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Formulation and Evaluation of Ethosomal Drug Delivery System of Clotrimazole using Poly Vinyl Alcohol Gel

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ABSTRACT

Ethosomes have been studied as a possible carrier for topical delivery of clotrimazole, an anti-fungal agent. The presence of ethanol in the aqueous compartment of the ethosomal vesicles favoured the encapsulation and enhanced its permeation via the skin because of the synergistic effect of ethanol vesicles and skin lipids. The dimensions of clotrimazole ethosomes depends on the composition and in particular, the mean size 223.31 nm decreases with increasing ethanol concentration(30 ml). Ethosome size exhibited limited dependence on phospholipid concentration.The ethosomal formulations with high phospholipid concentration had high entrapment efficiency(71.48). Very high amount of ethanol content had a lowering effect on drug entrapment in vesicles. Clotrimazole ethosomes showed sustained release of drug from the formulations over a specified period of time. An ideal or best formulation of ethosome is said to be one which gives high entrapment efficiency (ETH6). In this study entrapment efficiency is found to be ehanol-phospholipid concentration ratio dependent. Zeta potential study proved that the above formulation have an excellent stability. By these facts study can be concluded by saying ethosomes formed from optimum phospholipid and ethanol ratio is a promising carrier to improve the bioavailability of clotrimazole even for an extended period of time. These findings are very encouraging and confirms that ethosomes are a very promising carrier for the topical administration of Clotrimazole.

Keywords: Polyvinyl alcohol, Stratum corneum, Transmission electron microscopy, Differential scanning colorimetry, Phosphatidylcholine, Ethosomes

1. INTRODUCTION

The skin is used as portal of entry of drugs in dermal and transdermal delivery, for local and systemic treatment. [1] Because of the barrier properties of the outer layer of skin, in many cases, permeation enhancing agents are needed to achieve therapeutic levels of drug. [2] Classical liposomal systems were found to be effective at forming drug reservoir in the upper layers of the skin, for local skin therapy. [3] Ethosomal carriers, phospholipids vesicular system containing relatively high concentrations of alcohol, were effective at enhancing dermal and transdermal delivery of both hydrophilic and lipophillic drugs.[4-6]

Today lipid based drug delivery systems have drawn much attention from researchers as potential carriers of various bioactive molecules that could be used for therapeutic application[7]. Several liposome and ethosome based drugs have already been used with a great success,For example liposomes and ethosomes have been used to encapsulate colchines[8], tretinoin[9], enoxacin, estradiol[10], methotrexate[11], acyclovir, minoxidil[12], testosterone[13], bacitracin[14], lamivudine[15] for applications such as anticancer and antituburacular, antileishmanial, anti-inflammatory, hormonal drug and oral vaccines.

Dermal drug delivery refers to delivery of drugs to particular locations within the skin so that they exert a local effect. This type of dermal drug delivery approach is commonly used in the treatment of dermatological conditions such as skin cancer, psoriasis, eczema and microbial infections, where the disease is located in the skin. The skin has both benefits and limitations when compared with more conventional methods such as oral routes.

Topical delivery can be defined as the application of drug containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of general diseases with the intent of containing the pharamacological or other effect of the drug to the skin surface or with in the skin.

Topical formulation are intended to treat cutaneous disorders or cutaneous manifestations of general diseases while keeping pharmacological effects of the drug restricted to the intracutaneous regions of drug penetration and deposition. They deliver drug to local tissues or deeper regions somewhat distant from the application site. As little as 1% and usually no more than 15% of drug in dermatological formulation is bioavailable from topical formulations but still topical formulation allow one to achieve high local tissue drug level. Systemic uptake though unwelcome is unavoidable for most topical formulation but still regional concentration in the skin are higher than that which can be achieved by systemic administration ointments and creams are the most common topical formulations, gels foams, sprays, medicated powders, solutions and even medicated adhesive system also fall in this category. [16]

Dermal drug delivery system is a kind of topical application of drugs to the skin in the treatment of skin diseases. This has the advantage that high concentration of drugs can be localized at the site of action, reducing the systemic drug levels and therefore also reducing the systemic side effects.

