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# Anti-Inflammatory Flexible Liposomal Gel for Treatment of Rheumatoid Arthritis

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**ABSTRACT: Background:** Liposomes have been extensively investigated as drug delivery systems in the treatment of rheumatoid arthritis (RA). Low bioavailability, high clearance rates and limited selectivity of several important drugs used for RA treatment require high and frequent dosing to achieve sufficient therapeutic efficacy. **Aim:** This work was undertaken to formulate Aceclofenac loaded flexible liposomes as a topical nano carrier for the treatment of Rheumatoid arthritis as an alternative to simple Aceclofenac gel. **Method:** Different Aceclofenac loaded flexible liposomes were prepared by modified hand shaking technique. F1 to F6 formulations were prepared by using phosphotidylcholine as a vesicle forming polymer, chloroform and ethanol as solvents, Tween 80 and span 80 as edge activators. Flexible liposomes with different surfactants were prepared in the optimization process and formulations were optimized through in *vitro* diffusion studies, characterized for encapsulation efficiency, particle size, zeta potential and cumulative drug release. The best liposomal formulation was incorporated into different concentrations of Carbopol 934 base. Finally these gels were tested for characteristic properties. **Results:** Results revealed that liposomal with span 80 showed better entrapment efficiency, best cumulative drug release and zeta potential was found to be -23.5mV. Out of three gel formulations, TG2 showed maximum drug content, pH 6.9 and spreadability was found to be 24.05 g.cm/s. **Conclusion:** From the study it was concluded that Aceclofenac liposomal gel possessed better skin permeation potential, stability and ability as a self penetration enhancer.

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## INTRODUCTION:

Physiopathology of a wide variety of diseases involves inflammation. Even though it is a self protective response but over a prolonged period can lead unfavorably to the disease process. Consequently anti-inflammatory drugs are very widely used. The non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of minor pain and for the management of edema and tissue damage resulting from inflammatory joint disease (arthritis) [1]. As skin has a total surface area of 1.8 $m^2$ which makes it suitable for

dermal delivery of drugs [2,3]. Many researchers had reported that novel vesicular formulations were proved to be best when compared with conventional topical formulations [4-6].

Liposomes are bilayer phospholipid vesicles which can entrap both hydrophilic and lipophilic components. Liposomes synonyms are elastic liposomes, flexible liposomes, ultradeformable vesicles, highly deformable vesicles, elastic vesicles [7], made up of phospholipid component, single chain surfactant molecule and solvents like chloroform, ethanol [8-10]. In order to formulate more deformable elastic liposomes with high amounts of drug entrapment need to add appropriate concentrations of lipid and edge activators [11,12]. Its infrastructure possesses both hydrophilic and hydrophobic moieties together and it can entrap both types of drugs. They can act as carrier for low as well as high molecular weight drugs like analgesics, anesthetics, corticosteroids, sex hormones, anti cancer drugs and insulin [13].

It was evident from many researcher reports that high penetration capability of liposomes made them able to deliver a wide variety of therapeutic moieties into deeper skin layers or for transport of drugs into systemic circulation [14]. Potential advantages of liposomes are high entrapment efficiency, for lipophilic drug it is near to 90 %, suitable for both systemic as well as topical delivery of drug, protect the encapsulated drug from metabolic degradation, biodegradability and lack of toxicity [14-16].

Aceclofenac is a non-steroidal anti-inflammatory agent which possesses remarkable anti-inflammatory, analgesic, antipyretic properties and is used in the treatment of rheumatologic disorders [17,18]. It acts by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase. The main drawback of Aceclofenac is low aqueous solubility, leading to poor dissolution and insufficient oral bioavailability [19]. As Aceclofenac is a BCS-class II drug, undergoes first pass metabolism hence there is a need to search for an alternate delivery system. Transdermal delivery of Aceclofenac can increase its therapeutic effect by reducing side effects that are associated with conventional oral delivery of Aceclofenac. The objective of present study is to design, characterize and evaluate flexible liposomes of Aceclofenac for transdermal delivery.

## MATERIALS AND METHODS:

Lecithin was purchased from Lipidome Life Sciences from Lipoid group and Aceclofenac and Carbopol – 934 were purchased from Yarrow Chem, India. The ethanol was obtained from Changshu Yangyuan Chemical, China. Triethanolamine, Tween 80, Span 80 were purchased from Finar Ltd, India. Sodium hydroxide, Potassium dihydrogen phosphate was obtained from Thermo Fisher Scientific India Pvt. Ltd, India. All the materials used in this formulation study were of analytical and pharmaceutical grade.

### Pre-formulation studies:

The drug was identified by organoleptic properties, UV spectroscopy, IR spectroscopy, solubility and melting point determination of the drug. Organoleptic properties of Aceclofenac were studied by visual inspection by personal experiences.

Melting point determines the purity of the compound. Melting point of Aceclofenac was carried out by capillary method and DSC studies. Solubility determination of aceclofenac was carried out in water and organic solvents. The pure drug aceclofenac was estimated by using UV-Visible spectrophotometer by preparing a series of standard solutions ranging from 5 to 25 µg/ml using pH 7.4 phosphate buffer. The absorbance values were taken for each concentration at 275 nm wavelength. The plot was drawn by taking concentration in µg/ml on X-axis and absorbance on Y-axis. The FTIR spectrum of aceclofenac was determined by using the Cary 630 Agilent FTIR.

