PREPARATION AND CHARACTERIZATION OF NIOSOMAL GEL OF LIDOCAINE HCL FOR TRANSDERMAL IONTOPHORESIS

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Abstract

Nonionic surfactant vesicular systems, otherwise known as niosomes, are a novel and efficient approach to drug delivery. Their vesicular membrane is mainly composed of nonionic surfactants, cholesterol and charge inducer. I have selected Lidocaine HCl as drug candidate after extensive literature survey because Lidocaine HCl provide efficient anesthesia of the skin or of mucosal tissues in case of pain itching and burning associated with cutaneous inflammatory response to different agents and with minor surgical operations. But permeability of Lidocaine HCl through skin i.e. stratum corneum is very low, hence attempt made to improve the permeation of Lidocaine HCl across skin by synergistic effect of niosomes & iontophoresis. Niosomes also protects drug degradation which occurs due to electric current during iontophoresis. No niosomal system with iontophoresis for Lidocaine HCl has been formulated for local dermal anesthesia. In this work, niosomes are prepared by Rotary evaporation method & ether injection method, best method will be selected depending on entrapment efficiency & vesicle size. Different surfactants (Tweens and Spans) screened to select best surfactant depending on entrapment efficiency and vesicle size, span 60 optimized as best surfactant. 32 full factorial design was used to optimize the formula, the optimized batch was evaluated for Optical microscopy, vesicle size analysis and PDI, entrapment efficiency, in vitro release study, SEM analysis, zeta potential analysis and DSC study & best batch (F5) formulated in to gel. Permeation experiments for niosomal dispersion, simple marketed gel (Lignocaine HCl gel 2% I.P. i.e. LOX 2% Gel) & niosomal gel with or without iontophoresis using cellophane membrane was carried out and it shows that, niosomes and iontophoresis have synergistic effect on permeation flux. While performing iontophoresis of marketed gel it was observed that there is reduction in permeation of drug after 3 hr, it might be due to drug degradation due to electric current. To confirm this degradation, study was carried out on marketed gel and niosomal gel and results shows that niosomes offers protection of drug from electric current during iontophoresis.

Keywords: Niosomes, Transdermal iontophoresis, Permeation flux, etc

1. Introduction

Transdermal drug delivery offers benefits over other routes of administration, e.g., avoidance of hepatic first-pass metabolism, fewer side effects, and improved patient compliance. However, stratum corneum plays the major role in regulating the barrier function of the skin because of its unique nature, which creates an interstitial lipoidal environment. Several techniques have been developed to overcome this barrier including chemical (penetration enhancers) and physical techniques (iontophoresis, electrophoresis, and sonophoresis) or combination of both. The iontophoresis is a non-invasive technique which provides simplified therapeutic regimen (drug input kinetics can be modulated by the current profile) and leads to improved patient compliance. It is a promising delivery technique for the charged and uncharged molecules having high and low molecular weights.

The combination of novel carrier and iontophoresis could be utilized to synergize the delivery of drugs vis-à-vis to decrease the iontophoretic degradation of the free drug (Sanyog J et al. 2012). In similar type of approaches, Kulkarni et al. showed that encapsulation of neutral colchicine into positively charged liposomes significantly enhanced the iontophoretic flux by two- to threefolds. However, liposomes as a drug carrier suffer from certain disadvantages like chemical instability (phospholipid hydrolysis and fatty acid oxidation), variable purity of phospholipids and relatively higher cost. Niosomes, the nonionic surfactant-based vesicles that are essentially similar in properties to liposomes have been proposed as an alternative to liposomes. Niosomes are more stable and free from other shortcoming of liposomes. Recently, the transdermal delivery of certain drugs using niosomes has been envisaged and niosomes have proved to be superior transdermal nanocarriers.[13]

In the present study, an attempt has been made to enhance transdermal delivery of Lidocaine Hydrochloride by formulating it into niosomal gel which was further facilitated by application of iontophoresis. Both niosomes and iontophoresis enhance transdermal permeation by two different mechanisms and combination of both was thought to have synergistic effect that resulted in higher transdermal flux of Lidocaine Hydrochloride. Encapsulation in niosomes cannot only protect the drug from skin metabolism but also from degradation due to application of current during iontophoresis[10,1]

2. MATERIALS & METHODS

2.1 Raw material & chemical

The drug lidocain HCL kindly obtained from Cipla, Kurkumbh, Stearlyamine from TCI, and other chemical from Research Lab ,Mumbai.

2.2 Preformulation study

2.2.1 Characterization of lidocaine hydrochloride

2.2.1.1 FTIR spectroscopy

The FTIR spectrum of Lidocaine hydrochloride was recorded using FTIR spectrophotometer (Shimadzu, Japan) by KBr pellet technique. IR spectrum was recorded in the wavelength range 400 – 4000 cm^{-1} (Shimadzu, Japan).

2.2.1.2 DSC study

DSC thermogram of pure drug Lidocaine HCl was taken.

2.2.2 Drug excipients compatibility study[3]

2.2.2.1 FTIR spectroscopy

Drug excipients compatibility testing was performed by mixing drug with excipients in equal proportion. The drug polymer interaction was studied using FT-IR. Individual IR spectra of drug and combination of drug and excipients were taken.

2.2.2.2 Differential Scanning Calorimetry:

DSC studies were performed for pure drug-Lidocaine Hydrochloride and drug with physical mixture. The DSC measurements were performing on a DSC-differential scanning colorimeter with thermal analyzer. All accurately weighed samples (about 10 mg) were placed in a sealed aluminium pans, under nitrogen flow (20 ml/min) at a scanning rate of 20^0C per min from 100 to 300^0C. An empty aluminium pan was used as reference.

2.3 Formulation and development

2.3.1 Preparation of Niosomes[2]

Niosomes were prepared by two different methods i.e. thin film hydration and ether injection method to select the best method for further study.

2.3.1.1 Rotary Evaporation method (Thin Film Hydration method) :

Preliminary studies were done for optimizing speed of RBF during solvent removal process.

Procedure

1. Weighed quantity of non-ionic surfactant and Cholesterol were dissolved in 30 ml of solvent i.e. Chloroform &

methanol in 3:1 to form clear solution in 250ml round bottom flask.

2. Then by using rotary evaporator organic solvent was evaporated at 40^0c for 15 min.
3. After selection of suitable temperature, the film was hydrated with 20 ml distilled water containing drug at 60^0c at 50 rpm for 30 min to form white dispersion.
4. Then the solution was sonicated for 10 min. in bath sonicator.
5. Then the dispersion was kept overnight at room temperature for complete vesiculation.

2.3.1.2 Ether Injection

Preliminary studies were done for optimizing speed of magnetic stirrer to get a good Niosomal dispersion. Table 2.1: show three different formulations by Rotary Evaporation and Ether Injection method

Procedure

1. Weighed quantities of Soya lecithin and cholesterol were dissolved in 10 ml of diethyl ether to form a clear solution.
2. Drug was dissolved in 20 ml of distilled water and it was heated at 55-60^0c.
3. The lipid solution was added to drug solution slowly through 14 gauge needle with maximum speed continuous stirring.
4. Then the solution was sonicated for 10 min. in bath sonicator.
5. Then the dispersion was kept overnight at room temperature for complete vesiculation.

2.3.2 Selection of Method & Surfactant:

The best method was selected which showed greatest entrapment efficiency among all and also have optimum vesicle size.

Span-60 gives the highest entrapment efficiency and niosomes of optimum vesicle size was obtained, hence selected for further study.

2.4 Optimization of Formulation [1,2]

Film hydration method was selected for further study. To study the effect of variables on Niosome characteristics, different batches were prepared using 3^2 factorial design approaches. Amount of non-ionic surfactant and cholesterol were selected as two independent variables. Entrapment efficiency (EE) and % drug permeated were selected as dependent variables. Experimental trials were performed at 3 possible combinations higher, lower and middle. The resulting data were fitted into Design Expert® 8.0.1 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of non- ionic surfactant and cholesterol on dependent variables. The probable formulations using 3^2 factorial designs are shown in tables 2.2, 2.3, 2.4.

2.4.1 Determination of entrapment efficiency and % drug permeation[8]

2.4.1.1 Entrapment efficiency

The Niosomal suspension was centrifuged (Remi) at 5000 rpm for 30 min. Supernatant was removed and methanol was added to residue and sonicated for 10 min. to disrupt the Niosomes. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 262 nm. The entrapment efficiency was then calculated using following equation,

$$\text{Entrapment Efficiency} = \frac{\text{Entrapped Drug}}{\text{Total drug added}} \times 100$$

2.4.1.2 % Drug permeation

A Franz diffusion cell was used for diffusion studies. Cellophane membrane was mounted on the diffusion cell. The donor phase was consisting of 5 ml of F5 niosomal dispersion. The receptor compartment was consisting of 15 ml of Phosphate buffer saline 6.4 (PBS). The solution in the receptor compartment was continuously stirred at 250 rpm by means of Teflon coated magnetic bead. Samples (5 ml) were withdrawn from the receptor compartment at 30 minutes interval for a period of 4 hr. The withdrawal sample is analyzed by UV spectrophotometer at 262 nm. Fresh Phosphate buffer saline 6.4 (PBS) was added to replace the withdrawn sample volumes.

2.5 Vesicle size and PDI determination

The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mw using a Fourier lens [R-5] to a point at the center of multielement detector and a sample holding unit (Su cell). The sample was stirred using a stirrer before determining the vesicle size. The vesicle dispersions were diluted about 100 times in the deionized water. Diluted niosomal suspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce inter particles aggregation and laser beam was focused.

2.6 Addition of charge inducer

5 % w/w of stearyl amine was added i.e. (3.125mg) to total of the surfactant and cholesterol content in order to facilitate iontophoresis and to minimize the aggregation. It was observed that, there is increase in entrapment efficiency as well as vesicle size.

