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Stability Study of Griseofulvin in Non Aqueous Microemulsion System

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Abstract
New non aqueous based microemulsion allows the delivery of storage sensitive personal care actives in conventional cream, lotion and gel. Griseofulvin is an antifungal antibiotic drug micro emulsion system as the basic formulation for investigation. The quantity of griseofulvin and the accumulation of anhydrous micro emulsions at various temperatures were analyzed with poor aqueous solubility. It is thought to inhibit fungal cell mitosis and nuclear acid synthesis. Pharmaceutical and cosmetic micro emulsions are normally oil-in-water (o/w) or water-in-oil (w/o) systems; however, micro emulsions can be formulated with no aqueous phase to produce an anhydrous micro emulsion system. An anhydrous micro emulsion system could offer a stable vehicle for compounds that are sensitive to hydrolysis or oxidation. Therefore, to enhance the stability of griseofulvin in formulations, we chose the non aqueous, anhydrous through an established ultra violet (uv) method. The results indicated that Stability of griseofulvin in non aqueous microemulsion carried out at at temperature 5°C, 25°C and 40°C. Cream was stable at 5°C and 25°C. Stability studies were carried out at agitation, centrifugation, freeze thaw cycle, in that evaluated various parameter i.e phase separation, globule size, viscosity, drug content.

Keywords: Non-aqueous micro emulsion; Griseofulvin; stability

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INTRODUCTION

Griseofulvin is an antifungal substance typically produced by the growth of certain strains of *Penicillium griseofulvum*[1]. Griseofulvin is usually given systemically, beneficial responses in fungal skin infections have been reported with some topical formulations. Griseofulvin is also used as a veterinary antifungal drug. Griseofulvin's spectrum of activity is limited to dermatophytes and these fungi possess a prolonged energy-dependent transport system for the antibiotic. In contrast, in insensitive organisms, such as *C. albicans*, this system is replaced by a short-energy-independent transport system[2]. In the case of topical drug delivery, the diffusion takes place mainly through the stratum corneum (lipoidial barrier). The drug allows different path to permeate through stratum corneum. Owing to poor aqueous solubility griseofulvin cannot permeate through the skin to its less solubility in water than required for crossing the skin barrier[1,2] .

Definition of emulsion doesn't explain that which type of two immiscible liquids will be use in formulation. Conventionally water is one of the liquid phase in formulation of emulsion, but emulsion can be formulated without an aqueous phase to produce anhydrous, non aqueous or oil in oil emulsion. Such systems can replace conventional emulsion, that means there is no restriction regarding selection of two immiscible phases therefore it may be of oil-oil, oil-water/water oil or polar solvent-oil**.** Non-aqueous emulsions, however, could replace regular aqueous emulsions wherever the presence of water is undesirable; for example, incorporating drugs susceptible to hydrolysis. There have been only occasional reports on non-aqueous emulsion systems[3,4]. Non-aqueous emulsions may be of pharmaceutical or cosmetic value if they are composed primarily of edible, non-toxic ingredients and can be formulated to exhibit a wide range of physical properties[5,6]. Some possible uses might be as topical application bases for dermatological, particularly for labile drugs, as emollient bases for cosmetic preparations, or as nutrient preparations. Thus, they can be used alone or combined with cosmetic ingredients to form a number of over-the-counter (OTC) personal care products. The stabilization of emulsions composed of non-toxic, pharmaceutically relevant substances where olive oil or mineral oil were used as non-polar phase, glycerin as polar phase and conventional emulsifiers as stabilizing agents[6]. After comprehensive screening of existing low molecular emulsifiers, it was found that non-aqueous emulsions could be stabilized with amphiphilic surfactants which have attraction for the polar and the non-polar phase. Increasing the viscosity of the continuous phase improved emulsion stability[7,8,9].

EXPERIMENTAL

Materials

Griseofulvin (Mahaluxmi chemie, Hyderabad), Glycerin (Loba Chemical, Mumbai), Olive oil (Poona chemical laboratory, Pune), Glycerol mono stearate, (Research lab fine chemical industry, Mumbai) were received as gift sample. Methanol, Chloroform, distilled water, phosphate buffer pH (7.4) were also used throughout the study. All other chemicals and reagent were of analytical grade and were used without further purification.

Formulation of NAME, AME

Non aqueous microemulsion and aqueous microemulsion was prepared with the help of titration method (Table 1)[9, 10,26]. After preparing Microemulsion evaluated by agitation, centrifugation and freeze thaw cycle method using UV method.