### Preparation of liposomes:

Aceclofenac loaded liposomes were prepared by modified thin film hydration technique. Drug, Phosphatidylcholine (PC), and activator were dissolved in ethanol: chloroform (1:1) mixtures. Organic solvent was removed by evaporation while handshaking above lipid transition temperature (46 °C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent.

The formed film was then hydrated with a phosphate buffer (pH 7.4) with gentle shaking for 15 min at corresponding temperature. The liposome suspension further hydrated up to 1 h at 2 to 8 °C, scheme as represented in Fig 2. The resulting vesicles were swollen

for 2 h at room temperature. To prepare small vesicles; resulting Large lamellar vesicles (LMVs) were ultra sonicated (Probe Sonicator, Model AIPS 250,AIPS500, Aspire Inc, Karnataka, India) for 30 min at room temperature [20-23].

**Characterization of liposomes [24-28]:**

***Visual inspection*:**

The formulated liposomes were visually inspected for their color and homogeneity.

***Microscopic observation of prepared liposomes*:**

An optical microscope with camera attachment was used to observe the shape of prepared liposomes formulation.

***Surface morphology***:

Surface morphology of liposomes was determined by Scanning Electron Microscopy technique.

***Fourier Transforms Infrared Spectroscopy study*:**

Infra red (IR) spectroscopy was used to ensure the compatibility between the drug and the liposomes component. The IR spectra of Aceclofenac, a physical mixture with Span 80 and a physical mixture with Tween 80 were obtained by using a Fourier- transform infrared spectrophotometer, and then scanned from 4000 to 400 $cm^{-1}$.

***Entrapment efficiency*:**

The percentage entrapment efficiency (EE %) of Aceclofenac in liposomal formulation was performed by centrifugation method. Briefly, 1 ml of each sample of TRSs was centrifuged (TDL 60 B, Ultracentrifuge, Sri Vijaya Enterprises, Telangana) at 14,000 rpm for 60 min at $4^{o}$C. The precipitated TRSs were washed with PBS at pH 7.4 twice. Then, the clear fraction (supernatant) was separated each time from TRSs and assayed for free non-entrapped drug. The drug in the washed samples was determined spectrophotometrically (Cary 60 UV, UV-Visible spectrophotometer) at $\lambda_{max}$ of 275 nm using PBS as blank. It was found that other additives do not interfere with the UV absorption spectrum of Aceclofenac at the chosen $\lambda_{max}$. The entrapment efficiency was calculated by using the formula;

EE (%) = [(TDC – CUD)/TDC] × 100 ….(1)

Where, TDC is total drug concentration and CUD is concentration of unentrapped drug.

***Turbidity*:**

The turbidity of the drug in aqueous solution was measured using the Nephelometer.

***In-vitro drug release*:**

An *in-vitro* drug release study was performed using modified Franz diffusion cells. Dialysis membrane was placed between receptor and donor compartments. Liposomal formulation of Aceclofenac was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4 (30 ml). The diffusion cells were maintained at 37±0.5 °C with stirring at 100 rpm throughout the experiment. At different time intervals, 5 ml of aliquots were withdrawn from the receiver compartment through the side tube and analyzed for drug content at 275 nm by using the UV-Visible spectrophotometer.

***Differential Scanning Calorimetry*:**

Differential Scanning Calorimetry (DSC) studies for the optimized formulation were carried out. The study was carried out using a DSC instrument. This technique was used to evaluate interaction between Aceclofenac and span 80.

***Zeta potential***:

The particle size and Zeta potential of the liposomes were measured by Zetasizer.

***No. of vesicles per cubic mm*:**

This is an important parameter for optimizing the composition and other process variables. Non sonicated liposome formulations are diluted 5 times with 0.9 % sodium chloride solution. Haemocytometer and optical microscope were used for further study. The total number of liposomes per cubic mm (N) was calculated by using the following formula;

N = (TLC × DF × 4000)/ TSC …..(2)

Where, TLC is the total number of liposomes counted, DF is the dilution factor and TSC is the total number of squares counted.

***Stability studies*:**

Stability studies were performed for 3 months and it was found that the formulation justifies all the characterization parameters even after 90 days. A little grittiness was found in the formulation after 3 months slightly starts loosing homogeneity and viscosity. This indicates that the formulation is perfectly stable for 2 months. From the results, it was clearly evident that the liposome formulation, SF3, was proved to show good entrapment efficiency, zeta potential and particle size. Optimized formulation (SF3) was added to the

preformed gel base of different strength represented in Table 6.

**Preparation of Liposomal gel:**

Since liposomes had very low viscosity and cannot be retained at the site of application for sufficiently long time, the viscosity represented a significant function in elevating the drug retention in the stratum corneum and viable epidermis. In the present study, Carbopol has been chosen to augment the viscosity of liposomes so that developed liposomes - based gel provides better applicability of formulation. Carbopols considered being a safe and non-irritant gelling agent with no reports of their sensitivity in human on topical application.

For the gel preparation, the best available formulation was selected for *in vitro* observation. For this aim, Carbopol - 934 was first dispersed in water and kept to a side for sufficiently long time so that polymer chains become fully hydrated and completely swell. Further, polyethylene glycol-400 (15 % w/w) was added. To this, triethanolamine was added with continuous stirring until a transparent alkaline gel was found. Liposomes formulation was added to this preformed gel base formulation.

**Evaluation of Liposomal gel [29-32]:**

***Physical evaluation*:**

The physical parameters considered for the evaluation were visual appearance, color, odor, consistency, leak and phase separation.