2.7 Evaluation of Niosomes [2,12]

All F1-F9 formulations were evaluated for Entrapment Efficiency (EE) and % Drug permeated. Then optimization as applied based on results obtained and optimized batch were finalized. Finalized optimized batch (F5) was evaluated for following parameters.

2.7.1 Morphology by optical microscopy

The entire prepared Niosomes were observed under binocular compound microscope and motic microscope at 40X and 100x magnification for studying the vesicle size and shape.

2.7.2 Vesicle size and size distribution

The mean vesicle size and vesicle size distribution of the optimized batch was obtained by particle size analyzer (Sympatec HELOS, Germany (H1004)). The sample was stirred using a stirrer before determining the vesicle size. The vesicle dispersions were diluted about 100 times in the deionized water. Diluted niosomal suspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce inter particles aggregation and laser beam was focused.

2.7.3 Scanning electron microscopy

The morphology of the Niosomes was determined using a Scanning electron microscope (JSM-7600F, Japan). The samples were placed over a carbon paste coated stub and sputter coated with a thin layer of platinum prior to viewing.

2.7.4 Determination of zeta potential

Charge on drug loaded vesicles surface was determined using Zeta potential analyzer (Brookhaven instruments Corp. Analysis time was kept for 60 s and average zeta potential and charge on the Niosome was determined. Dispersion of Niosome in phosphate was used for analysis. Temperature was kept at 25^0C and 3 runs were carried out.

2.7.5 Differential scanning calorimetry

The phase transition temperature (Tc) of Niosomal bilayer membranes represents the rippled gel–liquid crystalline phase transition and it can be usefully measured by differential scanning calorimetry. The analyses were performed on 5 mg Niosomal samples sealed in standard aluminium pans. Thermograms were obtained at a scanning rate of 20°C/min. Each sample was scanned between 0°C to 250°C. The temperature of maximal excess heat capacity was defined as the phase transition temperature.

2.7.6 In vitro drug release studies[9]

The release of drug from Niosomal formulation was determined using the membrane diffusion technique. F5 and F9 batch was taken for comparative study. 5 ml of Niosomal dispersion was sealed in dialysis bag (Himedia) and attached to USP type II dissolution apparatus. The Niosomal dispersion was then immersed in 200 ml of PBS 6.4 at 37±0.5^{o}c at 50 rpm. The samples were withdrawn at scheduled intervals (replaced with equivalent amount of PBS pH 6.4) and analyzed for drug content by UV estimation at 274nm.

2.7.6.1 Data treatment:

In order to investigate the mode of release from Niosome, the release data were analyzed with the following mathematical kinetic models using PCP-DISSO – v2 software.

2.7.6.1.1 Korsmeyer Peppas Model: $Q=kpt^n$

Where

k_P is constant incorporating structural and geometric characteristics of the release device.

n is the release indicative of the mechanism of release.

This equation was further simplified and proposed by Ritger and Peppas

$M_t / M_{inf} = at^n$

Where, M_t / M_{inf} = fractional release of drug

a = constant depending on structural and geometric characteristics of the drug dosage form.
n = release exponent

The value of n indicates the drug release mechanism. For a slab the value n = 0.5 indicates Fickian diffusion and values of n between 0.5 and 1.0 or n = 1.0 indicate non- Fickian mechanism. In case of a cylinder n = 0.45 instead of 0.5, and 0.89 instead of 1.0. This model is used to analyze the release from polymeric dosage forms. Table: 2.5. Show Interpretation of diffusion release mechanisms from dosage forms

2.7.6 Entrapment Efficiency (EE)

The Niosomal suspension was centrifuged (Remi) at 5000 rpm for 30 min. Supernatant was removed and methanol was added to residue and sonicated for 10 min. to disrupt the Niosomes. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 262 nm. The entrapment efficiency was then calculated using following equation.

$$\text{Entrapment Efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug added}} \times 100$$

2.8 Formulation of niosomal gel

Procedure: A weighed amount of Carbopol 974 P (1% w/v) was dispersed at 500 rpm in deionized water and allowed to swell for 2 h. After the swelling, propylene glycol (5% w/v) was added and the mixture was neutralized up to pH 6.0 by dropwise addition of 10% w/v NaOH. Same procedures were followed for preparation of 0.5 % (w/v) and 2% (w/v) of carbopol gel. Niosomal residue (Approximately 170mg) of F5 batch were taken and dispersed in to 5 gm of 0.5%, 1% carbopol and 2% carbopol gel with slow speed of rotation i.e. 100 rpm.

2.9 Evaluation of niosomal gel

2.9.1 Consistency: Niosomal gels and marketed gels were checked for consistency such as fluidity, stiffy or firm mass of the gel.

2.9.2 Physical appearance and colour: Niosomal gel prepared from different carbopol conc. i.e. 0.5 % w/v, 1% w/v, 2%w/v and marketed gel were evaluated for physical appearance and colour.

2.9.3 Viscosity measurement: Viscosity of marketed gel containing lidocaine HCl (2 %w/v) and niosomal gels of different carbopol conc. i.e. 0.5,1 and 2 %w/v (containing residue of niosomal dispersion-F5) was measured using Brookfield Synchroelectric Viscometer (LVDV- II + Pro) with spindle no. 52 at 25^0C at 20 rpm.

2.9.4 pH determination: 2.5 gm of the niosomal gel were accurately weighed and dispersed in 25 ml of PBS 6.4. Then the pH of the dispersion was measured by using digital pH meter.

2.9.5 Drug content uniformity: Drug content uniformity of niosomal gel was determined by analyzing the drug concentration in the sample taken from four different points. The gel samples (2.5 gm) were dissolved in 50 ml PBS (pH 6.4) and stirred at 500 rpm to facilitate rupture of the vesicles. Drug content was determined using UV spectrophotometer at 262 nm.

2.9.6 Microscopic evaluations: Microscopic evaluations were performed in order to seen the any aggregation or lump formation after incorporating niosomal residue in to the carbopol gel.

2.10 Iontophoresis study

Generally the iontophoresis is classified in to anodal iontophoresis and cathodal iontophoresis. But, while preparation of the niosomes, the stearyl amine was used as positive charge inducer in order to minimize the aggregation and to facilitate iontophoresis. Hence anodal iontophoresis was used to observe the effect of iontophoresis on permeation.[6] 2.10.1 In-vitro Anodal trans-dermal Iontophoretic permeation study of continuous current

A Franz diffusion cell was used for diffusion studies. Cellophane membrane was mounted on the diffusion cell. Current of 1 mA was used to observe the effect of iontophoresis on the permeation rate. The donor phase was consisting of 5ml of niosomal dispersion. The receptor compartment was consisting of 15 ml of Phosphate buffer saline 6.4 (PBS). A constant direct current of 1 mA was applied using silver–silver chloride electrode. Silver wire of 4.0 cm was used as the anode and silver– silver chloride wire of 2.0 cm was used as the cathode. The anode was dipped in the donor solution and the cathode in the receptor solution, which was stirred using a Teflon-coated magnetic stirrer at 250 rpm. Samples (5ml) were withdrawn from the receptor compartment at 30min.interval for a period of 4 hr. The withdrawal sample is diluted suitably with PBS 6.4 and analyses by UV spectrophotometer at 262 nm. Fresh PBS 6.4 was added to replace the withdrawn sample volumes.

2.10.2 In vitro Anodal iontophoretic permeation studies of Lidocaine HCl to optimize current density

Optimization of the current density is essential for the maximum permeation of drug because the permeation of drug was directly affected by amount of current applied. So that permeation studies were carried out at three different current density 0.5, 1 and 1.5 mA /cm^2. The donor phase was consisting of 5ml of niosomal dispersion. The receptor compartment was consisting of 15 ml of Phosphate buffer saline 6.4 (PBS). A constant direct current of 1 mA was applied using silver–silver chloride electrode. Silver wire of 4.0 cm was used as the anode and silver– silver chloride wire of 2.0 cm was used as the cathode. The anode was dipped in the donor solution and the cathode in the receptor solution, which was stirred using a Teflon-coated magnetic stirrer at 250 rpm. Samples (5ml) were withdrawn from the receptor compartment at 30min.interval for a period of 4 hr. The withdrawal sample is diluted suitably with PBS 6.4 and analyses by UV spectrophotometer at 262 nm. Fresh PBS 6.4 was added to replace the withdrawn sample volumes. Same was carried out at different current density of 1 mA/cm2 and 1.5 mA /cm2 for optimization.

2.10.3 In vitro permeation studies of Lidocaine HCl to study the effect of pulse current[5]

This study was done by using the pulsatile current (1mA/cm2) instead of continuous current in the ON: OFF ratio of 1:1, 1:2, 2:1, 3:1, 4:1. Remaining study was same as above.

2.11 Iontophoretic permeation comparison study:

Permeation study of 1 % Niosomal gel, Niosomal dispersion (F5) and Marketed gel was carried out in order to observe the comparative effect of iontophoresis on the permeation flux. Niosomal dispersion has slightly high permeation rate than the Niosomal gel, it confirms that the formulating niosomal dispersion in to the gel doesn't have much more influence on the permeation rate. While performing iontophoresis study of marketed gel, it was observed that there is decrease in drug permeation after 3 hr of iontophoresis treatment & this effect was observed might be because of drug degradation due to electric current. To confirm this, drug degradation study was carried out.

2.12 Drug degradation comparative study of marketed gel & Niosomal gel:

2.12.1 Drug degradation study of marketed gel: Significant reduction was noted in the amount of drug permeated (marketed gel) after 3 hr with iontophoresis.

