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## FORMULATION CHARACTERIZATION AND EVALUATION OF NEW TOPICAL 5-FU BY DRUG ENTRAPMENT IN OLEIC ACID VESICLES.

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### ABSTRACT

Due to the lower risk of systemic side effects topical treatment of skin disease appears favourable, yet the stratum corneum counteracts the penetration of xenobiotics into viable skin. Fatty acids have been widely used as adjuvant, vehicles in drug delivery viz penetration enhancers in topical delivery and in polymeric micelles to provide sustained release. However, the present investigation aims at exploring the potential of fatty acid vesicles for the topical delivery of 5-FU. Vesicles were prepared by film hydration method using oleic acid as a fatty acid principal component. Developed vesicles were characterized for size, size distribution, shape, *In vitro* release, pH dependent and storage stability, skin and ex-vivo skin permeation. Optical microscopy and TEM studies confirmed vesicular dispersion of fatty acid. The vesicles possessed higher entrapment efficiency ( $64.0 \pm 4.2\%$ ) with optimum vesicle size and homogeneity in regard to size distribution ( $PDI = 0.234 \pm 0.016$ ) at 7:3 oleic acid-to-5-FU ratio. *In vitro* drug release study suggested sustained release of drug from the vesicles. The vesicles were fairly stable at refrigerated conditions. *Ex-vivo* skin permeation and Confocal microscopic studies suggested that oleic acid vesicles penetrate the stratum corneum and retain the drug accumulated in the epidermal part of the skin. On the basis of sustained release behavior and skin retention it can be inferred that oleic acid vesicles can serve as a potential carrier for the topical localized delivery of bioactives.

**Key words:** Oleic acid, 5-FU, CLSM

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## INTRODUCTION

5-Fluorouracil (5-FU) is effective in the topical treatment of a number of disease states that are characterized by uncontrolled proliferation of various skin cell populations. However, in each instance less than optimal delivery of 5-FU using conventional preparations has necessitated the use of more drastic measures to obtain therapeutic results. As examples, light curettage of basal cell carcinomas (BCC), which physically removes the topical barrier to absorption, reduces the 5-year cumulative recurrence rate after topical 5-FU treatment from 21% to a more clinically acceptable 6% (Epstein, 1985). Similarly, topical 5-FU treatment of psoriasis is only effective if applied under occlusion<sup>1</sup>.

The advantages of transdermal drug delivery systems are well-documented<sup>2</sup>. An increasing number of drugs are being added to the list of therapeutic agents that can be delivered into systemic circulation, in clinically effective concentrations, via the skin portal<sup>3</sup>. It has been documented and reported that unsaturated fatty acids such as oleic acid and linoleic acid have a tendency to form vesicles in the aqueous environment<sup>4</sup>. After about a decade saturated fatty acids with carbon atoms in the range of 8 –12 were also found to under self-assemblage into vesicles in a pH dependent manner<sup>5</sup>. Fatty acids being highly soluble tend to partition into artificial as well as natural membranes quite rapidly<sup>6</sup>. It has also been investigated that fatty acid vesicles enhance the absorption of therapeutic molecules through the GIT, probably by forming mixed micelles or through chylomicron(s), thus increasing the bioavailability of the molecules<sup>7</sup>. It has been reported that free fatty acids act as penetration enhancers for the bioactives through the stratum corneum<sup>8</sup>. However, skin permeation property of fatty acids varies with the chain length and branching. The penetration enhancement effect of fatty acid increases with an increase in the chain length; however, it follows the relation only up to C18. The skin permeation property of unsaturated fatty acids is higher than the corresponding saturated fatty acid. Further, fatty acid(s) containing C is double bond exhibited higher penetration potential as compared to Trans form<sup>9</sup>. The major limiting property of free fatty acids in their use as a penetration enhancer is their skin irritation characteristics. The problem of skin irritation, however, could be addressed by using fatty acid vesicles as drug bearing carriers. It has been shown that bilayer membrane possesses a fusogenic tendency due to its capability to lower the phase transition temperature of the lipids in the biological membrane. The vesicular membrane fuses with skin lipid bilayers, releasing its contents. Thus, it is hypothesized that fatty acid vesicles will act as a suitable carrier to enhance the penetration of bioactive agents through the stratum corneum with reduced toxicity.