***Homogeneity*:**

The homogeneity of the liposomal gel formulations were tested by visual appearance after the gels have been introduced into their containers. Furthermore, a small quantity of each gel formulation is pressed between the thumb and the index finger, and the consistency of the gel was noticed whether homogeneous or not.

***Determination of pH*:**

The pH measurement was carried out by using a calibrated digital type pH meter. About 50 g of gel formulation was weighed and transferred in 10 ml of beaker and by dipping the glass electrode completely in to the gel so as to cover the electrodes, the pH of the gel was measured by using the digital pH meter (LI 120 pH meter, Elico pH meter, India).

***Drug content*:**

Liposomal gel of 1 g was mixed with 100 ml of ethyl alcohol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 275 nm by using UV-Visible spectrophotometer and drug content was calculated.

***In-vitro drug diffusion study*:**

An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane was placed between receptor and donor compartments. Liposomal gel of aceclofenac was placed in the donor compartment and the receptor compartment was filled with a phosphate buffer, pH 7.4 (30 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 100 rpm throughout the experiment. At different time intervals, 5 ml of aliquots were withdrawn from the receiver compartment through the side tube and analyzed for drug content at 275 nm by UV-Visible spectrophotometer.

***Stability studies*:**

The formulated liposomal gels were divided into 3 groups. These 3 groups of liposomal gel formulation were filled into aluminum collapsible tubes and stored at room temperature, 37±5 °C and 4 °C. The liposomal gel formulation was stored for a period of three months. Samples were withdrawn every month for a period of three months and assessed for the drug content. At the end of the third month they were evaluated for physical parameters and integrity of the product.

***Ex-vivo permeation studies*:**

Animal skin was used to carry out these studies. We did use goat skin. Flux values and permeability coefficient values were determined. The drug permeation study was performed using modified Franz diffusion cell. The procedure was the same as per the drug diffusion study mentioned above.

**RESULTS AND DISCUSSION:**

**Organoleptic evaluation:**

Organoleptic properties of Aceclofenac were studied and as represented in Table 1. The color and odor of the drug were white and odorless. The drug was crystalline in nature.

**Table 1. Organoleptic properties of Aceclofenac.**

| Sl. No. | Properties | Inference |
|---|---|---|
| 1 | Description | Crystalline powder |
| 2 | Odour | Odourless |
| 3 | Colour | White |

**Melting point determination:**

The DSC thermogram of pure drug Aceclofenac was shown in Fig 1. DSC study showed a sharp endothermic peak at 157.29 °C with a melting enthalpy of 101.72 J/g indicating that Aceclofenac was presented in crystalline form. The drug was melted at 150 °C by capillary method.

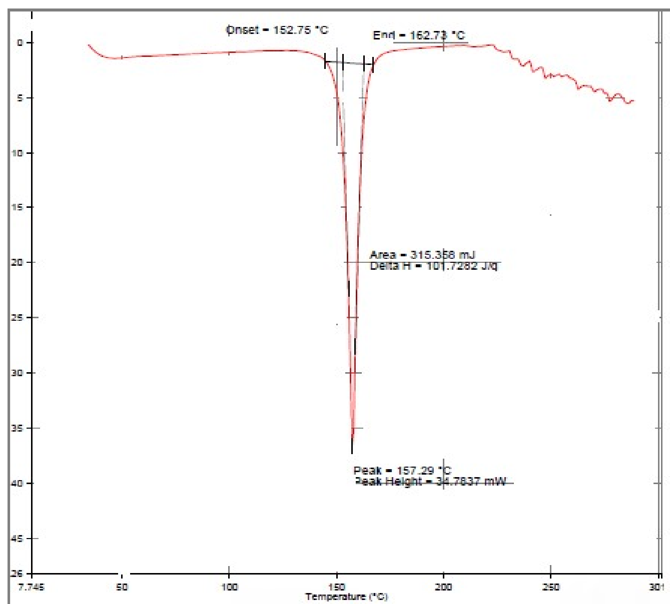


**Fig 1. DSC thermogram of Aceclofenac.**

**Solubility**:

Solubility determination of aceclofenac was carried out in water and organic solvents. Being a BCS class II drug, aceclofenac shows poor solubility. It was found that aceclofenac is practically insoluble in water, but soluble in organic solvents like ethanol/methanol. The above estimation done was beneficial for further studies.

**UV spectroscopy:**

It was observed that there is an increase in the concentration corresponding to an increase in the absorbance and it obeys the Beer-Lambert's law from the linearity of the calibration curve.

**Table 2. Concentration and absorbance values of aceclofenac in pH 7.4 phosphate buffer.**

| Sl. No. | Concentration (µg/ml) | Absorbance at 275 nm |
|---|---|---|
| 1 | 5 | 0.1651 |
| 2 | 10 | 0.2852 |
| 3 | 15 | 0.4201 |
| 4 | 20 | 0.5691 |
| 5 | 25 | 0.7203 |