Procedure-

1) 20 ml of marketed gel (1gm) dispersion in PBS 6.4 was directly exposed to current 1 mA and pulse current (3:1) by dipping the anodic electrode into the it.
2) After 3 hr of current application, the gel dispersion was analyzed for the drug content and it was observed that the drug content was reduced from 100% to 74.72%.

2.12.2 Drug degradation study of niosomal gel:

A similar experiment was also performed in presence of lidocaine hydrochloride loaded cationic niosomes.

Procedure –

1) 5 gm of niosomal gel dispersed in 20 ml of PBS 6.4 and exposed to current 1 mA and pulse current (3:1) by dipping the anodic electrode into the it, after 3 hr 80 ml of methanol added to rupture the vesicles.
2) Solution was filtered and then absorbance was taken.

It was found that drug content was reduced from 100 to 94.73 % after 3hr of current application, which indicates that niosomes offer protection to the drug from degradation due to current during iontophoresis.

2.13 STABILITY STUDY

It is the responsibility of the manufacturers to see that the medicine reaches the consumer in an active form. So the stability of pharmaceuticals is an important criterion. Wherever possible, commercial pharmaceutical products should have a shelf-life of 3 years. The potency should not fall below 95% under the recommended storage conditions and the product should still look and perform as it did when first manufactured.

Niosome stability can be subdivided into physical, chemical and biological stabilities, which are all inter-related. Generally, the shelf-life stability of Niosomes is determined by the physical and chemical stability (Uniformity of size distribution and encapsulation efficiency, and minimal degradation of all compounds, respectively).Since the period of stability testing can be as long as two years, it is time consuming and expensive. Therefore it is essential to devise a method that will help rapid prediction of long-term stability of dosage form.

The accelerated stability testing is defined as the validated method by which the product stability may be predicted by storage of the product under conditions that accelerate the change in defined and predictable manner.

Stability testing of formulation batch was carried out using ICH guidance Q1AR Stability Testing of New Drug Substances and Products to determine the stability of drug and carrier. And also to determine the physical stability of formulation under accelerated storage condition. The prepared Niosomal suspensions were placed in glass vial containers. The samples were kept at condition of refrigeration temperature (2-8°C), room temperature (25±2°C/60%±5%RH) and 45°C/75%±5%RH for a period of 1 month for their changes in:

- Appearance
- Entrapment efficiency
- Vesicle size

2.13.1 Permeation parameters calculations[7]

Steady state flux (Jss)

The cumulative amount of drug permeated per unit skin surface area plotted against time and the slope of the linear portion of the plot is estimated as steady-state flux (µg/cm2/hr).

Cumulative amount of drug permeated:

It was calculated by using following equation. Cumulative amount of drug permeated was calculated at the end of 4th hr. was given as Q4.

Total amount of drug permeated

Cumulative amount of drug permeated = ___________________________

Area of Permeation

Permeability coefficient (Kp)

It is calculated by following equation.

KP = JSS / Cd

Where:

KP = Permeability coefficient.

Jss = Steady State flux.

Cd = Initial concentration of drug in donor compartment.

3. RESULTS AND DISCUSSION

3.1 Preformulation Study

3.1.1 Characterization of LidocaineHCl

3.1.1.1 FTIR Spectroscopy

The results of FTIR spectrum of drug is given in Fig. 3.1.and the principal peaks in Table 3.1.

3.1.1.2 Differential Scanning Calorimetry analysis (DSC)

The DSC thermogram of given LidocaineHCl is shown in Fig. 3.2.The onset temperature is as reported in graph. The melting point of FF was 76-79 ^{0}C and DSC thermogram of LidocaineHCl shows endothermic melting peak at 84.29 ^{0}C.

3.1.2 Drug-Excipients compatibility study

Compatibility of excipients with the drug was confirmed by 1) FTIR study and 2) DSC study.

3.1.2.1 FTIR study-

FTIR spectra of drug and excipients was taken, it was observed that the all principal peaks shown by pure drug were observed in physical mixture so it confirms that there is no interactions in between drug and excipients. Show in Fig.3.3

3.1.2.2 DSC study-

The DSC thermogram of given drug + excipients is shown in Fig. 3.4. DSC thermogram of physical mixture shows endothermic peak at 75.39 ^{0}C and endothermic peak shown by LidocaineHCl at 84.31 ^{0}Ci.e. slight shifting of peak from 84.31 ^{0}C to 75.39 ^{0}C indicates that there is no interactions in between drug and excipients.

3.2 Formulation and development

Preparation methods of Niosomes and method selection: Niosomes prepared by two methods Rotary Evaporation method (Thin film hydration technique) and Ether injection method by using span 60 as non-ionic surfactant. Both the methods are compared on the basis of entrapment efficiency and particle size and it was found that the Rotary evaporation method gives best result as compared to Ether injection method, hence selected for further study. The results of the both the methods are given in following table 3.2.

3.2.1 Optimization of Rotary evaporation method-

3.2.1.1 Speed of rotation

At the **100 rpm** uniform film is formed, so this speed was selected for further study. Table 3.3

3.2.1.2 Amount of drug

It was observed that by increasing amount of drug added from 80mg to 100mg there was increase in entrapment efficiency. But addition of drug quantity above 100mg leads to slight increase in entrapment efficiency, so to avoid wastage of drug and to minimize drug leakage from the vesicle 100mg drug quantity had been fixed Table 3.4.

3.2.2 Screening of surfactants:

There are number of non-ionic surfactants available for the preparation of niosomes, among these tween 20, tween 40, tween 60 (Tweens) and span 20, span 40, span 60 (Spans) were screened to select best non-ionic surfactant depending on entrapment efficiency and vesicle size. The span 60 given the best results as compared to other tweens and spans, hence selected for further study. The results reported in following tables 3.5 & 3.6.

3.3. Optimization of formulation

Concentration of surfactant and cholesterol were found to be critical in preparation and stabilization of niosomes and hence both were selected as independent variables in the 3^2full factorialdesigns**.** Niosomes were prepared using thin film hydration technique and method was found to be well suited for the production of niosomes without aggregation as compared to ether injection method. Responses of different batches were obtained by using factorial design. Batches were formulated as per Table 2.4. in experimental part and evaluated mainly for entrapment efficiency and % drug permeated. These formulations also evaluated for vesicle size and PDI.

Niosomeswere prepared using film hydration technique and method was found to bewell suited for the production of niosomes without aggregation. Responses of different batches were obtained by using factorial design**.** table 3.7. shows Optimization batches of formulation F1-F9by3^2full factorial design Obtained data were subjected to multiple regression analysis using Design Expert® 0.8 Software and obtained data were fitted in following equation

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$$

Where Y is the dependent variable; b0 is the arithmetic average of all the quantitative outcomes of nine runs. b_1, b_2, b_{12} are the estimated coefficients computed from the observed experimental response values of Y and X_1 and X_2 are the coded levels of the independent variables. The interaction term (X_1X_2) shows how the response values change when two factors are simultaneously changed.

Statistical validity of the polynomials was established on the basis of analysis of variance (ANOVA) provision in the Design Expert software. Level of significance was considered at $p < 0.05$. The best-fitting mathematical model was selected based on the comparison of several statistical parameters, including the coefficient of variation (CV), the multiple correlation coefficient(R^2), the adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of squares(PRESS), provided by the software. PRESS indicates how well the model fits the data, and for the chosen model, it should be small relative to the other models under consideration. The 3-D response surface graphs and the 2-D contour plots were also generated by the Design Expert® software. These plots are very useful to see interaction effects of the factors on responses.

3.3.1 Entrapment Efficiency-

Entrapment of LidocaineHCl in niosomes was determined using the method elaborated in experimental work. Table 3.8. shows the result of theses determination. The percentage entrapment efficiency of different niosomal batches by 3^2 full factorial designs were found to be between ranges of 57.34 to 72.6. The maximum entrapment was observed in batch F5 i.e. 72.6 %.To understands the effect of surfactant and cholesterol concentration on entrapment efficiency (EE) fitted in equation. Fig: 3.5.show Entrapment efficiency (EE) of F1-F9 batches.

Final Equation in Terms of Coded Factors

Entrapment Efficiency =+71.61+ 0.96 x A-1.18 x B- 0.42 x A x B-6.16 x A^2 - 6.19 x B^2

Final equation in terms of **actual factors**

Entrapment efficiency =-52.50222 + 27.24333 x Conc of surfactant +196.56000 x Conc of cholesterol - 1.66000 x Conc of surfactant x Conc of cholesterol - 6.15667 x Conc of surfactant^2- 98.98667 x Conc of cholesterol^2.

3.3.1.1 Effect of Surfactant amount on entrapment efficiency:

Surfactant is an important component in the formation of niosomal vesicles and the variation in the concentration may affect the entrapment efficiency. In the present study, when the concentration of Span 60 was varied from 43mg to 129 mg, the maximum and minimum entrapment efficiency were found to be 72.60 % and 57.34 %, respectively. The data shows that the variation in the concentration of surfactant from 43mg to 86mg showed a significant increase in the entrapment efficiency, whereas the further increase in concentration from 86mg to 129mg decreased the entrapment efficiency. Initial increase in the concentration of surfactant may increase the number of niosomes formed; therefore, the volume of hydrophobic domain increases and hence increases in entrapment efficiency. However, the further increase in concentration showed decrease in entrapment efficiency, possibly due to formation of mixed micelles along with the niosomal vesicles with high concentration of surfactants, which may lead to lower entrapment efficiency. It is reported that size of micelles < 10nm, thus fewer amounts may be entrapped inside the vesicles. It may be that due to these reasons, it forms vesicles with low entrapment efficiency.

3.3.1.2 Effect of Cholesterol amount on entrapment efficiency:

The concentration of cholesterol plays an important role in the entrapment of drug in the vesicles. The variation in the concentration of cholesterol significantly affects the entrapment efficiency ($p<0.05$). The observed entrapment efficiency was increased significantly when cholesterol amount was increased from 28.99mg to 38.66mg, but further increase in the cholesterol decreased the entrapment efficiency.