Moreover, fatty acid vesicles seem advantageous as they are easy to prepare as well as cost effective<sup>10</sup>. The present study involves the use of oleic acid vesicles to encapsulate 5-FU and evaluates its potential as an alternative drug delivery system for effective topical application.

## MATERIALS AND METHODS

5-FU (lot # C5666A, purity > 99%) was generously donated by Alfa Aesar, A Johnson Matthey Company (Ward Hill, MA). Oleic acid was purchased from CDH (New Delhi, India). Sephadex G-50 and Dialysis membrane were purchased from Himedia (Mumbai, India). Carbopol 940 was purchased from Himedia (Mumbai, India). All other solvents used were of analytical grade, unless otherwise mentioned, and purchased from CDH.

### Preparation of Drug-Fatty Acid Vesicle Dispersion

Oleic acid vesicles were prepared by film hydration method, as reported earlier by<sup>11</sup> with slight modification. Briefly, oleic acid and 5-FU were dissolved in methanol in a round bottom flask followed by evaporation of solvent under vacuum using a rotary evaporator (Perfit equipments, Ambala, India) to remove even the last traces of organic solvent. The completely dried film in rota evaporator was left overnight for the removal of any possible traces of methanol and also to prevent the formation of emulsion due to the residual organic solvent. The dried film formed was then hydrated at ambient temperature for 1 h with PBS (pH 7.4). The prepared vesicular dispersion was sonicated to form the uniform size vesicular dispersion. Optimization was performed by varying the ratios of oleic acid and 5-FU (Table 1). Untrapped drug was estimated by using dialysis method (Molecular weight cut of 12,000-14,000, Himedia, Ltd.). Briefly, 1% w/v of Carbopol 940 was dispersed into purified water with the help of a vortex shaker (Tarsons, Kolkata, India) and allowed to hydrate for 4–5 h. The pH value of the gel was adjusted to 7.4 using triethanolamine. During preparation of the gel, the solution was agitated slowly to avoid any air entrapment. Plain drug gel was prepared by using an equivalent amount of 5-FU solution into the previously made carbopol gel in a 2:1 ratio under gentle mechanical mixing for 5 min.

### Vesicles shape

Fatty acid vesicles were visualized by using Moragagni 268D TEM with an accelerating voltage of 100kV. A drop of the sample was placed onto a carbon-coated copper grid to leave a thin film on the grid. Before the film dried on the grid, it was negatively stained with 1% phosphotungstic acid (PTA). A drop of the staining solution was added onto the film and the excess of the solution was drained off with a filter paper. The grid was allowed to air dry thoroughly and the samples were viewed on a transmission electron microscope.

Vesicles without sonication were also visualized by using an optical microscope. A thin film of FAV was spread on a slide and after placing cover slip it was observed under the optical microscope. (Figure 1)

### ***In Vitro* Vesicle Characterization**

#### **Determination of entrapment efficiency**

Entrapment Efficiency of 5-FU from the 5-FU-FAV1, 5-FU-FAV2, 5-FU-FAV3 formulations were investigated using dialysis method (Molecular weight cut of 12,000-14,000, HI Media, Ltd.). The prepared complex was dissolved in PBS (pH 7.4) at a concentration of 1mg/ml (the same concentration of 5-FU as 20 mg/ml pure drug solution). This solution (2 ml in volume) was transferred to a dialysis bag (size cut off = 2.5 nm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 40 ml PBS (pH 7.4). The outer phase was stirred continuously. At predetermined time intervals sample was withdrawn and replenished with same amount of receptor fluid. The absorbance of the outer phase was monitored at 260 nm using a spectrophotometer (Systronics Electronic Limited, Ahmedabad, India) in order to characterize the concentration of 5-FU. The entrapment efficiency calculated for various molar ratios is listed in Table 1.