Formulation Type	Drug (mg)	Oil (%)	S/Cos mix (%)	Glycerin (%)	Water (%)
NAME	500	59.32	29.85	15.25	_
Aqueous Formulation	500	59.32	29.85	_	15.25

Table 1: Compositions of NAME and AME used

Stability Test

Agitation test

5 gm of the non-aqueous cream was filled in a container and container was placed on a reciprocating shaker. The container was shaken at room temperature approximately 60 cycles per minute at for 24 hrs. After 24 hrs container was removed and cream was observed for any signs of phase separation[11].

Centrifugation test

Centrifugation test was performed immediately after formulation of the non-aqueous cream. 5 gm of non-aqueous cream was filled in a centrifuge tube. The tubes containing non-aqueous cream were subjected to centrifugation at 3500 rpm for 30 minutes. Cream was observed for any signs of phase separation after the test[12].

Freeze-thaw cycles

During this test non-aqueous cream was subjected to cyclic temperature testing. Three freeze-thaw cycles were carried out between freezer temperature (-10°C) and 25°C. Firstly, samples were stored in freezer for 48 hrs. After 48 hrs, samples were taken out from freezer and kept at 25°C for 48 hrs. Procedure was repeated for three times. After each storage period stability of cream was checked in terms of phase separation, cracking etc. Also viscosity of the formulation was measured before and after the freeze-thaw cycling[13].

Accelerated stability study

Investigation of physical changes in an optimized non-aqueous cream was performed by accelerated stability

study. Samples of optimized formulation were kept in refrigerator and programmable environmental chamber for three months at 5°C ± 2°C, 25°C and 60% RH and elevated temperature 40°C ± 2°C and 75 % RH ± 5 % RH. Samples were withdrawn at 1, 2 and 3 months from the time of placing sample into the chamber. At these time intervals formulation was monitored for changes in color, viscosity and drug content[14].

Sample Preparation and Ultra violet spectroscopy (UV) Analysis

For sample preparation, 0.25 g of emulsion was dissolved in 50 mL of methanol and then sonicated for 30 min in a water bath sonicator. The extracted sample was subsequently diluted for analysis in pure methanol. After dilution analyse the sample at UV Wavelength was set to 291 nm[15]. The amount of drug content in aqueous and non aqueous microemulsion was calculated (Figure 3.2).

RESULTS AND DISCUSSION

Agitation

Droplets of the emulsion exhibit brownian movement. It is believed that no coalescence of droplets takes place unless droplets impinge upon each other owing to their Brownian movement. Agitation can contribute to the energy with which two droplets impinge upon each other. After agitation on a reciprocating shaker for 24 hrs, there was no phase separation in non-aqueous cream which indicates it has good stability[16] and can withstand the mechanical forces during the transportation and handling.

Study parameter	Evaluation parameter of Trial Batches				
		I	II	II	Remark
Agitation (37±°C)	Phase separation	No	No	No	No phase separation occurs
	Viscosity (cPs)	11250 ±0.133	11239 ±0.1347	11231 ±0.1278	Viscosity slightly changes
Centrifugation (37±°C)	Phase separation	No	No	No	No phase separation occurs
	Viscosity (cPs)	11250 ±0.127	11210 ±0.1345	11164 ±013.74	Viscosity slightly changes

Table 3.2: Stability study of NAME by Agitation and Centrifugation

Centrifugation

Shelf life of emulsion under normal storage conditions can be predicted by observing the separation of the dispersed phase due to either creaming or coalescence when emulsion was an exposed to centrifugation. Stokes' law shows that creaming is function of gravity and increase in gravity accelerates separation[17]. Centrifugation test was carried out to examine the effect of gravity on the non-aqueous cream. Non-aqueous cream showed no phase separation after centrifugation for 30 minutes at 3500 rpm. This indicates that cream has a good stability over the gravitational forces.

Accelerated stability study

The chemical and physical stability of the non-aqueous cream was evaluated through accelerated stability studies. During studies, formulation was kept at three temperature conditions: low temperature (5°C), moderate temperature (25°C) and high temperature (40°C) [19, 20,21,22,23]. Formulation was monitored for changes in color, viscosity and drug content for the period of three months.

Stability study at 5°C

There was slight decrease in the drug content at 25°C. Drug content was decreased from 98.13±0.56 to 97.50±0.35% within three months. As seen in case of low temperature, there was initial increase in viscosity followed by decrease upto 9369 cPs. Greater decrease in viscosity was observed due to increase in temperature. Also there was slight change in the color of the formulation; color was changed from fresh white to dull white.

Stability study at 25°C

Drug content of the non-aqueous cream was found to be constant at 5°C. There was no significant change in the chemical composition of the formulation[24]. But there were some changes in the viscosity of the formulation. Initial viscosity of the formulation was found to be 11043 cPs up to 1 month, thereafter there was increase in the viscosity up to 129800 cPs, and globule size is slightly changes. Color of the formulation didn't change at the low temperature. So it was observed that non-aqueous cream was stable at low temperature.