The increase in entrapment efficiency shows that the cholesterol, which acts as the “vesicular cement” in the molecular cavities of surfactant bilayer, and abolishes the gel to sol transition, thereby forms less leak vesicles. Therefore, the increase in the rigidity decreases the permeability of the entrapped drug and hence improves the entrapment efficiency. However, when cholesterol amount was increased further from 38.66mg to 48.32mg, the opposite result occurred. The reason behind decreased entrapment efficiency may be due to the reason that a cholesterol molecule will compete with drug for the space within the bilayer, remove the drug from the bilayer and in addition to this will disrupt the vesicular membrane structure. figure 3.6 shows entrapment efficiency v/s conc. of Surfactant and conc. of Cholesterol.

Model summary statistics shows the appropriate model for data treatments. Following table 3.9.gives details related to standard deviation and R^2 value. Data were analyzed Statistically by one-way analysis of variance (ANOVA) using software.Table 3.10.shows ANOVA for response surface quadratic model.The Model **F-value 15.62** implies the model is **significant.** There is onlya 2.34% chance that a "Model F- Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.

3.3.2. % Drug permeated: The % of drug permeation of LidocaineHCl through cellophane membrane was determined by method elaborated in experimental part**.** The increased permeation flux due to increase in surfactant concentration may be due to the non-ionic surfactant present in it, which modifies the structural composition of stratum corneum and increases the thermodynamic activity of the drug as well as skin vesicular partitioning. The following table 3.11. & Fig 3.7., 3.7a ,3.7b and3.7c shows drug permeation profile of F1-F9 batches.

Model summary statistics shows the appropriate model for data treatments. Following table 3.12.gives details related to standard deviation and R^2 value.

To understand the effect of surfactant concentration on % drug permeated, coefficient observed for % drug permeation fitted in Eq.

Final equation in terms of coded factors

% Drug permeated =+59.95 +3.87 x A **-**0.76 x B Final equation in terms of actual factors

% Drug permeated =+ 55.23444 + 3.87333 x Conc of surfactant **-** 3.03333 x Conc of cholesterol.

Data were analyzed statistically by one-way analysis of variance (ANOVA) using software. Following table 3.13.shows ANOVA for response surface linear model. The Model **F-value of 46.73** implies the model is **significant.** There is only a 0.18% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. Fig: 3.8. show graphical representation of effect of factors on % Drug permeated by using response surface plot.

3.4 Vesicle size and PDI determination:

The vesicle size and size distribution of niosomes containing LidocaineHCl were determined using method described previously in experimental part.

Niosomes shows the vesicle sizes of all batches, are in between 985.15 nm to 1484.6 nm and PDI of all batches, which are in between 0.201 to 0.378. These were shown in figure no 3.9,3.10,3.11,3.12,3.13 ,3.14,3.15,3.16,3.17.

3.5 Addition of charge inducer

Positive charge inducer (stearyl amine-5%) was added in to the optimized formulation F5 to minimize the aggregation of the vesicles and for the purpose of iontophoresis. After addition of charge inducer, it was observed that there is increase in vesicle size (From 1400.97nm to 2113.07nm) as well as entrapment efficiency (From 72.6 % to 74.84%) but improvement in PDI (From 0.201 to 0.150). These were shown in fig 3.18

3.6 Evaluation of optimized formulation

3.6.1 Morphology by Optical microscopy

Niosomes were studied under 40X and 100X by optical microscopy (moticmicroscope).Under 100X magnification multilamellar vesicles were seen clearly. fig. 3.19 a & b shows images of niosomes:

3.6.2 Vesicle size analysis and PDI:

Vesicle size distribution curve of optimized batch show in fig 3.20

3.6.3 SEM analysis

The scanning electron micrograph of optimized formulation of niosomal dispersion shows spherical morphology and size in the nanodimensions in fig 3.21.

3.6.4 Zeta potential analysis:

The value of zeta potential was found to be 28.77 mV for optimized batch. It indicates prepared niosomes have sufficient surface charge to prevent aggregation of the vesicles. **In fig 3.22** and also Zeta potential measurement parameters in table 3.14

3.6.5 DSC study:

DSC results of freeze dried formulation shows that the absence of endothermic peak or shifting of peak of pure drug so it indicates that there is significant interaction between niosomes bilayer and pure drug and drug entrapped within the vesicle. The shifting ofpeak from 84.31 ^{0}C to 115.17 ^{0}C was observed, which is shown in following fig. 3.23.

3.6.6 In vitro release study

F5 and F9 showed sustained release of drug up to 6 hrs. F9 showed more sustained release than F5 due to more cholesterol content. Show in fig 3.24

3.6.6.1 Data treatment

Data treatment to drug release from F5 formulation showed that it followed **Korsmayer- Peppas** model.in fig. 3.25.shows drug release profile of optimized formulation. And Table: 3.15.show Drug release kinetics from LidocaineHCl niosomes

3.7 Formulation And Evaluation Of Nisomal Gel

3.7.1.Formulation of Niosomal gel:

Procedure: A weighed amount of Carbopol 974 P (1% w/v) was dispersed at 500 rpm in deionized water and allowed to swell for 2 h. After the swelling, propylene glycol (5% w/v) was added and the mixture was neutralized up to pH 6.0 by dropwise addition of 10% w/v NaOH.Same procedures were followed for preparation of 0.5 % (w/v) and 2% (w/v) of carbopol gel.Niosomal residue (Approximately 170mg) of F5 batch were taken and dispersed in to 5 gm of 0.5%, 1% carbopol and 2% carbopol gel with slow speed of rotation i.e. 100 rpm.

3.7.2. Evaluation of Niosomal gel:

3.7.2.1 Consistency:

Carbopol gel prepared with 0.5% w/vcarbopol was not of good consistency, it was fluidy in nature. Whereas gel with 2% w/vcarbopol was stiff. Carbopol gel (1% w/v) was found to be of good consistency. Hence 1 % carbopol gel was used to make niosomal gel.

3.7.2.2 Appearance and colour

Table: 3.16.show Appearance and colour of different gel formulations

3.7.2.3 Viscosity measurement:

Viscosity of marketed gel containing lidocaineHCl (2 %w/v) and niosomal gels of different

carbopol conc. i.e. 0.5,1 and 2 %w/v (containing residue ofniosomal dispersion-F5) was measured

using Brookfield Synchroelectric Viscometer (LVDV-II + Pro) with spindle no. 52 at 25^0C at 20 rpm.

Show in table 3.17

3.7.2.4 pH determination:

2.5 gm of the niosomal gel were accurately weighed and dispersed in 25 ml of PBS 6.4.Then the pH of the dispersion was measured by using digital pH meter. The pH of the gel were noted in the following table 3.18.

3.7.2.5 Drug content uniformity:

Drug content uniformity of niosomal gel was determined by analyzing the drug concentration in the sample taken from four different points. The gel samples (2.5 gm) were dissolved in 50 ml PBS (pH 6.4) and stirred at 500 rpm to facilitate rupture of the vesicles. Drug content was determined using UV spectrophotometer at 262 nm. show Table: 3.19.

3.7.2.6 Microscopic evaluations:

Microscopic evaluations were performed in order to seen the any aggregation or lump formation after incorporating niosomal residue in to the carbopol gel. The results suggests that there was no aggregation seen which is depicted in following fig.3.26a &b.

3.8 Iontophoresis study

Generally the iontophoresis is classified in to anodal iontophoresis and cathodaliontophoresis. But, while preparation of the niosomes,the stearyl amine was used as positive charge inducer in order to minimize the aggregation and to facilitate iontophoresis. Hence anodal iontophoresis was used to observe the effect of iontophoresis on permeation.

3.8.2 In-vitroAnodal trans-dermal Iontophoretic permeation study of continuous current

A Franz diffusion cell was used for diffusion studies& for this study 1 % Niosomal gel was used. Cellophane membrane was mounted on the diffusion cell. Current of 1 mA was used to observe the effect of iontophoresis on the permeation rate. This was depicted in following fig 3.27.

3.8.3 In vitroAnodal iontophoretic permeation studies of LidocaineHCl to optimize current density

Optimization of the current density is essential for the maximum permeation of drug because the permeation of drug was directly affected by amount of current applied. So that permeation studies were carried out at three different current density 0.5, 1 and 1.5 mA/cm^2 and 1 % niosomal gel was used for the study.

As seen in Fig.3.27. change in current density, will result change in permeation of LidocaineHCl. With the current density 0.5 mA/cm2, the flux was 2159.8µg/cm2/hr, while it was changes to 2488.12 µg/ cm^2/hr and 2418.37µg/cm2/hr for current density 1 and 1.5 mA/cm2 respectively (Table.3.20). There was significant increase in permeation when current density was increased from 0.5 to 1 mA/cm2. Since the maximum permeation was observed at 1 mA/cm2, thus for the further study same current density were used. show in fig. 28.

Jss= Steady state flux, Kp= Permeability coefficient, Er= Enhancement ratio, Q_4 = Cumulative amount permeated in 4 hr.

3.8.4 In vitropermeation studies of LidocaineHCl to study the effect of pulse current

Use of continuous direct current may result in permanent polarization of skin and saturation of skin surface due to drug, which can reduce the efficiency of iontophoretic delivery proportional to the length of direct current application. The buildup of this polarizable current and saturation of skin surface can be overcome by using pulsed direct current that is delivered periodically. Therefore, to further increase the permeation rate and the flux of LidocaineHCl across the skin, pulsed iontophoresis was performed.

