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Formulation Development of 5-Fluorouracil Transethosomes for Skin Cancer Therapy

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ABSTRACT

Transdermal drug delivery is an attractive alternative as it offers many advantages such as avoidance of first-pass metabolism, reduced dosing frequency, improved patient compliance etc. 5-fluorouracil, an anticancer drug, is considered as a gold standard for the treatment of skin cancers. The high elasticity of vesicular membranes allows transethosomes to squeeze them and pass through the pores which are much smaller in sizes as compared to their own sizes in stratum corneum. Out of the three methods namely classical mechanical dispersion, hot method and cold method, a cold method was selected for preparation of transethosomes. Phospholipon 90G was selected as the vesicle-forming unit for the formulation achieving higher entrapment efficiency and good stability as compared to the formulation using phospholipon 90H. The particle size was found in the range of 50nm to 110nm. Standard deviations were in the range of 70 to 90nm which suggested better polydispersity of the formulation. The average zeta potential was -46.19+ 15.3mV showing the good stability of the formulation. The entrapment efficiency of transethosomes ranged from 75.08% to 92.08%. The above results suggest that 5-Fluorouracil transethosomes could be a good career option for delivering the drug into the deeper skin layers and hence useful for the treatment of skin cancers.

INTRODUCTION:

5- fluorouracil is a hydrophilic antineoplastic agent with a plasma half-life of 10-20 mins. It is an established antineoplastic compound with activity against several premalignant conditions of the skin like squamous cell carcinomas and superficial basal cell carcinoma. However serious side effects are associated with conventional therapy including myelosuppression, hand-foot syndrome and gastrointestinal toxicities[1]. 5- fluorouracil has generally been administered through oral and parenteral route leading to rapid drug metabolism and erratic drug absorption from the gastrointestinal tract. Hence the development of alternative carrier via transdermal route is needed.

Skin cancer is a deadly disorder which shows the high incidence of cutaneous melanoma and nonmelanoma skin cancer. It is a serious health issue as the number increases 5 to 10% every year. The conventional pharmaceutical dosage forms which are widely administered transdermally are gels, creams, and ointments. Treatment options available are cryosurgery with liquid nitrogen, excisional surgery, laser surgery and also topical therapies like 5% of 5-fluorouracil, imiquimod and photodynamic therapy. Although these techniques are commonly used they result in scarring, and can be directed only on clinically apparent lesions. Due to their poor skin permeability, they are only suitable for topical drug delivery. The clinical use of 5-FU is limited to its stomatitis and myelotoxicity. Several efforts were made to reduce these side effects by encapsulating the drug in liposomes by using conventional methods which showed that the trapping efficiency of the water-soluble compound such as 5-FU, which is non- interacting with the bilayer is low[2]. Encapsulation efficiency and shelf life of the formulations can be enhanced by entrapment of vesicular formulation in gels. Transethosomes are lipid vesicles based on transfersomes and ethosomes. These vesicles were first introduced by song et al in 2012 and are characterized by having a high content of ethanol i.e up to 40% together with an edge activator. It contains advantages of both transfersomes and ethosomes. They have shown an irregular spherical shape and higher values in both vesicle elasticity and skin permeation studies.[3]They have the ability to encapsulate both hydrophilic and lipophilic drugs. They consist of phospholipids, surfactants such as sodium deoxycholate, sodium cholate, tween 80, span 80 which act as edge activators and destabilizes the lipid bilayers, and ethanol causes the rearrangement in the lipid bi-layer of these vesicles.

In this investigation, ultradeformable vesicles were developed to deliver 5-FU as an alternative vehicle for topical drug delivery to the oral conventional dosage form. 5-FU was the most suitable drug to deliver across the skin for the management of skin conditions. Ultra-deformability property of the vesicles makes this system a versatile carrier for systemic and topical delivery of the drug[1].Different surfactants in different concentration ratio with lipid have been tried to get enhanced drug permeation and drug deposition into the skin.