#### **Differential Scanning Calorimetry (DSC)**

The physical state of 5-FU inside the oleic acid was investigated by Differential Scanning Calorimetry (Shimadzu DSC-41, Japan). The samples were purged with dry nitrogen at a flow rate of 20 ml/min. The temperature was raised at 10 °C/ min

#### ***In vitro* drug release studies**

The *in vitro* release of 5-FU from different vesicular formulations was studied using locally fabricated Keshry Chein diffusion cell and through the cellophane membrane

#### ***In vitro* drug release using cellophane membrane**

*In vitro* release behavior of 5-FU from oleic acid was investigated using cellophane membrane (Molecular weight cut of 12,000-14,000, HI Media, Ltd.). Pure 5-FU was dissolved in PBS (pH 7.4.) at a concentration of 1 mg/ml and used as control. The prepared complex was dissolved in PBS (pH 7.4) at a concentration of 1 mg/ml (the same concentration of 5-FU as 1 mg/ml pure drug solution). This solution (2 ml in volume) was transferred to a dialysis bag (size cut off = 2.5 mm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 40 ml PBS (pH 7.4). The outer phase was stirred continuously. At predetermined time intervals sample was withdrawn and replenished with same amount of receptor fluid. The absorbance of the outer

phase was monitored at 260 nm spectrophotometrically in order to characterize the concentration of 5-FU (figure .2)

### **pH dependent stability**

The effect of pH on the stability and on the drug release behavior was monitored by incubating optimized vesicular dispersion with buffers of pH 8.5, 7.4, 6.5, and 5.5. At pre-determined time intervals the samples were withdrawn and centrifuged at 14,000 rpm for 30 min. The supernatant was analyzed for released free drug. The amount of drug leached was then calculated by the following formula:-

$$\% \text{ Drug diffused} = \frac{\text{Amount of free drug}}{\text{Total drug}} \times 100$$

Simultaneously, the incubated vesicles were observed for any change in morphology and size using an optical microscope. The studies were performed in triplicate.

### **Preparation of rat skin**

Albino rat 5-6 weeks old weighing 100-120g was sacrificed by chloroform inhalation. The hair of test animals were carefully trimmed short (<2mm) with a pair of scissors and the abdominal skin was separated from the underlying connective tissue using scalpel. The excised skin was placed on aluminium foil and the dermal side of the skin was gently teased off for any adhering fat and/ or subcutaneous tissue. The skin was then carefully checked through a magnifying glass to ensure that samples were free from any surface irregularities such as tiny holes or cervices in the portion that was used for transdermal permeation studies. The skin was washed with physiological buffer saline (pH 7.4) and freshly obtained skin was used in all experiments.

### **Skin permeation studies**

The *In vitro* skin permeation of 5-FU from different formulations was studied using locally fabricated diffusion cell. The effective permeation area of the diffusion cell was 2.303 cm<sup>2</sup>. The receptor compartment contains 22.5 ml PBS (pH 7.4). Albino abdomen rat skin was mounted between the donor and receptor compartments. The donor compartment was maintained at 37±1 °C with constant stirring at 125 rpm. The FAV-3 (2 ml) was applied to the epidermal surface of the rat skin. Samples were withdrawn through the sampling port of the diffusion cell at predetermined time intervals over 24 hrs and analyzed. The receptor phase was immediately replenished with an equal volume of fresh buffer. The *in vitro* drug release study of elastic liposomal formulation was repeated with a cellophane membrane by using the same method as

described above. Experiments were conducted to optimize the amount of 5-FU that can be incorporated into the vesicles and to optimize the FAV-3 formulation.

### **Determination of amount of drug deposited into the skin**

In this method the *in vitro* drug permeation study was performed in two stages using the same locally fabricated diffusion cell<sup>12</sup>. In the first stage PBS (pH 7.4) was used as the receptor medium and method as described above for skin permeation was carried out. FAV-3 (2ml) was applied to epidermal surface of rat skin. Samples were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 10 hrs and analyzed. The receptor phase was immediately replenished with equal volume of fresh buffer. At the end of 10 hrs the donor compartment was washed five times with warm receptor fluid. The second stage used 50% v/v ethanol as the receptor solution for a further period of 12 hrs and performed without any donor phase. During this stage an ethanolic receptor will diffuse into the skin disrupting the vesicular structure of any FAV-3 that may have penetrated and deposited in the tissue and thus releasing both FAV bound and free 5-FU for collection by receptor fluid. The amount of drug deposited was finally estimated by HPLC.

### **HPLC method for estimation of 5-FU**

The HPLC behavior of 5-FU extracted from human plasma was isocratically examined by using Genesis C18 analytical column. We have tested a number of mobile phases and found that (methanol : water, 97:3, %v/v) with pH adjusted to 3.5 gave excellent separation of the compounds of interest in a run time of 10 min. The mobile phase reported here in (methanol: water, 97:3, %v/v) was optimized for a rapid and interference-free chromatogram. The selected chromatographic conditions provided optimum resolution of 5-FU. The retention time was found to be 4.0min for 5-FU.