Fungal diseases are called mycoses and those affecting humans can be divided into four groups based on the level of penetration into the body tissues

1. Superficial mycoses are caused by fungi that grow only on the surface of the skin or hair.
2. Cutaneous mycoses or dermatophytosis include such infections as athlete's foot and ringworm, in which growth occurs only in the superficial layers of skin, nails, or hair.
3. Subcutaneous mycoses penetrate below the skin to involve the subcutaneous, connective, and bone tissue.
4. Systemic or deep mycoses are able to infect internal organs and become widely disseminated throughout the body. This type is often fatal. [17]

Candidiasis or thrush is a fungal infection (mycosis) of any of the candida species, of which candida albicans is the most common. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. Candida infections of the latter category are also referred to as Candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant and AIDS patients.

Most candidial infections are treatable and result in minimal complications such as redness, itching and discomfort, though complication may be severe or fatal if left untreated in certain populations. In immunocompetent persons, candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity(thrush) the pharynx or esophagus, the gastro intestinal tract, the urinary bladder, or the genitalia (vagina or penis).

Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These “soft vesicles” represents novel vesicular carrier for enhanced delivery through skin. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.[18-19]

2. MATERIALS AND METHODS

Clotrimazole was obtained as a gift sample from Algen Healthcare Ltd Delhi and other chemicals like Lecithin,Propylene glycol,Methanol,N-Octanol,Poly vinyl alcohol,Sodium lauryl sulphate and Acetone was purchased from CDH.

2.1 Methods used for preparation of PVA gel of Clotrimazole ethosomes

2.1.1 Preparation of Ethosomes of Clotrimazole

The method which is selected for the preparation of Ethosomes of Clotrimazole is Cold method. This is the most

common method of ethosome preparation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mechanical stirrer. Propylene glycol or other polyol was added during stirring. [20]

The mixture is heated to 30ºC at water bath. The water heated to 30ºC in a separate vessel is added in form of fine stream to the mixture which is then stirred for five minutes in a covered vessel. The vesicle size of the ethosomal vesicle can be decreased to desired extent using Sonication method. Finally the formulation is stored under refrigeration.

2.1.2 Preparation of PVA gel of Clotrimazole ethosomes

For preparation of PVA gel base, 20 gram of poly vinyl alcohol (PVA) was dispersed in 980 ml of distilled water, containing 0.001% of phenyl mercuric nitrate, using a magnetic stirrer. The ethosomal suspension was centrifuged at 2000 rpm for 20 minutes, and the pellets obtained were incorporated the prepared gel base to get 1 % (w/w) clotrimazole in the gel base. [20]

2.2 Evaluation of Prepared Formulation

Clotrimazole ethosomes were prepared by cold method and then selected optimized formulations were subjected to various characterization studies to find out the efficacy and stability of different formulations.

2.2.1 Vesicle size and shape

The effect of phospholipid and ethanol concentration on the size distribution of ethosome vesicles was investigated using Malvern mastersizer. To determine the size of ethosomal formulation Malvern mastersizer was used. The average vesicle size of different formulations was determined and is summarized in table 1.

Photographs of ethosomes negatively stained with phosphotungstic acid as observed by Transmission electron microscopy showed round vesicles. Since Phosphotungstic acid does not allow for visualization of lamellae, Micrographs confirmed that clotrimazole ethosomes have only few (1-3) lamellae. It was found that ethosomes are multilamellar vesicles with mean size of 150-300 nm.

2.2.2 Morphological characterization of vesicles

For initial characterization of vesicles, the prepared vesicles were first observed under 400X magnification to check the formation of vesicles. Some unevenness of vesicles was observed under the study may be due to drying process under normal environment condition. The photograph of ethosomes is shown (figure 1). The particles found to be uniform in size and shape.

To confirm the lamellarity of ethosomal vesicles at higher concentration of ethanol, the ethosomes were subjected to transmission electron microscopy after negative staining by phosphotungstic acid (figure 2). The presence of bilayer vesicles was confirmed in the ethosomal system by electron microscopy.

2.2.3 Entrapment efficiency

Various ethosomal formulations of clotrimazole using phosphatidylcholine and ethanol were prepared and were observed as spherical vesicles with smooth surface discrete and separate with no aggregation than they were subjected to sonication., the ability of vesicles for entrapping the drug was investigated by ultracentrifugation.