Analytical method development of 5- Fluorouracil by U.V Spectroscopy

10 mg of 5-FU was dissolved in 10 ml of methanol to give a stock solution A of concentration 1 mg/ml. From stock solution A, 1 ml was withdrawn and diluted to 10 ml with methanol to give a stock solution B of concentration 100 µg/ ml. From stock solution B, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml were subsequently withdrawn and diluted to 10 ml with methanol to give solutions of concentrations 2 µg/ ml, 4 µg/ ml, 6 µg/ ml, 8 µg/ ml, 10 µg/ ml, 12 µg/ ml, 14 µg/ ml, 16 µg/ ml respectively. Thus standard solutions with concentrations ranging from 2-18µg/ ml were obtained. The absorbance values of these solutions were read at 266 nm, in triplicate on a Jasco V-550 spectrophotometer against methanol as blank. These absorbance values were used to prepare the standard plot.

Drug- Excipient Compatibility Studies

Compatibility of KT with the excipients which would be present in the final formulation was studied by Differential Scanning Calorimetry and FTIR.

Differential Scanning Calorimetry (DSC) Studies

The DSC analysis of 5-FU, sodium cholate, phospholipon 90G, and the excipients and 5-FU loaded TEL formulation was carried out using the Mettler DSC 60 computerized with Mettler Toledo Star software system (Mettler, Switzerland). The instrument was calibrated with indium standard. Accurately weighed samples were placed in aluminum sample pans. Thermograms were obtained by heating the sample at a constant rate of 10ºC/minute. A dry purge of nitrogen gas (20ml/min) was used for all runs. Samples were heated from 30ºC – 300ºC. Scans were obtained from the samples. The melting point and peak maxima were observed in the DSC graphs.

Fourier Transform Infra-Red (FT-IR) studies

FT-IR measurements of KT and other excipients were obtained on FT-IR Spectrometer in the 500-4000cm^{-1} range and major absorption bands were recorded. The presence and absence of this bands and appearance of any new band were observed in the IR absorption spectrum.

MATERIALS AND METHODS:

MATERIALS:

5-fluorouracil was a generous gift sample from Naprod life science Pvt Ltd, Mumbai. Phospholipon 90G [phosphatidylcholine(PC)] was a gift sample obtained from Lipoid (Ludwigshafen, Germany). Sodium cholate was obtained from S.D. Fine chemicals. All other chemicals used were of analytical grade.

METHODS:

Transethosomes can be prepared by:

Hot method

Cold method

Hot method

Phospholipon 90G was dissolved in water by heating in water bath at 40^{o}C. Ethanol was heated to 40^{o}C. The drug was dissolved in water. Sodium cholate was dissolved in ethanol. An aqueous phase was added to ethanolic phase with constant stirring at 700rpm. The temperature was maintained at 40^{o}C throughout the preparation. Size reduction was done with the probe sonicator for 5 mins.

Cold method

Phospholipon 90G was dissolved in ethanol in a conical flask with constant stirring at 700 rpm. The temperature of this alcoholic mixture was maintained at 30°C. drug and sodium deoxycholate was dissolved in water and was maintained at 30°C in a separate vessel. This aqueous phase was then added to the alcoholic phase slowly in a fine stream with constant stirring at 700 rpm in a closed vessel. It was stirred for additional 5 min. The system was kept

at 30°C throughout the preparation. Size reduction was done by probe sonication for 5 min at RT.

Selection of a method for preparation of transethosomes was based on percent entrapment efficiency (%EE). However, a cold method was found to give more stable transethosomal formulations. Hence this technique was chosen for formulation development.

Selection of phospholipid

Phosphatidylcholine (PC) of two different grades was utilized to formulate transethosomes

1. Phospholipon 90G

2. Phospholipon 90H

Phospholipon90G and 90H were selected for transethosomal formulations. Based on %EE suitable surfactant was selected. Based on the entrapment efficiency sodium cholate was selected for further studies.

Selection of surfactant

In order to find the best surfactant with enhanced permeation properties, different surfactants were utilized. Sodium cholate, Span 80, Tween 80 were selected. Based on the %EE suitable surfactant was selected. Based on the entrapment efficiency sodium cholate was selected for further studies.