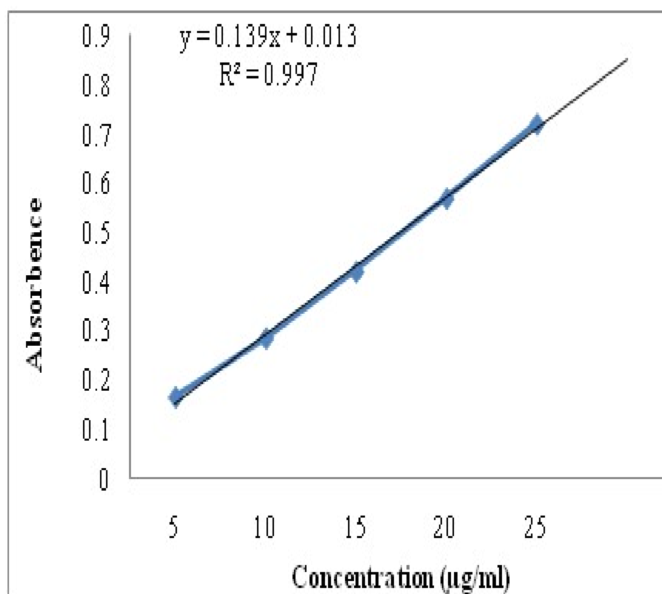


**Fig 2. Calibration curve of aceclefenac.**

The peaks showed sharp absorption maxima at 275 nm at pH 7.4 phosphate buffers. The drug was estimated by calibration curve using pH 7.4 phosphate buffer. The $\lambda_{max}$ of the drug was estimated by U.V. spectroscopy data was represented in Table 2 and Fig 2.

**Preparation of Liposome:**

The liposomal suspension was successfully prepared which was homogeneous, clear without any foreign particle as represented in Fig 3.

**Fig 3. Liposomal suspension.**

**Microscopic observation of prepared liposomes:**

A drop of liposomal formulation was placed over a glass slide and observed under a microscope**.** Photomicrograph of liposomes was depicted in Fig 4.

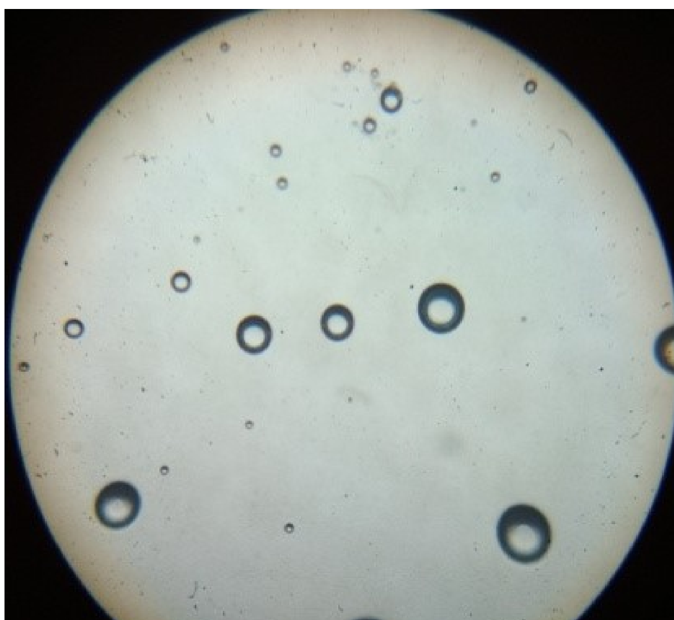
**Fig 4. Microscopic observation of liposomes.**

**Entrapment efficiency**

The percentage entrapment of liposomes was found to be 43.33, 68.06 and 85.66 % for TF1, TF2, TF3 and 40.14, 59.98 and 71.56 % respectively for formulations SF1, SF2, SF3. The entrapment data is given in Table 3

**Table 3. The evaluation parameters of liposome formulations.**

| FC | PS (nm) | PDI | ZP (mV) | EE (%) | DR (%) |
|---|---|---|---|---|---|
| SF1 | 255 | 0.270 | -14.6 | 43.33±0.2 | 77.1 |
| SF2 | 243 | 0.491 | -12.8 | 68.06±0.5 | 73.6 |
| SF3 | 235 | 0.180 | -15.3 | 85.66±0.1 | 89.6 |
| TF1 | 440 | 0.780 | -11.5 | 40.14±0.7 | 78.9 |
| TF2 | 447 | 0.490 | -12.8 | 59.98±0.5 | 81.2 |
| TF3 | 144 | 0.599 | -14.2 | 71.56±0.2 | 75.6 |

**FC – Formulation code, PS - Particle size, PDI - Polydispersity index, ZP - Zeta Potential, EE - Entrapement efficiency and DR- Drug release.**

Sonication brings about size reduction by breaking large liposomes to smaller ones and in doing so, leakage of small quantities of drug from the liposomes occurs. Hence sonication time was optimized to 30 min, and further reduction in the size by increasing sonication time was not attempted. The liposomal suspension is shown in Fig 3.

**Zeta potential**:

The Zeta potential data of the liposomes is Table 3 and Fig 5. The zeta potential of all liposomal formulations was found to be in the ranges of – 11.5 to 15.3 mV, signifying that the liposomes might be stable.