### **Statistical analysis**

All the results are expressed as mean  $\pm$  SD. Statistical analysis was carried out employing the Student's t-test using InStat 2.1 Software. The value of  $p < 0.01$  was considered as significant.

## **RESULTS AND DISCUSSION**

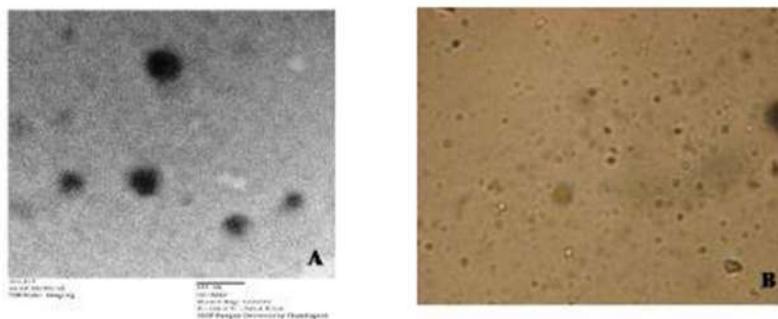
### **Fatty acid Vesicle Characterization**

Multilamellar oleic acid vesicles were prepared by film hydration method by varying oleic acid-to-5FU ratios. Hydration was affected at ambient temperature for 1 h to enable complete hydration. The speed of rotation influences the thickness and uniformity of the film. The rotation speed of 120 rpm was observed to yield a uniform thin lipid film while lower and higher rate of

rotation resulted in perceptible non-vesicular aggregated artifacts. The developed formulations were further characterized for size, entrapment efficiency, and zeta potential studies (Table 1). The studies carried out demonstrated no significant difference in the entrapment efficiency; however the mean entrapment efficiency of the vesicles increased with an increase in the molar quantity of 5-FU up to 7:3 oleic acid-to-5FU ratio (64.0±4.2%). Beyond this ratio no further increase in drug entrapment was recorded. The vesicular sizes were obtained in the range of 400 nm to 1µm. The oleic acid vesicles dispersions obtained were mostly poly disperse; however, at 7:3 oleic acid-to-5-FU ratios the dispersity was recorded to be  $0.534 \pm 0.026$ . The photomicrographs depict the spherical nature of the oleic acid vesicles (Figure 1A). Further, in addition the TEM study conducted confirmed the ultra structure of oleic acid vesicles which revealed multi lamellarity of vesicles (Figure 1B).

**Table.1 Size and entrapment efficiency of the prepared oleic acid vesicles.**

Formulation code	Oleic acid: 5-FU	Entrapment efficiency	Particle Size(nm)
FAV-1	9:1	(39.4±2.1)	505 ± 15
FAV-2	8:2	(45.4±2.1)	523±12
FAV-3	7:3	(68.0±4.2%),	632±17
FAV-4	6:4	(55.4±2.7)	531±16
FAV-5	5:5	(48.4±2.4)	404±13



**Figure 1. Vesicles morphology of optimized oleic acid vesicles by (A) TEM and (B) optical microscopy (400× magnification)**

### Preparation of vesicles

The fatty acid vesicles were evaluated to assess their efficacy in delivering the bioactives to and through stratum corneum of the skin. The drug 5-FU was used as a model drug. The major consideration in the formulation of fatty acid vesicles is pH of the formulation; this being a