Entrapment efficiency was studied for all the seven formulations to find the concentration of drug in the vesicles. The entrapment efficiency was found to be higher with formulation ETH6, which had an optimum ethanol: phospholipid ratio to provide a higher entrapment of clotrimazole (table 2).

2.2.4 Zeta potential analysis

Zeta potential is defined as the electric potential of the vesicle including its ionic atmosphere (Stern layer). The charge on the ethosomal vesicles is an important parameter that can influence both vesicular properties such as stability, as well as skin- vesicle interactions. The formulation ETH7 which was subjected to zeta potential analysis had a zeta value -6.34mv, which is a measure of net charge on ethosomes. This negative charge on the surface of vesicles produces a repulsive force between vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension.

2.2.5 In vitro release studies

In-Vitro release studies showed extent and rate of drug release. The *in–vitro* permeation study was also conducted for ethosomal formulation using Diffusion cell.

The release profile was conducted for four formulations as well as for PVA gel of clotrimazole ethosomes. Most of the formulations were found to have a linear release and formulations showed 55% release with in a period of 8 Hours (table 3).

2.2.6 Drug-phospholipid interaction by differential scanning calorimetry

Differential scanning calorimetry was performed by using DSC-60.The DSC thermograms of pure drug clotrimazole, lecithin and formulation are shown below. Pure clotrimazole showed a sharp endotherm at 144.88ºC corresponding to its melting point/Transition temperature. There is no appreciable change in the melting endotherm of the physical mixture of clotrimazole and

lecithin (formulation) compared to pure clotrimazole (figure 3, 4, 5).

2.2.7 Mechanism of Drug release

The mechanism of drug release was determined by fitting the release data to the various kinetic equations such as zero order, first order, higuchi's and kosermeyer-Peppa's finding the R^2 values of the release profile corresponding to each model.

Correlation value of Higuchi's plot revealed that the mechanism of drug release is diffusion. The correlation value of zero order plots and correlation value of Higuchi's plot presented in table 5. The *in vitro* kinetic data subjected to (peppa's model), all the value ranges from 1 to 2.0751 revealed the fact that the drug release follows a super case II transport diffusion. The slope value for each formula presented in table 5.

Table 1: Vesicle size of different Ethosomal formulation

S.No	Formulation code	Vesicle size (nm)
1.	ETH1	223.31±0.20
2.	ETH2	322.58±0.25
3.	ETH3	193.94±0.15
4.	ETH4	219.01±0.16
5.	ETH5	172.52±0.20
6.	ETH6	159.25±0.21
7.	ETH7	90.17±0.24

Table 2: Drug entrapment efficiency of different batches of ethosomes

Sample code	Percent drug entrapped
ETH1	58.5%
ETH2	43.5%
ETH3	43.0%
ETH4	60.4%
ETH5	51.0%
ETH6	71.4%
ETH7	27.5%

Table 3: *In vitro* release profile of selected formulations

TIME(HOURS)	CUMULATIVE % RELEASE ETH1	CUMULATIVE % RELEASE ETH4	CUMULATIVE % RELEASE ETH5	CUMULATIVE % RELEASE ETH6	CUMULATIVE % RELEASE PVA GEL
0	0	0	0	0	0
1	6.893	7.198	7.943	9.453	6.132
2	11.461	14.303	11.593	14.956	11.270
3	17.677	18.384	16.572	18.437	17.563
4	24.111	25.976	23.222	26.936	23.106
5	30.034	33.988	29.089	36.981	28.773
6	35.382	42.450	34.845	44.003	39.141
7	40.326	47.409	40.393	50.106	44.030
8	44.422	55.384	43.666	56.813	50.145

Table 4: DSC data of Clotrimazole

S.NO	SAMPLE	MELTING POINT
1	Clotrimazole	144.88º C
2	Lecithin	189.52º C
3	Formulation	142.39º C

Table 5: Drug release kinetic data

Formulations	Zero order		Higuchi's		Peppa's	
	Slope	Correlation	Slope	Correlation	Slope	Correlation
PVA gel	6.2095	0.9962	16.054	0.951316	1.9816	0.93208
ETH 4	6.8583	0.9981	17.763	0.95411	2.0388	0.91861
ETH6	7.1897	0.9961	18.632	0.95244	2.0629	0.90044
ETH1	5.6828	0.9961	14.811	0.9653	1.9461	0.91510
ETH5	5.7959	0.9963	15.024	0.9534	1.9480	0.90810

3. RESULT AND DISCUSSION

Clotrimazole ethosomes were prepared by cold method and then selected optimized formulations were subjected to various characterization studies.