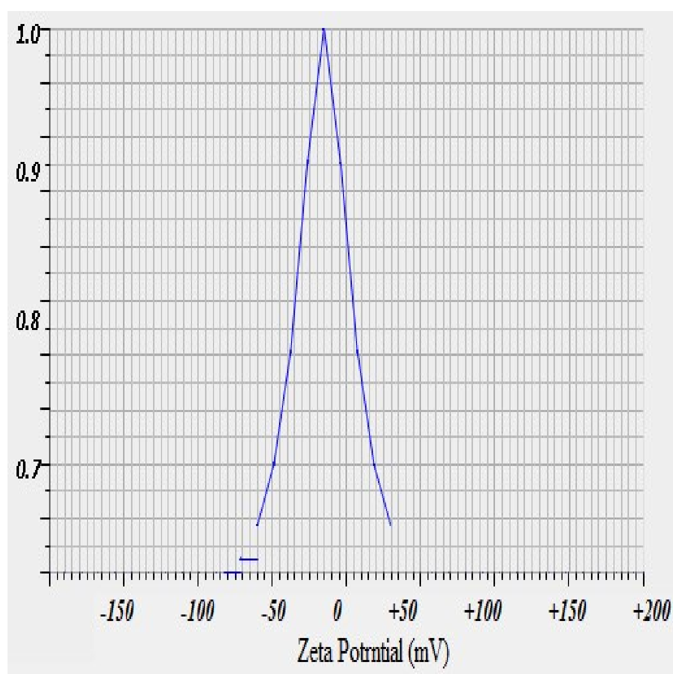

**Fig 5. Zeta potential of optimized liposomal formulation.**

**DSC studies:**

The DSC thermogram of Aceclofenac showed a sharp endothermic peak at 157.29 °C and corresponding to its melting point shown in Fig 6. The similar results were reported in literature. The DSC thermogram of phosphaotidylcholine endothermic peaks at 150.1 °C with a melting enthalpy of 2.24 J/g. In the DSC thermogram of optimized formulation endothermic peak at 150.89 °C. It did not show any characteristic changes in the drug and polymers. There are no extra peaks, indicating the compatibility between drug and excipients. So, the combinations can be used for further studies.

**X-ray Diffraction analysis:**

An XRD pattern of pure drug aceclofenac was shown in Fig 7. The crystalline nature of the drug was demonstrated by the characteristic XRD pattern with sharp peaks appearing at 2θ equivalent at 11, 15, 17, 25, 29 and 31°. The XRD results reveal that X-rays diffraction pattern of pure drug aceclofenac was clearly in crystalline form as represented in Fig 7.

The X-ray diffractogram of optimized formulation SF3 showed some peaks at 15.281, 17.51, 19.3, 23.58 and 27.89° with reduced intensity when compared to pure drug. Absence of aceclofenac peaks in the liquisolid XRD indicated that the drug was converted from crystalline form to amorphous form and was supported by DSC.