critical factor that controls the degree of ionization of fatty acid (14, 15) and is hence responsible for the formation of vesicle. The fatty acid (oleic acid) assembled into vesicles only if pH equals the pKa of the acid (8.5). At this pH, ~ 50% of the carboxylic acid is ionized and transforms into ionized amphiphile(s) with a tendency to form vesicles/aggregates. The acid is present as ionic  $\text{RCOO}^-$  as well as neutral  $\text{RCOOH}$  species. In such conditions each ionized group is stabilized through a strong hydrogen bond formed with the neutral molecules. The negative charge present on the ionized carboxylic group is shared between two adjacent fatty acid molecules, i.e. ionized and unionized, and thus results in the formation of typical dimmers. The hydrophobic hydrocarbon chain of so formed dimmers protects itself from the aqueous compartment and thus orients to form an enclosed bilayer structure that minimizes the interaction between the hydrocarbon chain and water. The ratio of protonated and deprotonated group seems also critical in the process of vesiculation. This is possible only if the concentration of the fatty acid in aqueous dispersion exceeds the critical vesicle concentration (CVC), which is reportedly 80 mM for oleic acid (16). The stability of the vesicles is attributed to the strong hydrogen bond-based interactions between the protonated and deprotonated groups viz  $\text{RCOO}^- \text{HOOCR}$  (17, 18, 19, 20) the effect of drug: oleic acid ration on the encapsulation of the 5-FU was studied. It was seen that the drug bearing capacity of the oleic acid vesicles depends upon the molar ratio of oleic acid and 5-FU. The entrapment efficiency increased up to oleic acid : drug molar ratio 7:3, beyond this ratio further increase in the amount of drug reduced the degree of drug encapsulation. This may be ascribed to the drug saturation in the bilayer domain. Further addition of drug could have destabilized the vesicle membrane leading to the leakage of content. Since the 7:3 vesicles showed the best physico-chemical characteristics (high homogeneity, high entrapment efficiency), the respective formulation was selected for further studies.

### **Drug release studies**

The study provided an insight on the release pattern of the drug from the vesicles. Release studies carried out with all the formulations of oleic acid vesicle dispersions have shown that almost 40–50–60% of the encapsulated drug was released from the vesicles within 24 h (Figure 2). The kinetic analysis of the release data was carried out by plotting a graph between cumulative drug releases vs. square root of time. It suggested a linear correlation of drug release with time. Furthermore, in order to evaluate the effect of drug release from vesicles, the release was compared with that of 1% 5-FU solution. It was observed that almost the entire drug was recovered in the acceptor compartment within 4 h.

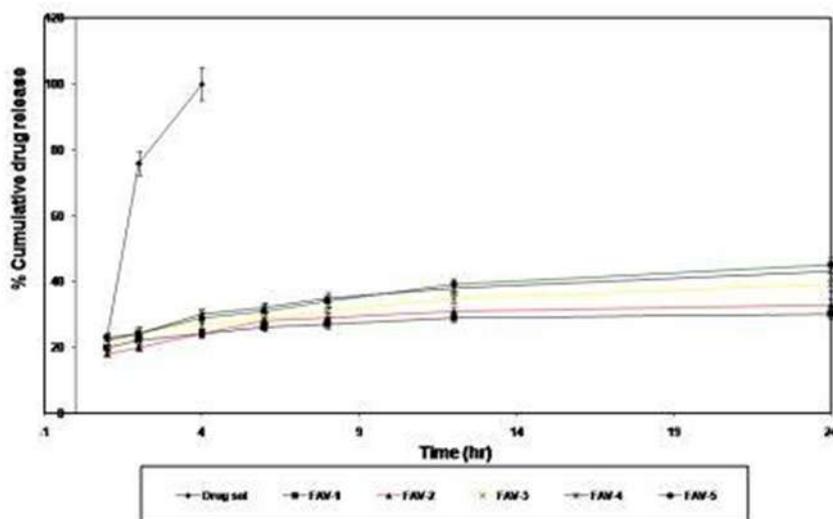


Fig.2: *In vitro* drug release 5-FU from oleic acid vesicles formulations

### pH-dependent stability

The study demonstrated the pH-dependent nature of the oleic acid vesicles. It also provided useful information on topical drug delivery potential and characteristics of oleic acid vesicles since the pH of skin is 5.5. It was observed that the release from vesicles is highly pH-dependent and on lowering the pH from 8.5 to 5.5, only 20% of the drug remained in vesicles to be released after 8 h of incubation in buffer of pH 5.5 as compared to residual drug estimated, i.e. 71% at pH 8.5 (Figure 3). The differences in drug diffusion recorded at pH 8.5 and 7.4 were not significant ( $p > 0.01$ ). Therefore, further studies were continued by adjusting the pH of vesicles suspension to 7.4, since higher pH values may cause skin irritation and may not be acceptable for topical application. Simultaneously, morphological changes in vesicles size and shape were also observed with changing pH. The results displayed an increase in the size of the vesicles at low pH values (Figure 4).