As shown in Fig. 3.19. the permeation profile of LidocaineHCl at pulsed iontophoresis of ON:OFF pulse ratios 1:1, 1:2, 2:1, 3:1 and 4:1 was performed and 1 % niosomal gel was used for the study. The flux was significantly increased at the pulse rate 3:1 with a flux of 2856.85µg/cm^2/hr as compared to other. The use of pulse current allows the skin to depolarize and return to its initial electric condition when the current phase is put off for a fraction of time. Therefore the best result obtained by using pulsatile current with on: off ratio 3:1. Table: 3.21. show Effect of pulse current on the Permeation parameters

Jss= Steady state flux, Kp= Permeability coefficient, Er= Enhancement ratio, Q_4 = Cumulative amount permeated in 4 hr. Fig: 3.29.show In vitro permeation studies to study the effect of pulsatile current. Iontophoretic permeation comparison study.

To observe the effect of iontophoresis on the transdermal permeation of the drug as compared to passive diffusion of the drug, the all the permeation profiles of 1% Niosomal gel and Marketed gel are studied for passive permeation and iontophoretic permeation.

The following fig. 3.30 shows the comparative permeation profiles of the niosomal gel & marketed gel. It was observed that the passive diffusion results in flux of 2012.41µg/ cm^2/hr and 1898.24µg/ cm^2/hrfor niosomal gel and marketed gel respectively and on:off (3:1) time was used. But iontophoretic permeation of 5520.04 µg/ cm^2/hr and 3792.9µg/ cm^2/hrin case of niosomal gel and marketed gel respectively Table. 3.22. While performing iontophoresis study of marketed gel, it was observed that there is decrease in drug permeation after 3 hr of iontophoresis treatment & this effect was observed might be because of drug degradation due to electric current. To confirm this, drug degradation study was carried out.

3.8.5 Drug degradation comparative study of marketed gel &Niosomal gel

Significant reduction was noted in the amount of drug permeated (marketed gel) after 3 hr with iontophoresis& to confirm this experiment was carried out. Results shows that there was reduction of drug permeation from 100% to 74.72%i.e. 25.28 % drug degradation. In case of niosomal gel there was reduction of drug permeation from 100 % to 94.73 % i.e. 5.27 % drug egradation. So, it concludes that niosomes protects drug degradation which occurs due to electric current during iontophoresis study.

3.9. Stability study

Stability studies of one month were conducted for optimized F5 batch with respectto the niosomes ability to retain an entrapped drug during a defined time period. Asexplained in experimental part, all samples kept at different temperature wereevaluated for appearance, entrapment efficiencies and vesicle size. Batch at 45°C/75%±5%RHshowed high drug leakage and entrapped drug was found to be only 46.28%. Batch at25±2°C/ 60%±5%RH and refrigeration temperature (2-8°C) shownless leakage andentrapped drug was found to be 64.53 % and 71.98 % respectively. Drug leakage atelevated temperatures may be resulted due to of chemical degradation (oxidation andhydrolysis) of lipids in the bilayers, leading to defects in membrane packing.Thus, earlier reports of the low-temperature stability of liposomal products may beattributed to the gel-state lipid membranes that help to hold drug molecules in placeand thus show high drug retention.Slight increase in vesicle size occurred due to aggregation of niosomes during storage. Table 3.23.shows the stability results.

4. SUMMARY AND CONCLUSION

Nonionic surfactant vesicular systems also known as niosomes are a novel and efficient approach to drug delivery. Their vesicular membrane is mainly composed of nonionic surfactants, cholesterol and charge inducer. I have selected Lidocaine HCl as drug candidate after extensive literature survey because Lidocaine HCl provide efficient anesthesia of the skin or of mucosal tissues in case of pain itching and burning associated with cutaneous inflammatory response to different agents and with minor surgical operations. But permeability of lidocaine through skin i.e. stratum corneum is very low, hence attempt made to improve the permeation of Lidocaine HCl across skin by synergistic effect of niosomes & iontophoresis. Niosomal system stabilized by addition of positive charge inducer i.e. stearyl amine, the charge inducer also used to facilitate iontophoresis. Then for the purpose of application on to skin, niosomal dispersion was converted in to suitable dosage form i.e. gel. Permeation experiments for Simple marketed gel (Lignocaine HCl gel 2% I.P. i.e. LOX 2% Gel) & Niosomal gel with or without iontophoresis using cellophane membrane was carried out to observe the effect of iontophoresis. The iontophoresis technique was optimized for parameters like current density, pulse ratio i.e. ON: OFF time in sec. Niosomes also protects drug degradation which occurs due to electric current during iontophoresis. No niosomal system with iontophoresis for Lidocaine HCl has been formulated for local dermal anesthesia.

In the Preformulation study of drug physicochemical properties like appearance, colour, odour and melting point, U.V. study, solubility study, FTIR study, DSC study was carried out and it confirms the authentification of drug. The interaction between drug and excipient was confirmed by FTIR study and DSC study; it was found that there is no interaction between drug and excipients. Niosomes were prepared by Rotary evaporation method and Ether injection method by using span 60 as non-ionic surfactant, Rotary evaporation method was selected for further study depending on optimum vesicle size production and maximum entrapment efficiency. The tween 20, tween 40, tween 60 (Tweens) and span 20, span 40, span 60 (Spans) were screened to select best non-ionic surfactant depending on entrapment efficiency and vesicle size. The span 60 given the best results as compared to other tweens and spans, hence selected for further study. Concentration of surfactant and cholesterol were found to be critical in preparation and stabilization of niosomes and hence both were selected as independent variables in the 3^2 full factorial designs and Entrapment efficiency and % drug permeated were selected as dependent variables. F5 batch was found to be optimized batch because it gives maximum entrapment efficiency i.e. 72.6 % and optimum drug permeation i.e. 63.89 %. Further, positive charge inducer (stearyl amine - 5% w/w) was added in to the optimized formulation F5 to minimize the aggregation of the vesicles and to facilitate iontophoresis. After addition of charge inducer, it was observed that there is increase in vesicle size (From 1400.97nm to 2113.07nm) as well as entrapment efficiency (From 72.6 % to 74.84%) but improvement in PDI (From 0.201 to 0.150). Optimized batch F5 evaluated for optical microscopy, Vesicle size analysis and PDI, SEM analysis, Zeta potential analysis, DSC study and In vitro release study. Optical microscopy shows that there is no aggregation of the niosomes, SEM analysis shows spherical structure of niosomes, Zeta potential value 28.77mV indicates niosomes have sufficient charge to prevent aggregation, DSC study shows minor shifting of endothermic peak so it concludes that there is no interactions between the formulation components and In-vitro release rate of Lidocaine HCl from niosomes showed 97.24 % of drug release.

This batch is formulated in to Niosomal gel by using Carbopol 974 P NF in three different conc. i.e. 0.5% w/v, 1 % w/v and 2% w/v, the 1% w/v Carbopol (depending on consistency) was found to be suitable for preparation of Niosomal gel.

Iontophoresis was applied for Niosomal gel to observe the effect of iontophoresis. During iontophoresis study the parameters like current density and ON: OFF time (sec) was optimized. The current density 0.5mA, 1mA and 1.5 mA were used; current density of 1 mA was selected for further study and in case of ON: OFF time, the ratio of 3:1 was selected at 1mA. The permeation experiments for Simple marketed gel (Lignocaine HCl gel 2% I.P. i.e. LOX 2% Gel) & Niosomal gel with or without iontophoresis using cellophane membrane was carried out by using optimized iontophoretic parameters. In case of niosomal gel it shows that, pulse iontophoresis is more effective than continuous iontophoresis but in case marketed gel it was observed that there is reduction in permeation of drug after 3 hr, drug degradation might be occur due to electric current. To confirm this degradation, reduction in concentration of marketed gel and niosomal gel was determined. Results shows that niosomes offers protection of drug from electric current during iontophoresis.

Present study concludes that, niosomal gel of Lidocaine HCl for transdermal iontophoresis was prepared and characterized with respect to permeation study. Niosomal gel of Lidocaine HCl was prepared and compared with the marketed formulation of Lidocaine gel (2%) with respect to transdermal flux and results shows that higher transdermal flux than the marketed gel. So niosomes and iontophoresis have synergistic effect on the transdermal permeation flux.