Surfactant concentration was varied to study the effect of surfactant concentration on particle size, entrapment efficiency and stability of the formulation.

Sr. No.		**FI**	**FII**	**FIII**	**FIV**	**FV**
1	Drug	100mg	100mg	100mg	100mg	100mg
2	Phospholipon 90G	900mg	900mg	900mg	900mg	900mg
3	Sodium cholate	90mg	80mg	40mg	120mg	105mg
4	Ethanol	30% w/v	30% w/v	30% w/v	30% w/v	30% w/v
5	Water	q.s 20ml	q.s 20ml	q.s 20ml	q.s 20ml	q.s 20ml

FI showed the particle size of 89nm, entrapment efficiency of 88% and was found to be stable. Hence surfactant concentration of 90mg was selected for optimization.

Phospholipid concentration was varied to study the effect of phospholipid concentration on particle size, entrapment efficiency and stability of the formulation.

Sr. No.		FI	FII	FII	FIV	FV
1	Drug	100mg	100mg	100mg	100mg	100mg
2	Phospholipon 90G	900mg	800mg	400mg	1200mg	600mg
3	Sodium cholate	90mg	90mg	90mg	90mg	90mg
4	Ethanol	30% w/v	30% w/v	30% w/v	30% w/v	30% w/v
5	Water	q.s 20ml	q.s 20ml	q.s 20ml	q.s 20ml	q.s 20ml

FI showed particle size as 85nm, entrapment efficiency as 86.09% and was found to be stable. Hence phospholipid concentration of 900mg was selected for optimization.

Table 1 & 2: Formulation development of transethosomes

Preparation of gel:

The formulations had low viscosity. In order to achieve the desired rheological characteristics and texture for transdermal applications, the formulation was converted into a gel. Gelling agents like Carbopol Ultrex 10 and Carbopol 940 were used to evaluate their ability to gel the formulations. Based on the compatibility with the vesicular formulation, aesthetic appeal, feel and ease of spreadability, Carbopol Ultrez 10 was selected as the gelling agent. Gels of different concentrations from 0.5-1%w/w were prepared. 1%w/w of Ultrez 10 was selected to prepare transethosomal gels. 1% carbopol was sprinkled into the water and soaked for 30 mins. The transethosomal dispersion was added to this swollen gel to give the total drug concentration of 0.5%w/w. triethanolamine was added to adjust the pH to 7 and then the remaining water was added to give a total weight of 10g. a gel was thoroughly dispersed using an overhead stirrer until the desired consistency and spreadability was formed.

Vesicular characterization

The particle size and zeta potential of freshly prepared transethosomes were determined by nanoparticle tracking analysis (NTA 3.1) using Nanosight NS500 with automated sample introduction, the computer-controlled motorized stage with CCD camera and red (638nm) laser. Drug entrapment efficiency of the formulations was determined by ultracentrifugation method. The vesicles were separated by ultracentrifugation at 15000 rpm for 90 mins at a temperature of 4ºC. The supernatant liquid was separated and diluted with methanol and the

amount of drug was quantified spectrophotometrically at 266nm. Entrapment efficiency was determined by the following equation.

% Entrapment efficiency= Qt-Qs/Qt×100

Where,

Qt is the amount of the drug added

Qs is the amount of drug found in the supernatant

Morphology of the vesicles was examined using TEM. The formulations were negatively stained with 2% w/v aqueous solution of phosphotungstic acid on a carbon coated copper grid. The grid was examined under transmission electron microscope (Philips CM 200) with the resolution of 2.4Å at accelerating voltage of 200kV.