To determine the size of ethosomal formulation Malvern master sizer was used which showed vesicle size in the range of 150-300 nm. This support the easily penetration of the vesicles at the time of topical administration .

For morphological characterization of vesicles, the prepared vesicles were first observed under 400X magnification by electron microscopy to check vesicles. Some unevenness of vesicles was observed under the study may be due to drying process under normal environment condition. The particles found to be uniform in size and shape and The presence of bilayer vesicles was confirmed in the ethosomal system by electron microscopy .

Entrapment efficiency was studied for all the seven formulations to find the concentration of drug in the vesicles. The entrapment efficiency was found to be higher in formulation ETH6, which had an optimum ethanol: phospholipid ratio to provide a higher entrapment of Drug.

The optimized formulation ETH7 was subjected to zeta potential analysis and it revealed a zeta value -6.34mv, which is a measure of net charge on ethosomes. This negative charge on the surface of vesicles produces a repulsive force between vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed formulation.

In-Vitro release studies showed extent and rate of drug release and it was done by using diffusion cell . It was observed through study that majority of formulation exhibited a linear release and formulations showed 55% release with in a period of 8 Hours.

Differential scanning calorimetry was performed to check the interaction between the drug and other excipients of the formulation and no sign of the interaction was observed in the DSC study before 150°C.After performing all the evaluation a conclusion could be drawn out that formulation ETH6 showed good entrapment efficiency ,Cumulative percent drug release ,Vesicle size as well as zero order release ,Though ETH7 revealed some similar result but its entrapment efficiency was far away from the expected .

4. CONCLUSION

It can be concluded by performing the above evaluation parameters that the objective of the study that is Formulation and evaluation of ethosomal drug delivery system of Clotrimazole using PVA gel was fulfilled and from the electron microscopy size and morphological aspects of ethosomes were successfully studied, Similarly through entrapment efficiency ,in vitro drug release and differential scanning calorimetry other parameters of the formulation were also studied and they were found to be promising and within the passable ranges.

5. ACKNOWLEDGEMENT

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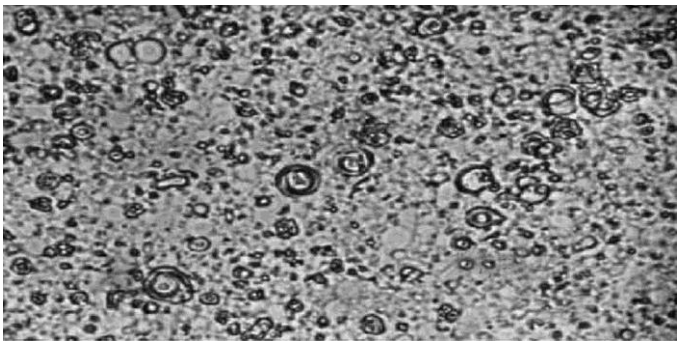