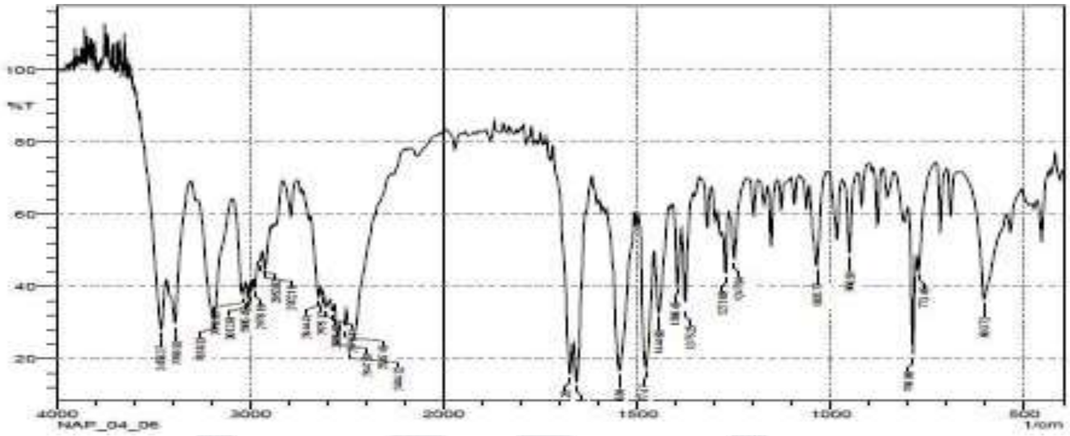

Fig:3.1. FTIR spectra of LidocaineHCl

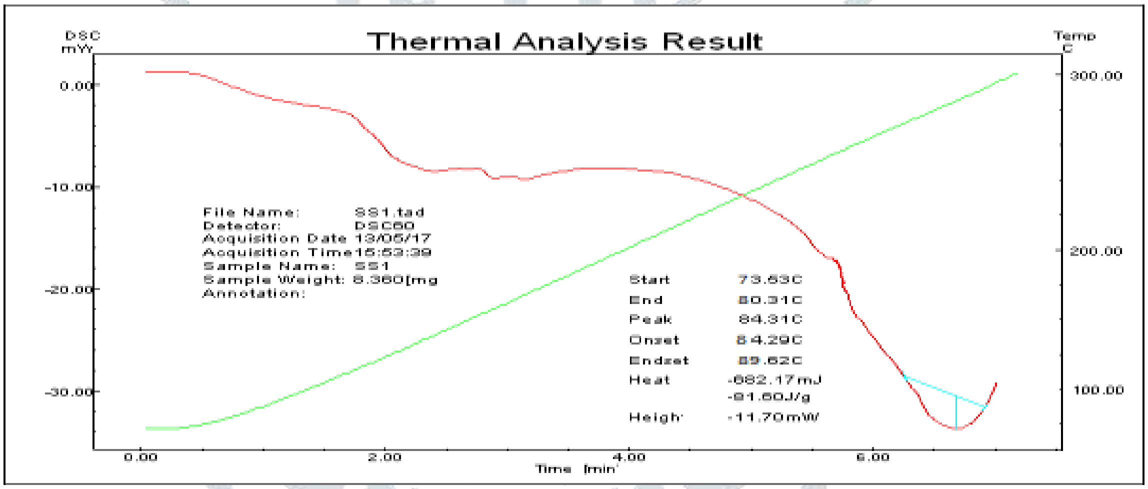

Fig: 3.2. DSC thermogram of LidocaineHCl.

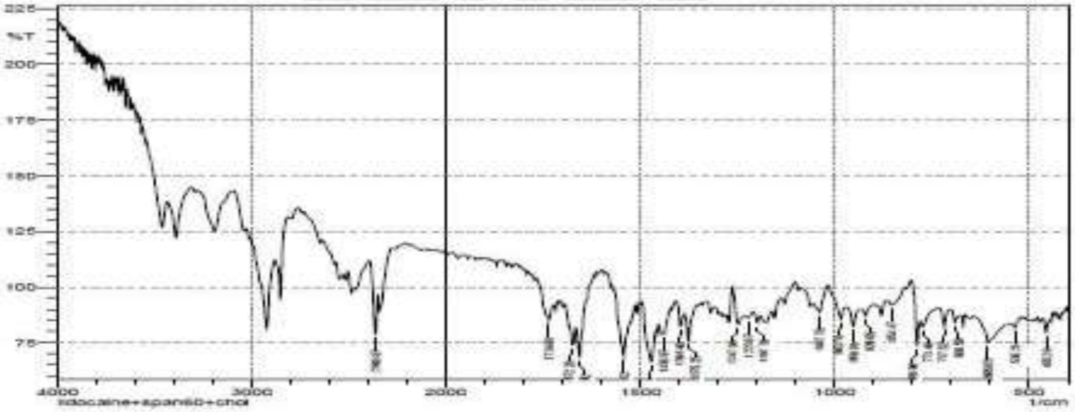

Fig: 3.3. FTIR spectra of drug + physical mixture.

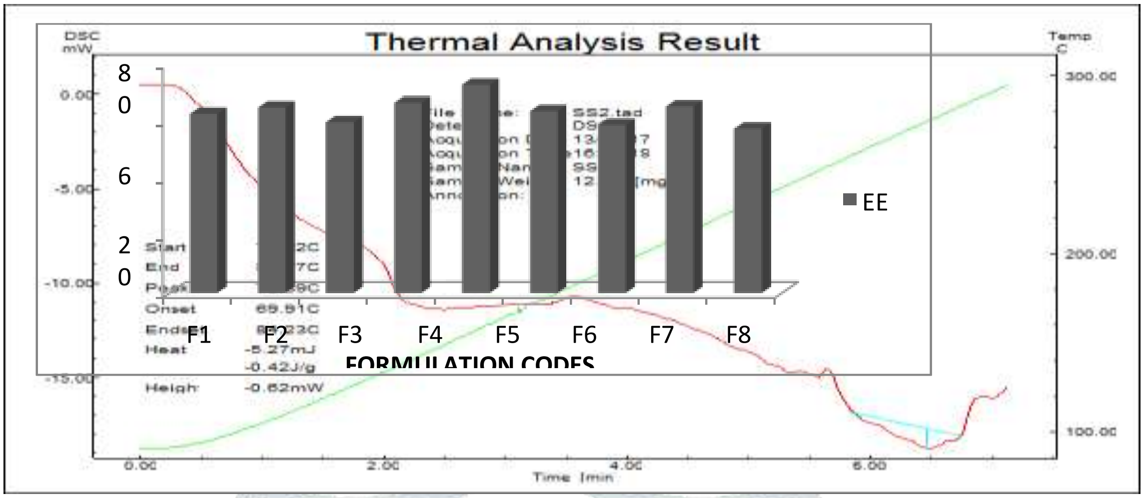

Fig: 3.4. DSC thermogram of Drug + Physical mixture.

% EE

Fig: 3.5. Entrapment efficiency (EE) of F1-F9 batches

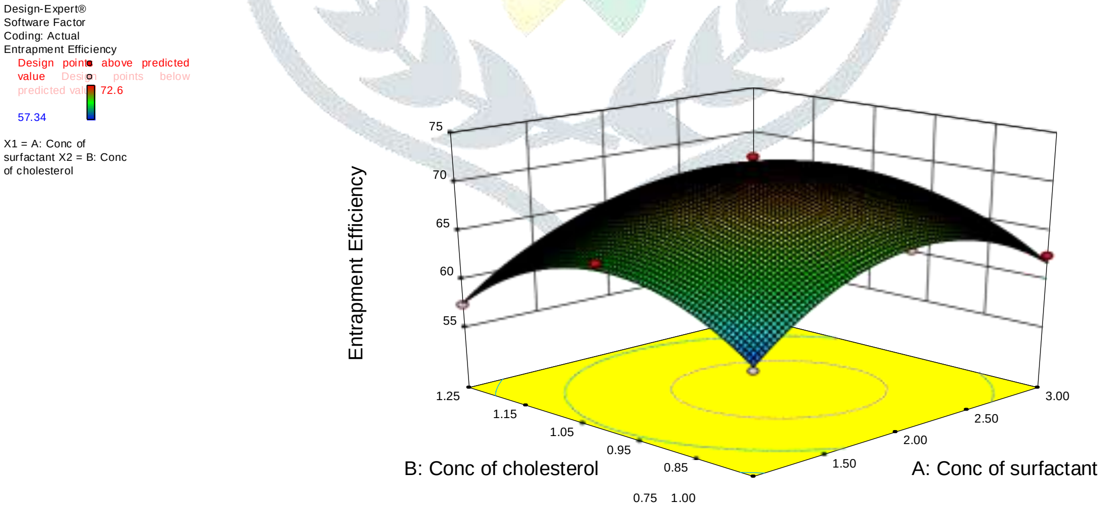

Fig: 3.6. Graphical representation of effect of factors on % Entrapment efficiency by using response surface plot