RESULTS AND DISCUSSION:

1. Preparation of standard plot of 5-FU in methanol

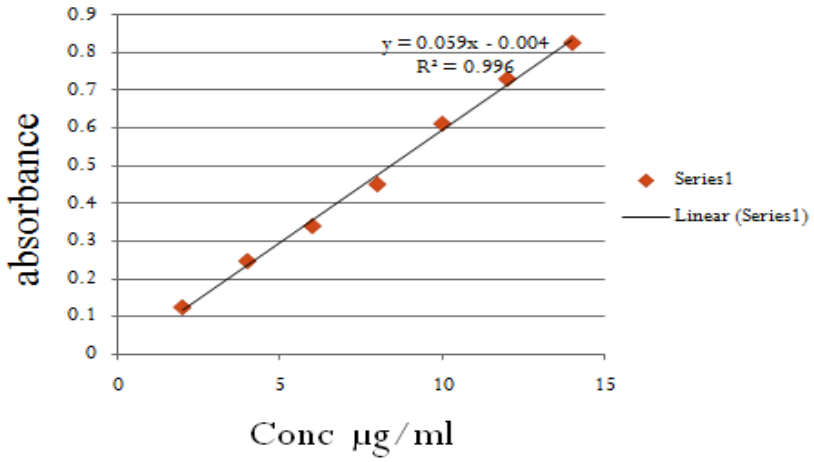

Fig 1: Standard plot of 5-FU in methanol

The standard plot was constructed for 5-FU in methanol. The R^2 value of standard plot for methanol was 0.996 which indicates a good linear relationship.

The equation for the straight line would be beneficial for the conversion of absorbance data into

concentration, as would be required in the subsequent studies.