### In-vitro performance of oleic acid vesicles

By comparing the release from various oleic acid vesicles based formulations (prepared using different oleic acid and 5-FU molar ratios), it was deduced that there was no considerable difference in the release in terms of kinetic pattern, although drug release exhibited a concentration-dependent behavior. These results are in agreement with the release kinetic reported by other research workers who documented that drug release from vesicles is affected by diffusion (21, 22). Further, by comparing the drug release data of oleic acid vesicles with that

of 5-FU solution it was concluded that the release of 5-FU from the vesicle was slow, controlled, and uniform as compared to 5-FU solution. The pH-dependent stability behavior substantiates that drug diffusion across the skin may increase with a decrease in the pH of the vesicles dispersion. Thus, the increased diffusion of drug from the vesicles at low pH may have resulted due to decreased stability of the vesicles at lower pH. This further suggests that vesicles tend to fuse when they are exposed to low pH. This particularly holds for the pH that is lower than physiological pH.

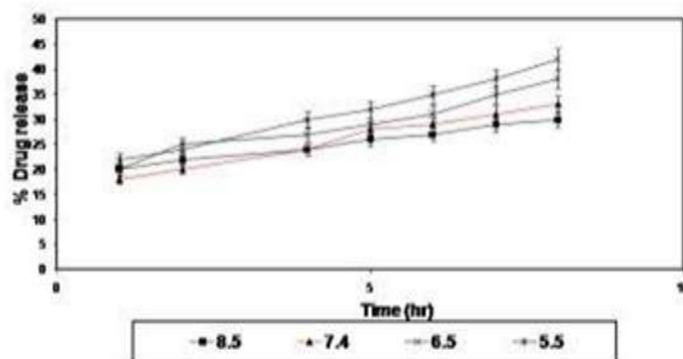


Fig.3 pH dependent release behavior of drug from oleic acid vesicles dispersion.

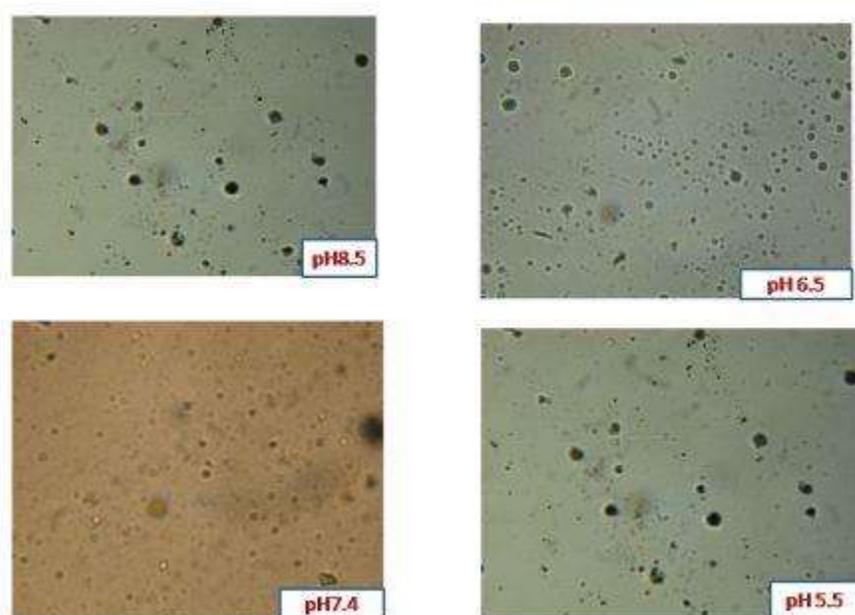


Figure 4. Photomicrograph of oleic acid vesicles dispersion incubated at different pH (400x magnification).