Fig: 3.7. Drug permeation profiles of F1-F9 batches

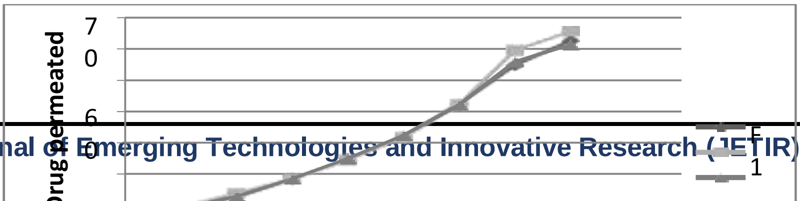

Fig:3.7.a) % Drug permeated from F1-F3

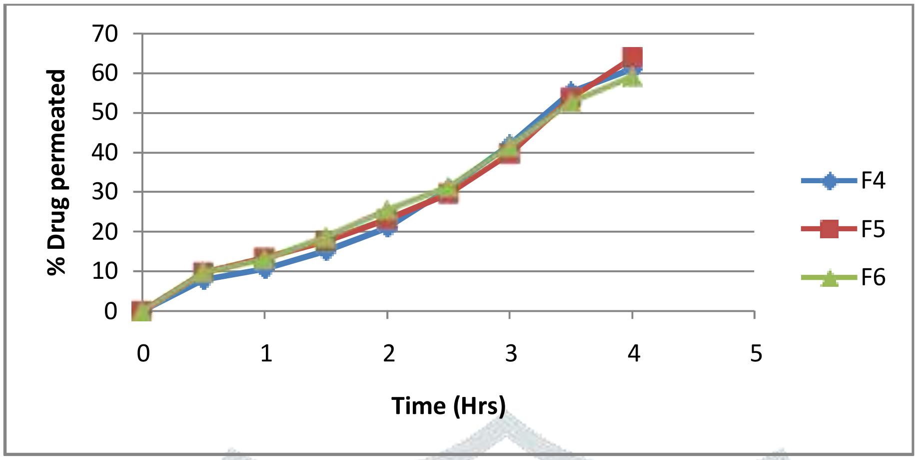

Figure:3.7.b) % Drug permeated from F4-F6

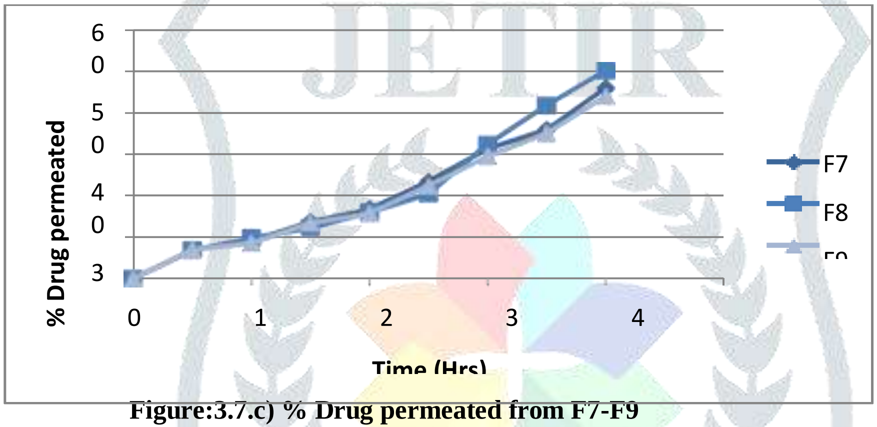

Figure:3.7.c) % Drug permeated from F7-F9

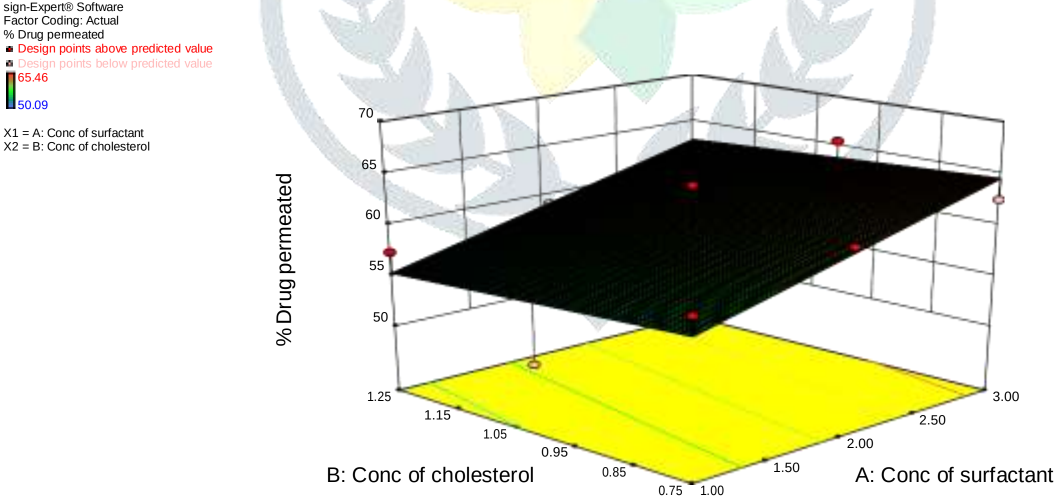

Fig: 3.8. Graphical representation of effect of factors on % Drug permeated by using response surface plot

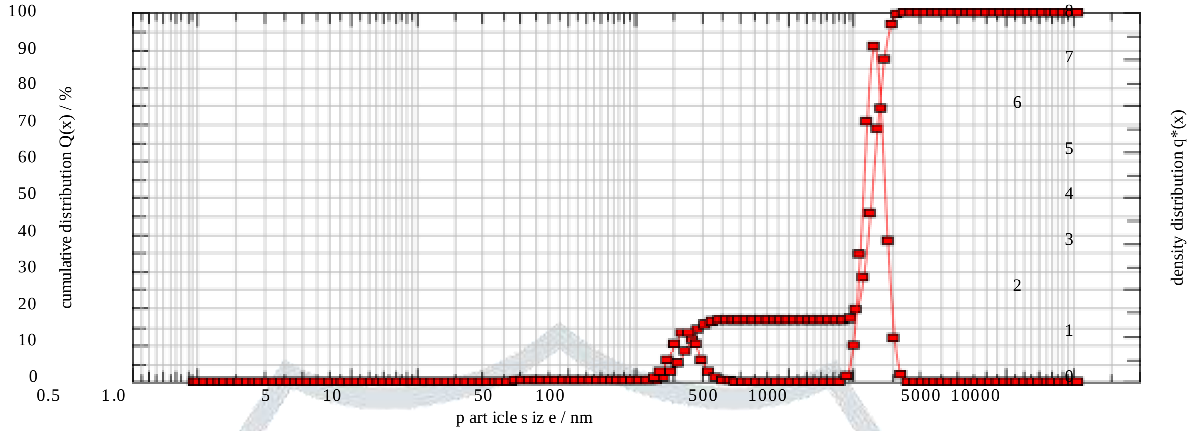

Fig: 3.9. Vesicle size distribution curve of F1

x_{10}= 1072.96 nm x_{50} = 1194.42 nm x_{90} =1392.10 nm PDI = 0.268

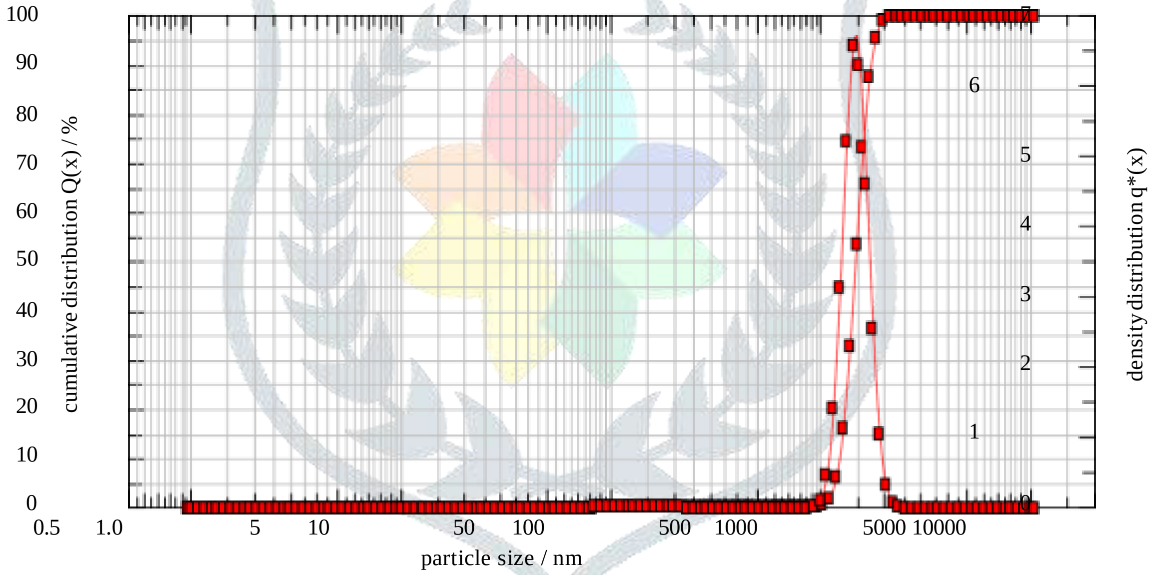

Figure: 3.10. Vesicle size distribution curve of F2

x_{10} = 1210.18 nm x_{50}= 1447.51 nm x_{90}= 1731.10 nm PDI = 0.360

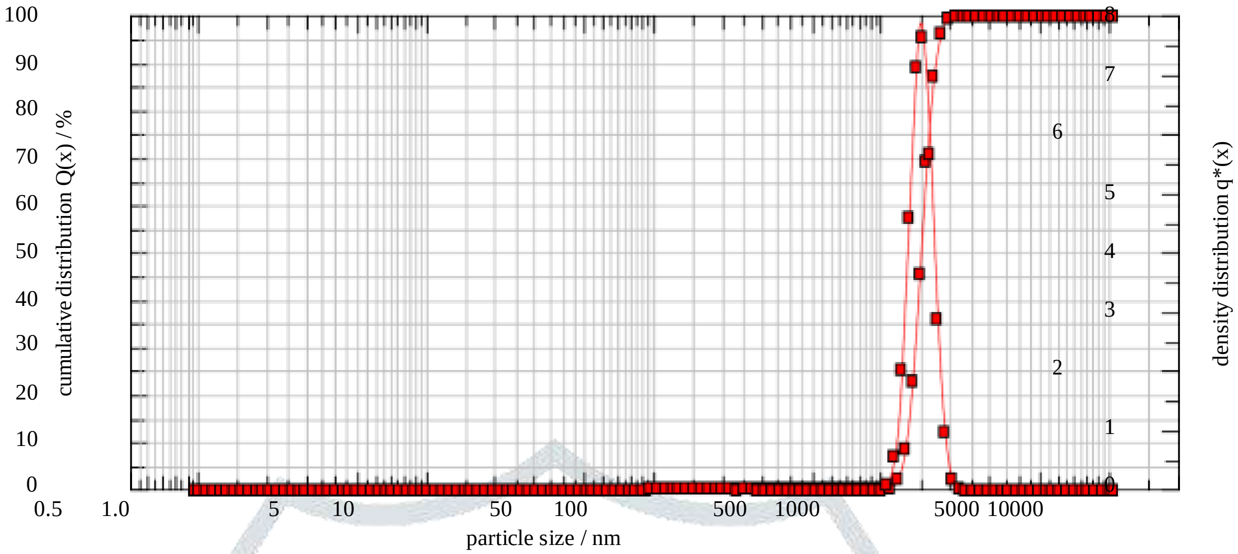

Figure: 3.11. Vesicle size distribution curve of F3 x_{10}= 1276.21 nm x_{50}= 1484.64 nm x_{9}= 1729.85 nmPDI = 0.305