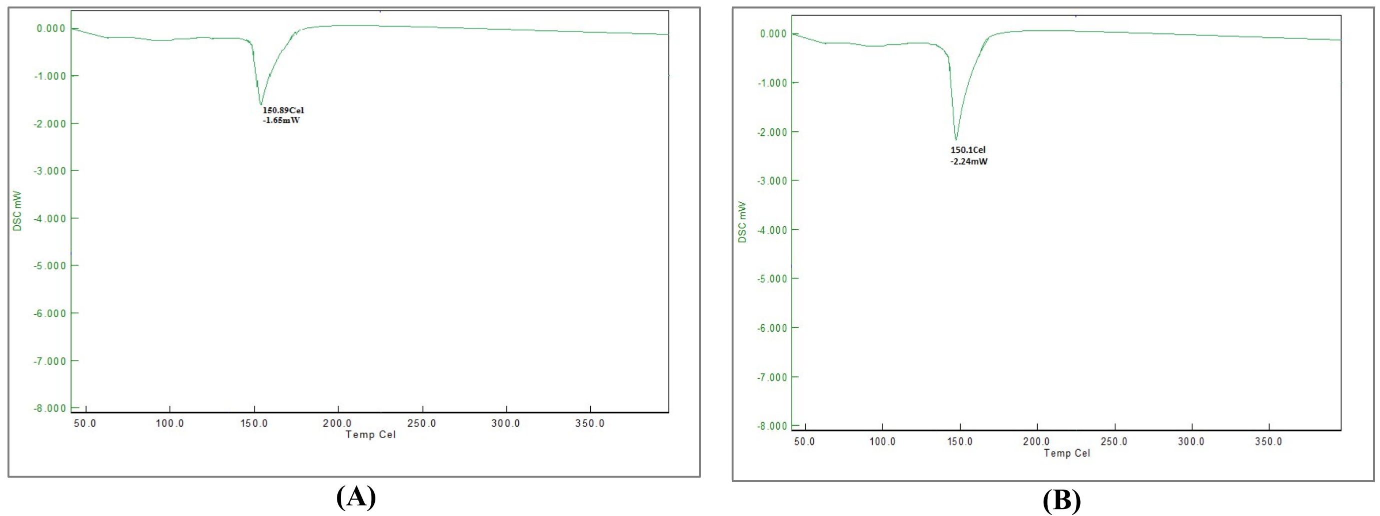


**Fig 6. DSC Chromatogram of aceclofenac (A) and optimized liposomal formulation SF3 (B).**

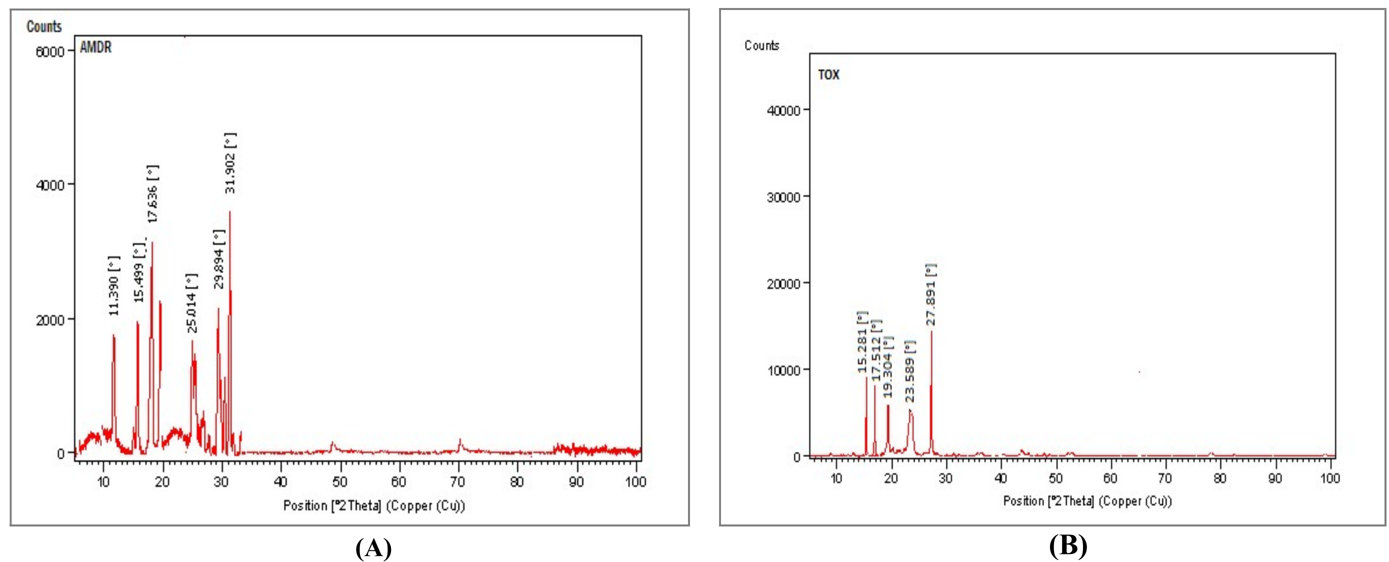


**Fig 7. XRD study data of aceclofenac (A) and optimized liposomal formulation SF3 (B).**

**Table 4. Cumulative % drug release data of the formulations TF1 to SF3 with pH 7.4 phosphate buffer.**

| Time (h) | TF1 | TF2 | TF3 | SF1 | SF2 | SF3 |
|---|---|---|---|---|---|---|
| 0 | 0.23±0.5 | 0.21±0.7 | 0.34±0.5 | 0.57±0.6 | 0.67±0.3 | 0.92±0.9 |
| 1 | 2.86±0.6 | 3.21±0.2 | 3.85±0.7 | 4.98±0.2 | 5.56±0.7 | 8.92±0.3 |
| 2 | 8.99±0.7 | 7.91±0.4 | 6.90±0.6 | 11.23±0.7 | 14.41±0.2 | 18.01±0.7 |
| 3 | 15.96±0.4 | 16.32±0.8 | 18.92±0.8 | 17.92±0.9 | 21.21±0.8 | 29.31±0.6 |
| 4 | 21.93±0.9 | 22.91±0.2 | 24.91±0.4 | 25.91±0.2 | 28.91±0.3 | 34.14±0.4 |
| 5 | 25.84±0.1 | 27.32±0.4 | 27.81±0.9 | 30.21±0.4 | 33.41±0.5 | 40.15±0.4 |
| 6 | 33.53±0.4 | 34.72±0.3 | 36.61±0.4 | 36.71±0.1 | 39.82±0.7 | 46.71±0.8 |
| 7 | 40.14±0.3 | 44.31±0.5 | 45.01±0.2 | 45.81±0.5 | 46.72±0.2 | 52.01±0.7 |
| 8 | 49.12±0.9 | 50.21±0.9 | 50.91±0.8 | 52.81±0.7 | 58.31±0.7 | 59.92±0.4 |
| 9 | 55.17±0.3 | 57.29±0.6 | 59.61±0.3 | 60.72±0.3 | 63.46±0.9 | 67.31±0.8 |
| 10 | 63.87±0.4 | 67.29±0.2 | 69.71±0.2 | 67.60±0.3 | 69.57±0.5 | 72.71±0.2 |
| 11 | 70.92±0.9 | 72.31±0.4 | 73.89±0.6 | 74.82±0.1 | 78.32±0.1 | 80.82±0.8 |
| 12 | 77.63±0.2 | 81.71±0.2 | 84.01±0.2 | 87.92±0.9 | 89.93±0.2 | 94.92±0.9 |

**Where all the values are presented as mean ± SD (n=3). TF1, TF2, TF3 - Liposome with Tween 80, SP1, SP2, SP3- Liposomes with span 80.**

**Table 5. The FTIR-Interpretation data of Aceclofenac and optimized liposome formulation.**

| Sample | Functional groups (1/cm) | | | | | |
|---|---|---|---|---|---|---|
| | OH Alcoholic | -CH- Aromatic | C=O aldehyde | N-H amine | C=C (Aromatic rings) | C=C (Alkenes) |
| Aceclofenac | - | 2972.6 | 1714.6 | 3317.3 | 1589.7 | 1654.9 |
| Lecithin | 3591.3 | 2916.2 | 1735.1 | 3367.6 | - | - |
| Tween 80 | - | 2857.0 | 1735.1 | - | - | - |
| Span 80 | 3570.8 | 2926.0 | 1701.5 | 3438.5 | 1541.3 | - |
| Optimized formulation | 3589.4 | - | 1733.2 | 3336.0 | - | 1636.3 |

***In-vitro* drug release:**

An *in-vitro* drug release study was performed using Franz diffusion cells. All the formulations prepared with Span 80 and Tween 80 were studied to determine the release profile of the drug by modified thin film method at pH 7.4 phosphate buffer. The *In-vitro* drug release data is given in Table 4 and Fig 8. All liposomal suspension released drug in sustained manner that is almost all drug liposomal formulation released the drug in satisfactory manner that is the formulation TF1, TF2, TF3, SF1, SF2 and SF3 released the drug 77.63, 81.71, 84.01, 87.92, 89.93 and 94.92 % respectively.