Figure 1: Photograph of Ethosomes

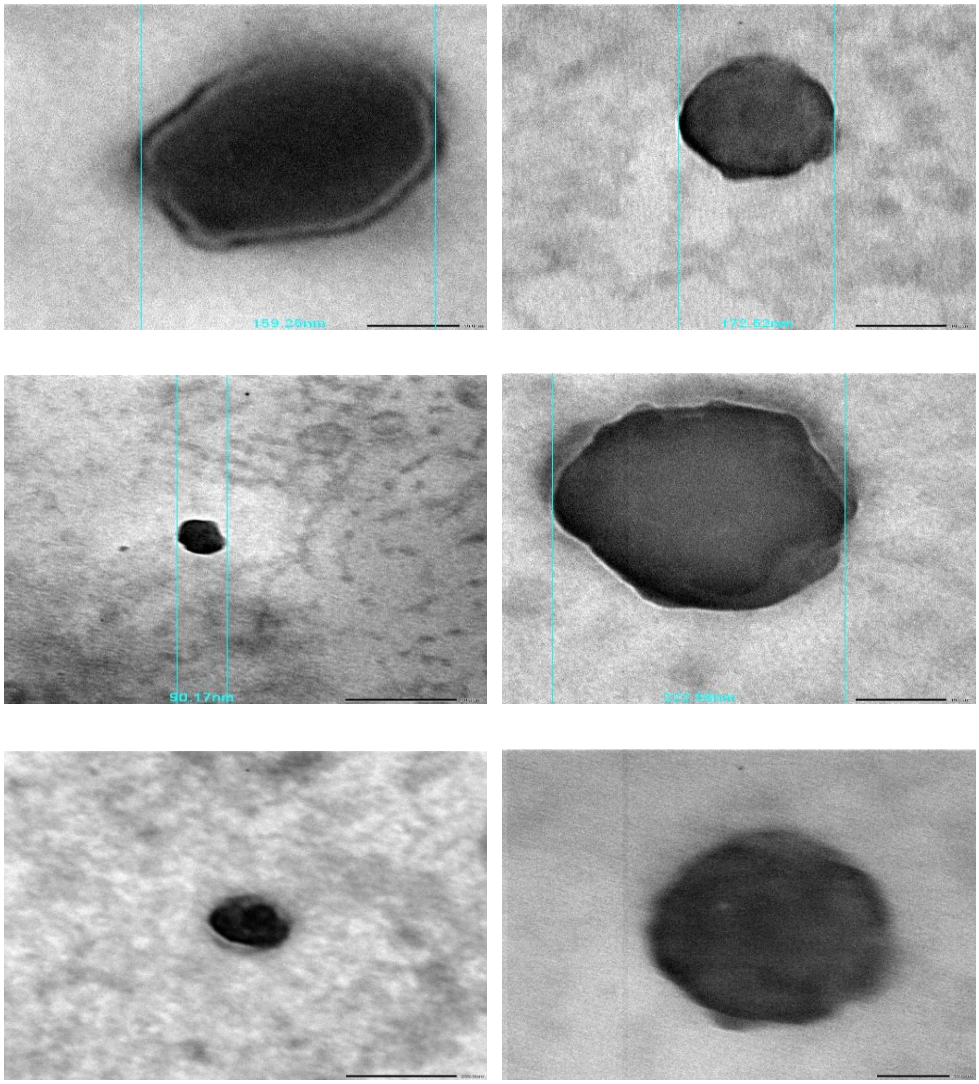

Figure 2: Transmission electron micrographs of ethosome formulation

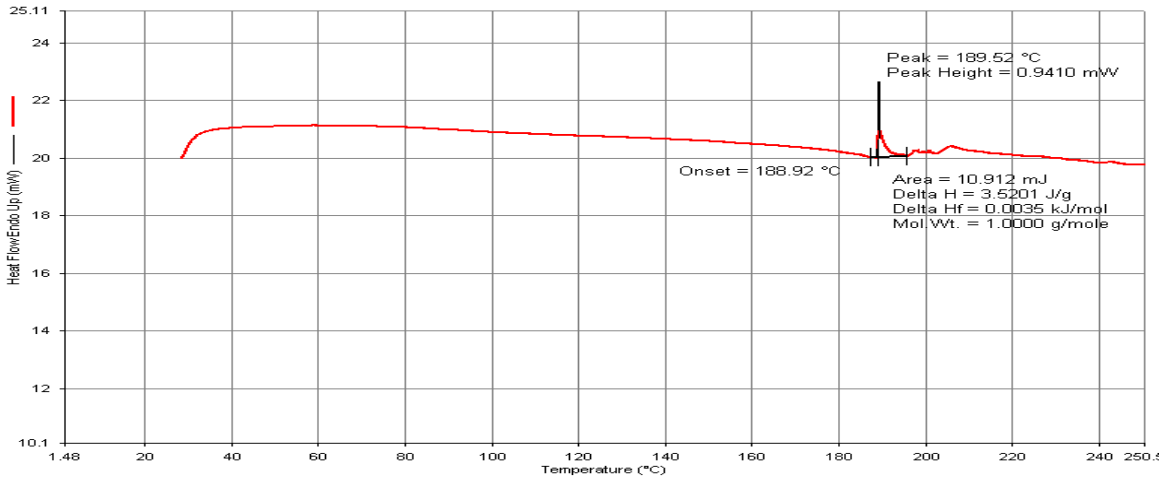