Stability study at 40°C

It was observed that non-aqueous cream was very sensitive to the high temperature. A distinct phase separation occurred within a week[25]. So stability study at high temperature was terminated. From stability studies it was evident that non-aqueous cream was stable at low and moderate temperatures. All this study comparing with the aqueous formulation was indicative.

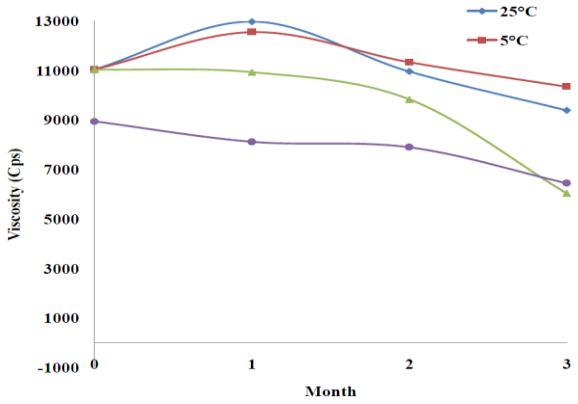

Figure 3.1: Viscosity changes after stability study

Tempecture	Duration (Months)	Appearance	Viscosity (cPs)	Globule size (nm)	Drug content (%)	Stability / Phase separation
NAME (25°C)	0	White	11043 ± 0.93	69 ±0.34	98.75 ±0.13	No phase separation
	1	White	12980 ± 0.86	67 ±0.91	98.13 ±0.13	
	2	White	10963 ± 0.76	64 ±0.78	98.13 ±0.13	
	3	White	9396 ± 0.34	61 ±0.46	97.50 ±0.13	
NAME (5°C)	0	White	11043 ± 0.67	74 ±0.23	98.13 ±0.13	No phase separation
	1	White	12560 ± 0.87	71 ±0.98	98.52 ±0.12	
	2	White	11345 ± 0.54	64 ±0.43	98.44 ±0.11	
	3	Dull White	10351 ± 0.12	62 ±0.89	98.03 ±0.11	
NAME (40°C)	0	White	11043 ± 0.99	56 ±0.74	98.35 ±0.13	Phase separation within 2 month
	1	White	10934 ± 0.98	43 ±0.71	96.23 ±0.12	
	2	Dull white	9835 ± 0.84	41 ±0.65	94.89 ±0.11	
	3	Dull white	6035 ± 0.67	39 ±0.67	93.56 ±0.10	
Aqueous cream	0	White	8953 ± 0.34	56 ±0.54	99.89 ±0.13	No phase separation
	1	White	8123 ± 0.97	51 ±0.34	98.45 ±0.12	
	2	Dull white	7903 ± 0.56	47 ±0.32	98.10 ±0.13	
	3	Brown	6452 ± 0.45	45 ±0.14	97.45 ±0.13	

Table 3.3: Stability studies of non-aqueous cream at 25°C and 5°C

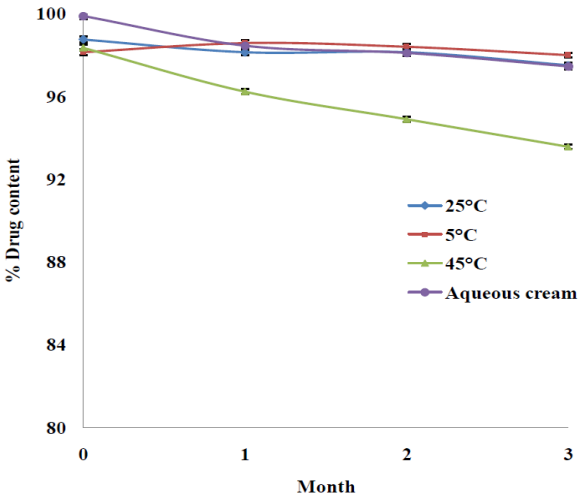

Figure 3.2: Drug content changes after stability study

CONCLUSION

The stability of griseofulvin in non aqueous microemulsion system was examined therefore, in the first part of the study; we used two Glycerine-in-olive oil anhydrous emulsions formulations containing griseofulvin. Secondly, all parameter were compared with aqueous micro emulsion of Griseofulvin. The stability study of all NAME and AME formulations were determined at various temperatures conditions. The non aqueous microemulsion were mostly stable at 25°C and 5°C Thus, the difference in the stability of griseofulvin in these non aqueous micro emulsions will be revealed by this study.

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