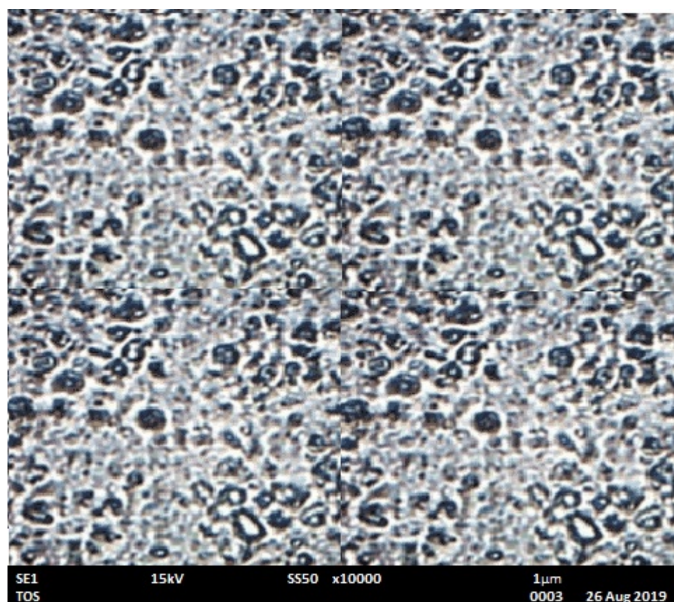


**Fig 10. SEM photographs of Optimized formulation SF3.**

**FTIR studies:**

The FTIR spectrum of pure drug aceclofenac Fig 5. The figure shows characteristics bonds at 3317.3 $cm^{-1}$ (N-H stretching), 1714.6 $cm^{-1}$ (CO stretching), 2972.6 (C-H stretching), 1589.7 $cm^{-1}$ (Skelton vibration of aromatic CC stretching), 1654.9 $cm^{-1}$ (C=C Alkenes), 1256.1 $cm^{-1}$. The literature reported the similar FTIR values for pure drug aceclofenac. These results confirm that the drug aceclofenac is pure, FTIR was done for remaining excipients of research study represented in Table 5 and Fig 9.

**Surface morphology**:

Surface morphology of liposomes was determined and SEM images obtained were represented in Fig 10.

**Evaluation of Liposomal gel:**

Optimized formulation (SF3) was added to the preformed gel base of different strength represented in Table 6. The aceclofenac liposomal gels were successfully prepared as represented in Fig 10.

***Determination of pH*:**

The pH of gels is given in Table 7. The pH was found within the skin pH range, demonstrating that the manufactured aceclofenac liposomal gel would be non-toxic, non-irritant and compatible to the skin.

**Table 6. Composition of different liposomal gels by using carbopol gel base.**

| Optimized formula | Formulation code | Carbopol-934 (%) |
|---|---|---|
| SF3 | TG1 | 0.5 |
| | TG2 | 1.0 |
| | TG3 | 2.0 |

**Liposomal gel formulations- TG1, TG2 and TG3.**

**Table 7. The pH and drug content of liposomal gel formulations.**

| LGF | DC (%) | pH |
|---|---|---|
| TG1 | 80.02 | 6.5 |
| TG2 | 95.28 | 6.8 |
| TG3 | 90.08 | 6.9 |

**LGF - Liposomal gel formulation.**

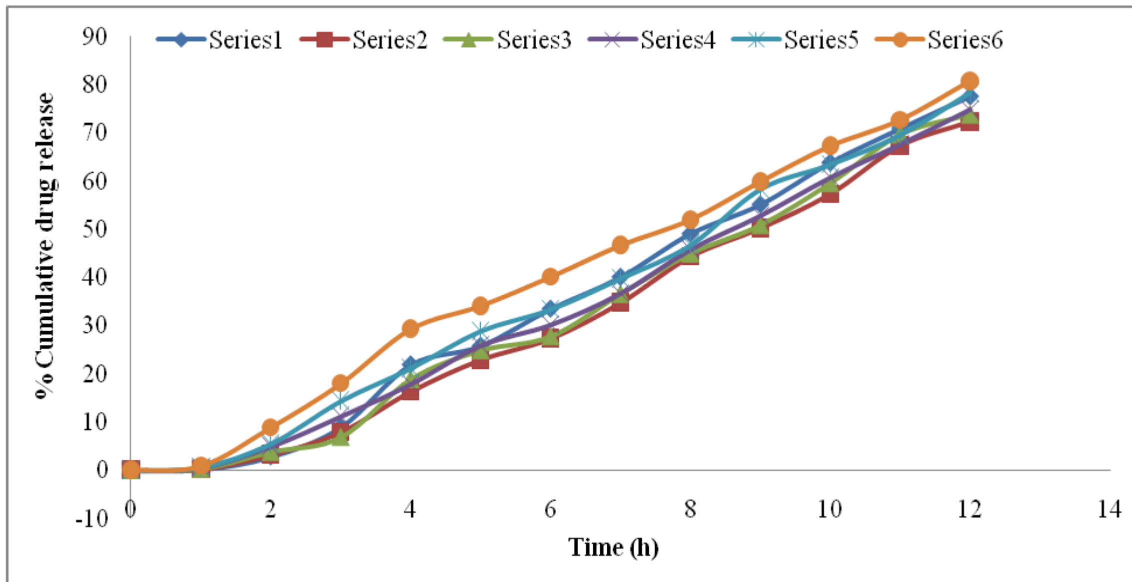


**Fig 8. Cumulative % drug release formulations TF1 to SF3 IN pH 7.4 phosphate buffer. Series 1 to 6 corresponds to TF1, TF2, TF3, SF1, SF2 and SF3.**

**Fig 9. The FTIR chromatogram of pure drug Aceclofenac (A), Tween 80 (B), phosphotidyl choline (C), Span 80 (D) and optimized liposome formulation (E).**

***Homogeneity*:**

The prepared aceclofenac liposomal gel was found to be homogeneous as evident from Fig 11. No such gritty foreign particle was found in the gel.