Figure 3: DSC Thermogram of lecithin

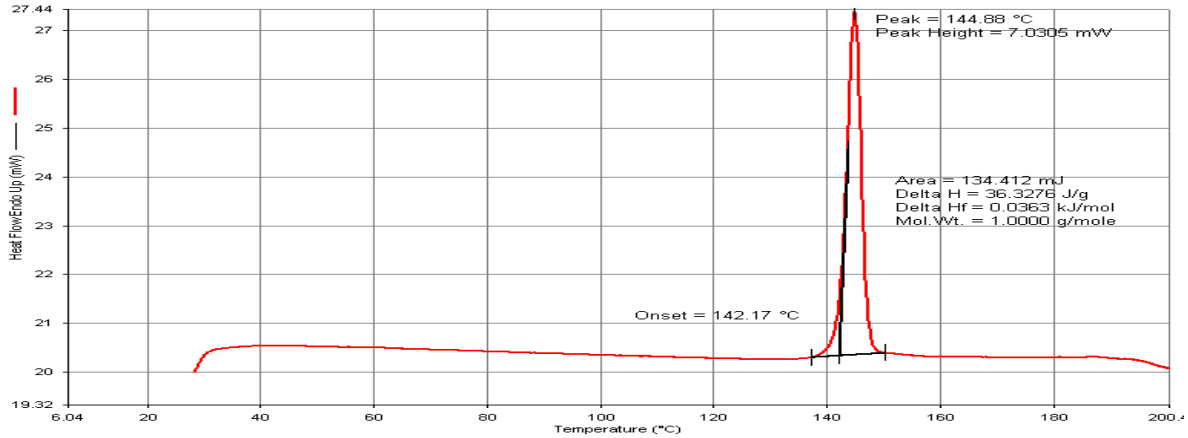

Figure 4: DSC Thermogram of pure clotrimazole

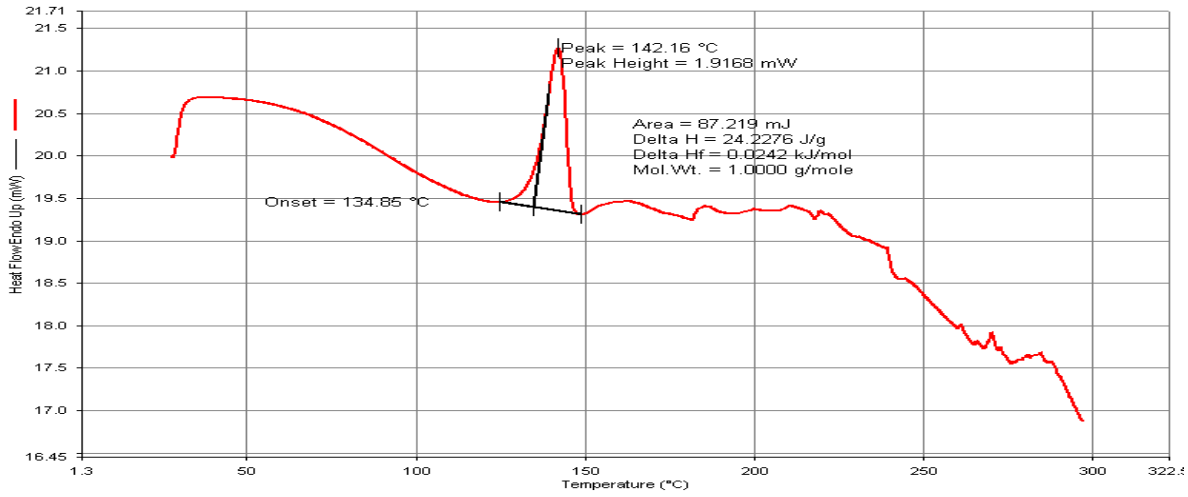

Figure 5: DSC Thermogram of formulation