2. Drug-excipient compatibility studies

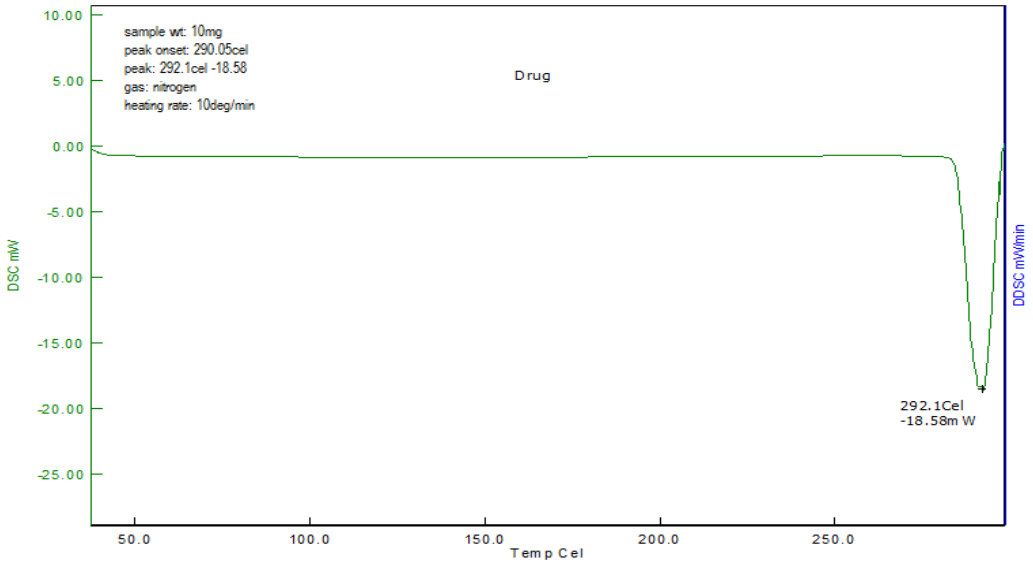

Fig 2: DSC Spectra of 5-FU

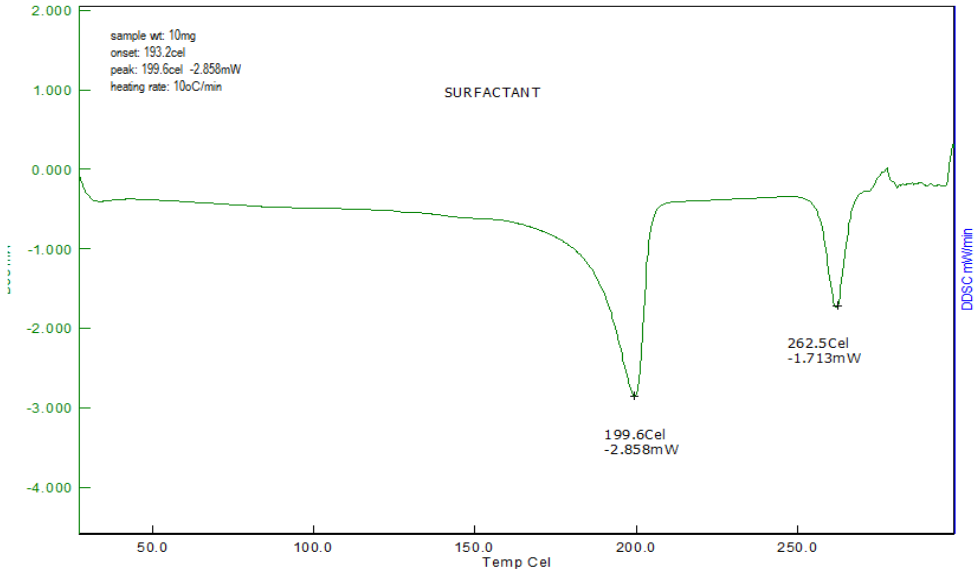

Fig 3: DSC Spectra of sodium cholate

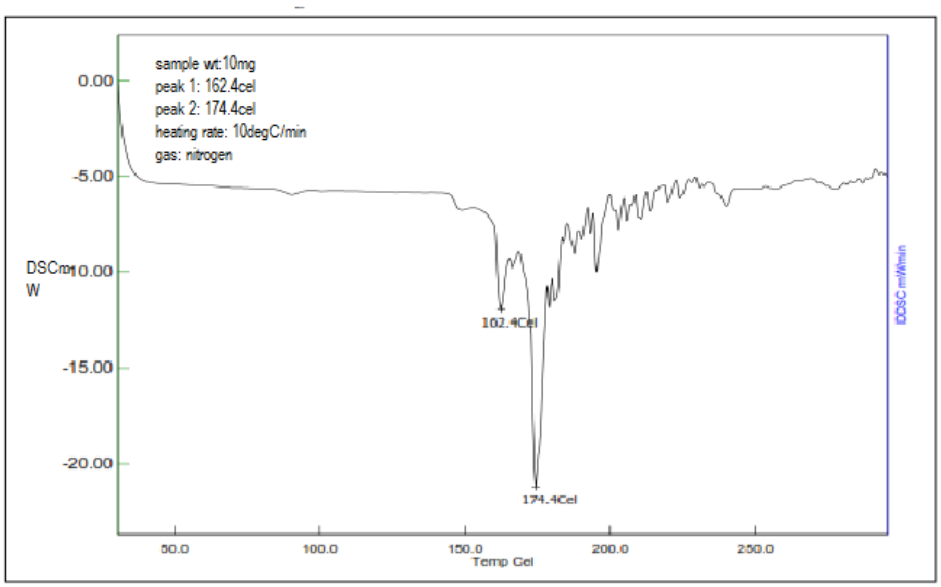

Fig 4: DSC Spectra of phospholipon 90G

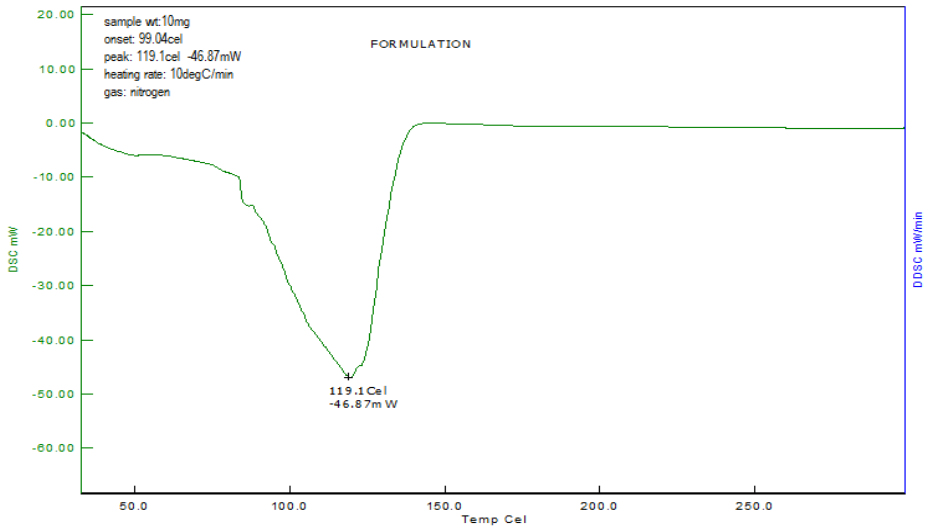

Fig 5: DSC Spectra of 5-FU Loaded Transethosomes

DSC is a highly useful means of detecting drug-excipients interactions (Lopes *et al.*, 2006; Duan *et al.*, 2011). Thermal behavior of 5-FU, Phospholipon 90G, Sodium cholate and the formulation of TEL were studied using DSC. The DSC studies showed the endothermic peak of 5-FU at 292.1ºC. The thermogram of the formulation was almost the overlap of each individual component. The thermogram of the TEL shows broadening curve and a single peak at 119.1ºC. This may be due to the melting of the lipid component and their interaction with 5-fluorouracil. This suggests that the drug has been entrapped into the lipid vesicles and the formulation is stable.

3. Physicochemical characterization

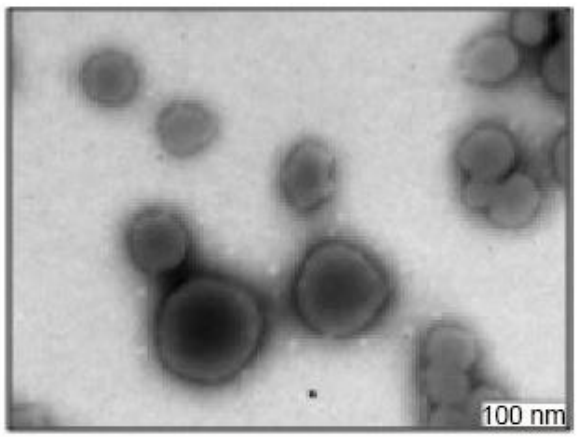

Fig 6: TEM image of 5-FU Transethosomes

TEM images showed that the transethosomes are spherical shaped vesicles. Visualization by TEM showed that they are unilamellar vesicular structure, and this confirms the existence of vesicular structure at the higher concentration of ethanol and edge activator.

Table 2: Physicochemical properties of the vesicular formulations

Sr. No.	Formulation	Size (nm)	Zeta potential (mV)	% Entrapment efficiency
1	F I	52	-40.76	92.06
2	F II	69	-46.19	88.22
3	F III	97	-55.67	75.08
4	F IV	49	-52.87	85.01
5	F V	61	-51.66	89.56