**Fig 11. Liposomal gel.**

***In-vitro drug release*:**

The *in vitro* drug release data is presented in Table 8 and Fig 12. The study revealed that all three gel formulations released the drug in a more controlled and constant manner. In 12 h of study the gels TG1, TG2 and TG3 released drugs 70.62, 84.11 and 73.09 % of drugs were released.

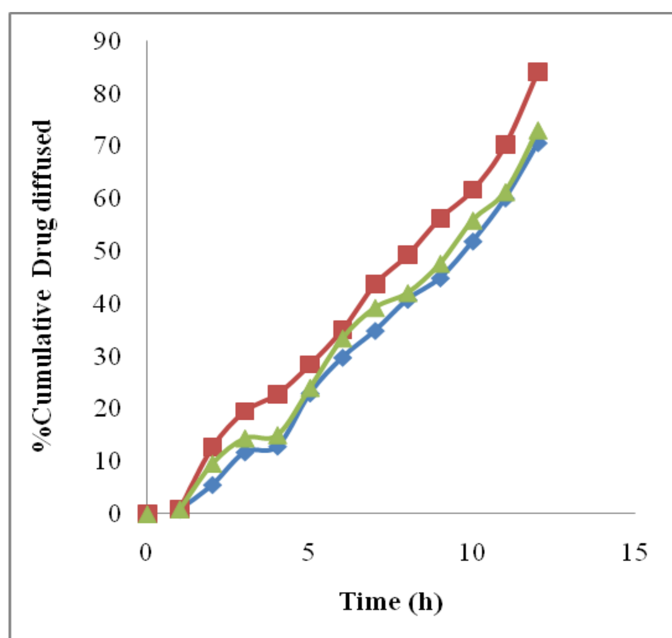


**Fig 12. Cumulative % drug release Aceclofenac liposomal gel formulations TG1- TG3 in phosphate buffer.**

**Table 8. The cumulative % drug release data of the liposomal gel formulations TG1 to TG3 with pH phosphate buffer.**

| Time (h) | TG1 | TG2 | TG3 |
|---|---|---|---|
| 0.5 | 0.65±0.3 | 0.93±0.5 | 0.73±0.2 |
| 1 | 2.72±0.4 | 7.32±0.5 | 5.01±0.2 |
| 2 | 6.55±0.3 | 10.72±0.5 | 9.61±0.4 |
| 3 | 11.76±0.5 | 15.54±0.3 | 14.43±0.3 |
| 4 | 14.86±0.7 | 22.85±0.6 | 17.06±0.7 |
| 5 | 22.97±0.8 | 28.45±0.5 | 24.05±0.9 |
| 6 | 29.78±1.2 | 35.09±0.9 | 33.46±1.2 |
| 7 | 34.88±1.4 | 43.74±0.8 | 39.27±1.5 |
| 8 | 40.80±1.2 | 49.47±1.3 | 42.08±0.4 |
| 9 | 44.90±0.9 | 56.26±0.9 | 47.69±0.7 |
| 10 | 51.90±0.7 | 62.63±1.5 | 55.91±0.9 |
| 11 | 60.09±1.3 | 75.22±1.2 | 61.32±1.5 |
| 12 | 70.62±0.6 | 84.11±1.3 | 73.09±1.3 |

**Where all the values are presented as mean ± SD (n=3).**

***Ex vivo drug permeability study*:**

The drug permeability coefficient data is given in Table 9. The liposomal gel of aceclofenac exhibited good permeability coefficient in comparison with liposomal suspension.

**Table 9. Flux and permeability coefficient values.**

| Samples | Flux ($mg/cm^2/h$) | $K_p$ |
|---|---|---|
| Liposomal gel | 0.017±0.01 | 0.00480032 |
| Drug suspension | 0.006±0.0004 | 0.00210011 |

***Stability studies*:**

From the stability study it was evident that the prepared gels were stable within the 3 month in different storage conditions, as the color, odour, pH and drug content was not changed significantly.

## CONCLUSION:

Aceclofenac was incorporated into liposomal formulation as an attempt to reduce the side effects that are associated with oral administration of aceclofenac and enhance its bioavailability after transdermal administration. Results had clearly shown that good entrapment efficiency, zeta potential and drug release behavior of the liposomes, were mainly dependent on concentration of edge activator and type of edge activator. Maximum drug entrapment and drug release were found in SF3 liposome formulation. Optimized SF3 formulation was incorporated into different

concentrations of gel bases and out of those formulations TG2 was found to have good spreadability coefficient, pH, drug content and drug release and had also shown good flux values. Thus the aceclofenac liposomal gel could be successfully use for topical delivery of aceclofenac for safe and effective management of any shorts of pain and arthritis.

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