### Skin permeation study

The skin permeation study was conducted on optimized formulation prepared at a 7:3 fatty acid: drug ratio (pH 7.4) (highest entrapment efficiency and more uniform sized vesicles). To normalize the effect of pH on skin permeation, the plain pH of drug gel was also adjusted to pH

7.4. A significant increase in the skin permeation of 5-FU was recorded from oleic acid vesicle dispersion in comparison to plain gel (Figure 5). The amount of 5-FU permeated from the plain carbopol gel was 19.83%. The drug penetration following application of an equivalent amount of drug in vesicular dispersion was significantly high, i.e. 35.8%. The permeation parameters were calculated by plotting a curve between cumulative amounts of drug permeated per unit area ( $\mu\text{g}/\text{cm}^2$ ) vs. time. The flux was obtained from the slope of the linear portion of the graph. The transdermal permeation rate constants obtained were higher for oleic acid vesicle dispersions ( $21 \pm 1.68 \mu\text{g}/\text{h}/\text{cm}^2$ ) than the plain drug gel ( $3.5 \pm 0.6 \mu\text{g}/\text{h}/\text{cm}^2$ ). It has also been observed that drug retained in the skin was more in the case of vesicular dispersion  $25.94 \pm 1.64$  as compared to plain drug gel ( $4.06 \pm 0.74\%$ ) (Table 2). The CLSM study was also conducted to confirm the skin penetration of the fatty acid vesicles. The bright fluorescent intensity of oleic acid vesicles was detected in the stratum corneum and epidermis up to a thickness of 55–60  $\mu\text{m}$  (Figure 6). However, as the thickness of skin increases, the fluorescent intensity tends to decrease.

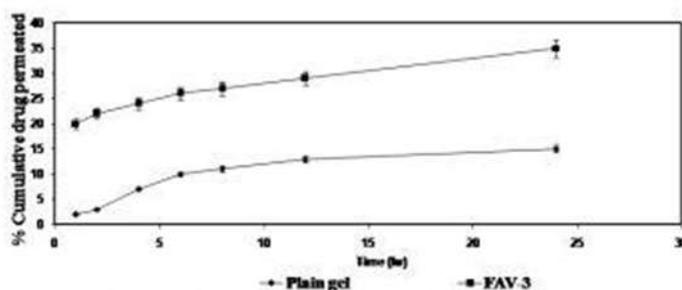


Figure 5 pH dependent release behavior of drug from oleic acid vesicles dispersion.

**Table 2 Skin permeation and retention of 5-FU from various formulations**

Formulation	Percentage drug retained in skin	Transdermal flux $J_{ss}$ ( $\mu\text{g}/\text{h}/\text{cm}^2$ )
Optimized vesicular preparation (pH 7.4)	$25.95 \pm 1.64$	$21 \pm 1.68$
Plain drug gel (pH 7.4)	$4.06 \pm 0.74$	$3.5 \pm 0.6$

### Ex-vivo assessment of drug accumulation and permeation

The oleic acid vesicles provided good skin permeation property as 36% of the 5-FU permeated across the skin. Comparatively lower permeation from the plain gel can be accounted for by the hydrophilic nature of 5-FU. The permeation parameters such as transdermal flux and the amount of drug retained calculated were higher in the case of oleic acid vesicles as compared to plain

drug gel. Thus, fatty acid vesicles have adequate permeation and penetration enhancing property. They may be effective in the treatment of deep localized fungal infections.

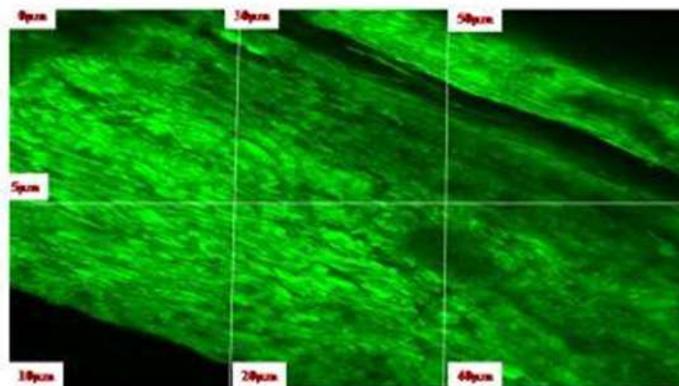


Figure 6. Confocal microscopic image of skin showing penetration behavior of oleic acid vesicles along the thickness of rat skin