Standard deviations were in the range of 65-72 nm which suggested better polydispersity of the formulation. Ethanol causes a modification in the net charge of the system and confers it with some degree of stearic stabilization that may lead to decrease in mean vesicle size[4]. The charge of the transethosomal vesicles is an important parameter as it can influence vesicular properties such as stability. Zeta potential of vesicles showed negative values, which may be due to the presence of edge activator[5]. Encapsulation efficiency represents drug to lipid mass ratio, which explains EE as a function of total drug concentration. Percent drug entrapment of the formulations ranged from 75.08% to 92.06%. Increase in ethanol concentration increases the drug entrapment due to increased fluidity of the vesicular membrane. Further increase in ethanol concentration decreases the drug entrapment as vesicle

membrane becomes leakier[6]. Depending on the stability, particle size, zeta potential and entrapment efficiency the most suitable formulation was found to be FI which will be further used for optimization.

CONCLUSION:

Many nanoscale colloidal carriers have been proposed as topical delivery vehicles for treatment of skin cancers, and tranethosomes represent desired nanovesicle with improved deformability, which enhances drug permeability and deep skin targeting. The results obtained from this study indicate that new phospholipid carrier transethosomes which consist of the high concentration of ethanol and edge activator enhance the permeation of 5-Fluorouracil. The results showed that phospholipon 90G was better phospholipid than phospholipon 90H. Thus the developed transethosomal formulation could be the potential carrier for 5-Fluorouracil and other similar drugs especially due to their simple production and ease of scale up.

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