8060±45.83
100	4136±52.91	4262±54.15
200	4655±60.84	2233±28.58
200	4246±59.98	2147±30.45
100	3570±50.88	3554±50.48
40	2831±9.37	7077±23.39
20	2343±10.75	11560±52.92
10	1840±6.44	18710±65.57
8	1620±3.23	20050±40.00
5	1321±2.53	26100±50.00
4	1164±1.44	29090±36.06
2	896.5±1.81	44030±88.88
1	676.3±0.85	69200±86.60
Yield value 1000 (D/cm²)		
Hysteresis loop area 10 (D/sec cm²)		
Farrow's no. 0.221		

*All values are expressed as mean±SD (n=3)

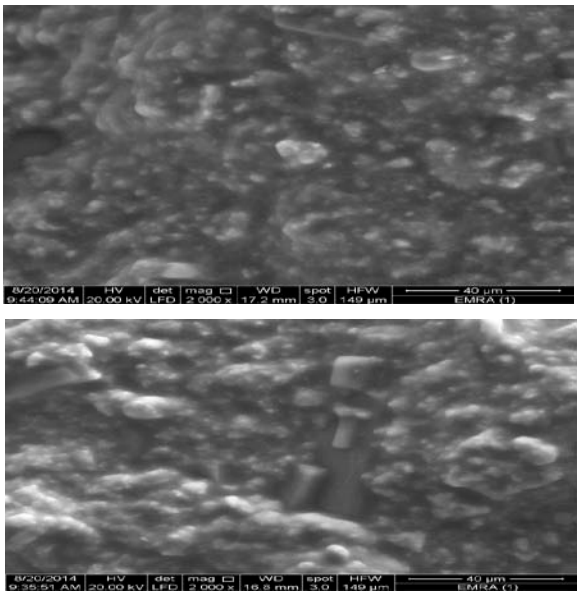

Fig. 7: The Proniosomes for permethrin 5 % micro emulsion-based hydrogel (PMG) under scanning electron microscope

PMG shows better stability than that of the topical powder although they consist of the same permethrin proniosomes. Yet, this may be attributed to that free permethrin (Hydrophobic) partitioning within oil phase of the micro emulsion, thus increasing its stability [17].

CONCLUSION

Preparation No 19 had comparatively good entrapment efficiency (55.57 %) and can be used for preparing topical dosage forms. Residual solvent determination for such preparation was found to be zero. This had an acceptable safety to the patient according to USP 32 acceptance criteria and so was selected for preparing topical permethrin powder and micro emulsion-based hydrogel. PTP stability testing showed that it was physically stable, but the assay of permethrin was 66.34 % after six months storage at accelerated conditions. Such results indicate that PTP is not stable under such conditions, and may not be stable enough during the shelf life. PMG stability testing showed that the product was stable physically, and the assay of permethrin was 96.50 % after six months storage at accelerated stability conditions showing that it may be stable along its shelf life. The clinical study indicated that PMG was more effective as scabicide than permethrin lotion while PTP is not effective. The difference in activity between (PMG) and (PTP) may be attributed to better spreadability, adhesion, viscosity, extrusion and hydration factor in the case of PMG [26].

CONFLICT OF INTERESTS

Declared None

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