### Differential Scanning Calorimetry (DSC)

The DSC thermo grams corresponding to 5-FU, Oleic acid, physical mixture and 5-FU-oleic acid are shown in Figure 7. The DSC curve of 5-FU showed a single melting peak at 284.1 °C (Figure 7A). The oleic acid thermogram displayed an endothermic peak at 309.6 °C (Figure 7B). For physical mixture the peak was detectable at the melting point of 5- FU (Figure7 C). However, no characteristic peak of 5-FU was observed in DSC of drug-loaded oleic acid vesicles, but it showed that the small peak was at 196.9 °C. This suggests that the drug was molecularly dispersed in the polymer matrix (Figure7 D). There is no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymer systems loaded with drug<sup>13</sup>. The reduction of height and sharpness of the endotherm peak is due to the presence of polymers in the nanoparticles. Since there was no shift in the Tg of the polymer, it can be concluded that there is no significant reaction occurring between the drug and the polymer

### Safety profile of the formulation

The study was conducted to test the safety and skin irritation caused by formulations. The fatty acid vesicles caused negligible erythematic manifestations. On account of self-assembly the oleic acid in vesicles have reduced exposure of the acidic functions (-COOH) (the triggering factor for the erythematic events)<sup>23</sup> with the stratum corneum resulting in reduced erythematic episodes. In fact, the hydrogen bonding between the protonated and deprotonated groups reduces the polarity of the COO<sup>-</sup> responsible for irritation<sup>24</sup>. It was found that oleic acid vesicles demonstrated skin tolerance, indicating their possible clinical potential in regard to patient acceptance and therapeutic efficacy.

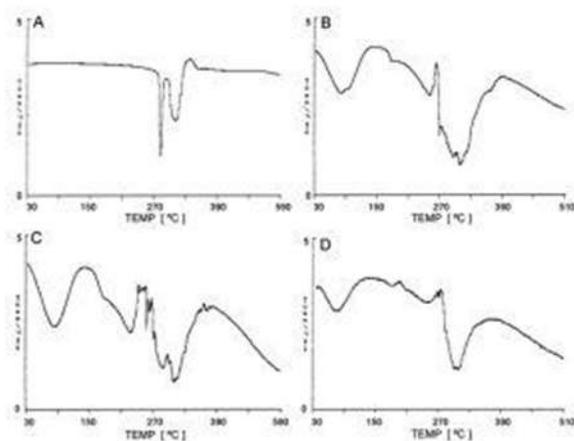


Fig. 7 DSC thermograms of 5-FU (A), Oleic acid(B), physical mixture of 5-FU, oleic acid (C),PU-FAV 3 (D).

### Stability Studies

Stability of the product may be defined as the capability of a particular formulation to remain with the physical, chemical, therapeutic and toxicological specifications. The optimized formulation (FAV-3) was selected for stability study on the basis of its *In vitro* performance and stored in tightly closed glass vials at room temperature and in refrigerator ( $4\pm 2^\circ\text{C}$ ). Following parameters were evaluated at different time intervals (20, 40 and 60 days): The formulations were stored in 10ml glass vials at refrigeration temperature ( $4\pm 2^\circ\text{C}$ ) and room temperature for a period of 2 months. The samples were analyzed at predetermined time intervals visually and under optical microscope for the change in consistency and appearance of drug crystals. Vesicle size and size distribution was determined at definitive time intervals for a period of 2 months using stage eyepiece micrometer and haemocytometer respectively as described earlier.

### Physical stability

The magnitude of drug retained within the vesicles ultimately governs the shelf-life of the formulation. The fatty acid-based vesicles formulations when stored at ambient and refrigerated conditions have shown that the vesicles are stable at  $4 \pm 1^\circ\text{C}$ . This is because the vesicles sizes at this temperature remains stable and unchanged, thus the leakage from the vesicles was minimal. The increase in size indicates inter-vesicular fusion. At ambient temperature ( $28^\circ\text{C}$ ), the phase transition temperature of oleic acid is exceeded, hence they tend to fuse<sup>24,25</sup>. The drug leakage studies carried out also suggested better stability of fatty vesicles at refrigerated conditions.

## CONCLUSION

From the above preliminary studies it has been concluded that fatty acid vesicles can serve as potential carriers for the delivery of therapeutic molecules for the treatment of skin diseases. They are cost effective and therapeutically viable. Sustained release behavior and drug retention in the deeper part of skin might be beneficial for the long-term effects of drugs. The oleic acid vesicles seemingly fuse with the skin and release the contents. They are seen to penetrate intact and to form drug depots in the skin. The fatty acid in addition may serve as a penetration enhancer, thus by circumventing the stratum corneum barrier potential they may lead to better permeation of the drug molecules.

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