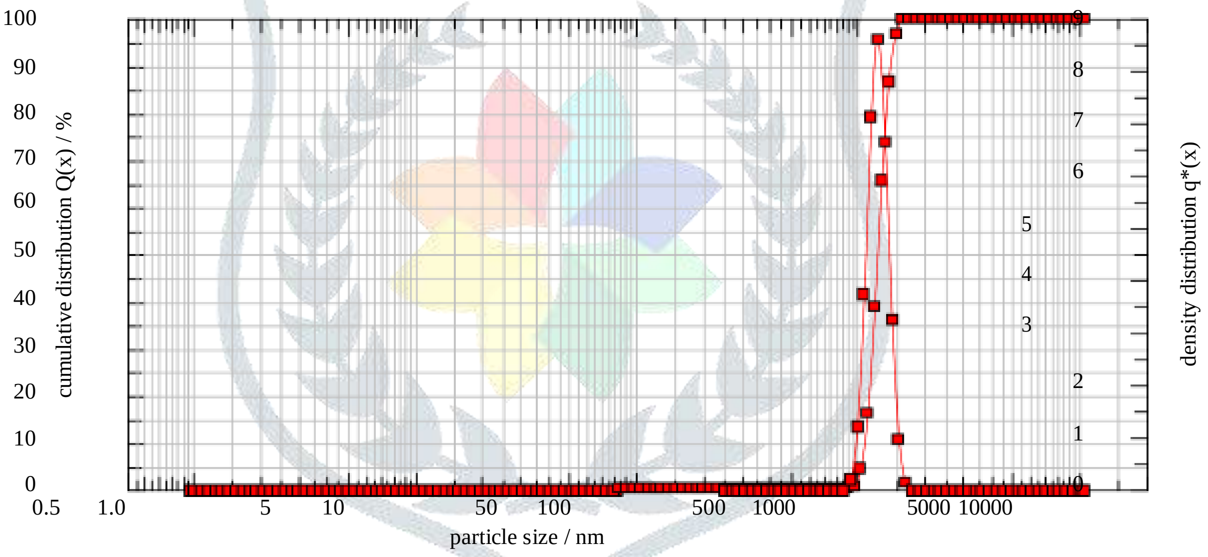

Figure: 3.12. Vesicle size distribution curve of F4

x_{10}= 1054.57 nm x_{50} =1214.51 nm x_{90} =1395.93 nm PDI = 0.281

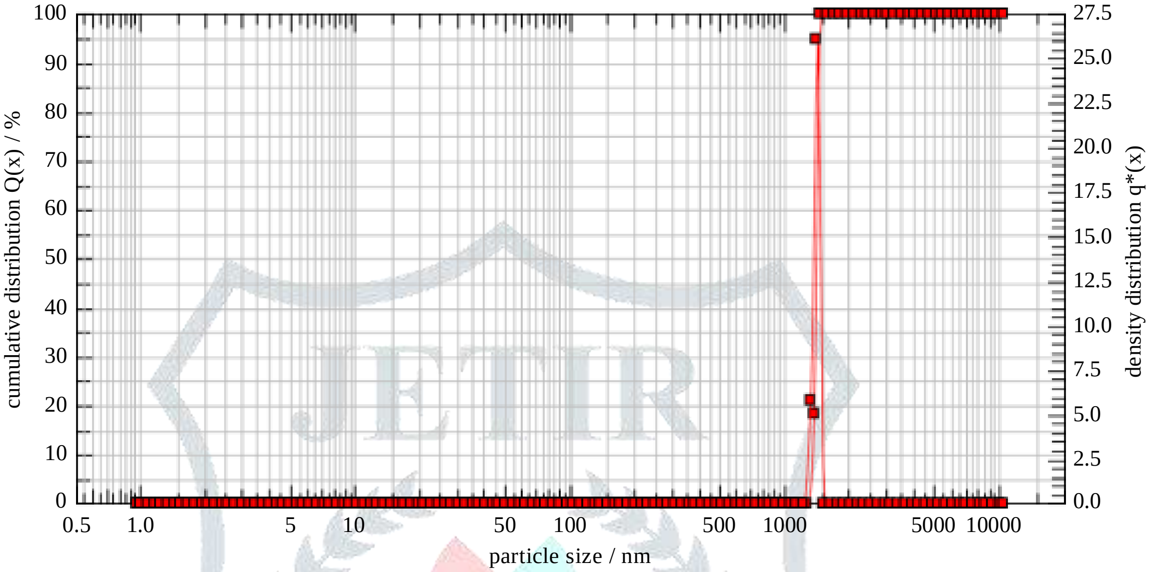

Figure: 3.13. Vesicle size distribution curve of F5

x_{10}= 1368.75 nm x_{50} =1400.97 nm x_{90} =1450.89 nm PDI = 0.201

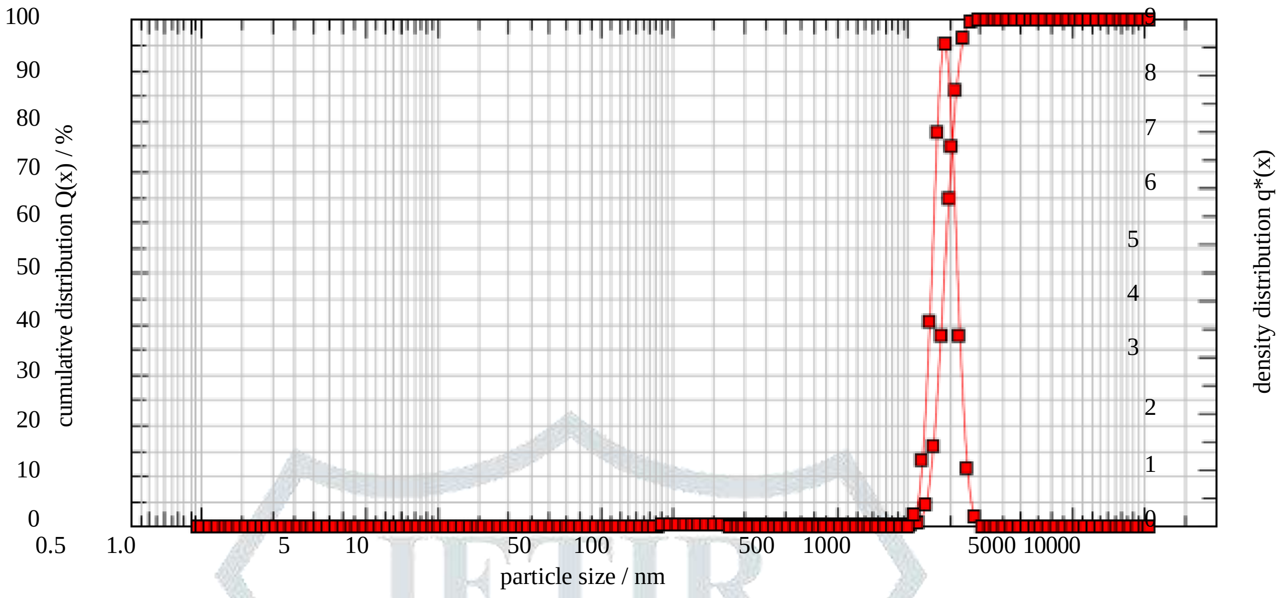

Figure: 3.14. Vesicle size distribution curve of F6

x_{10} = 1221.84 nm x_{50} =1407.91 nm x_{90}= 1620.94 nm PDI = 0.283

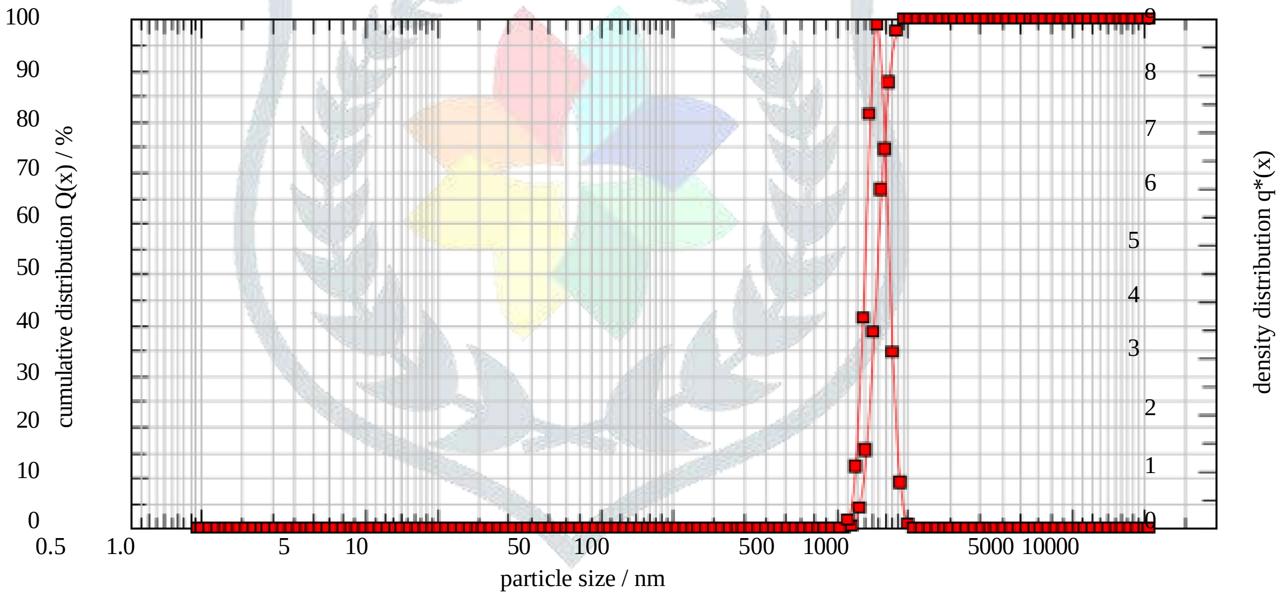

Figure: 3.15. Vesicle size distribution curve of F7

x_{10} = 877.53 nm x_{50} =985.15 nm x_{90} =1102.88 nm PDI = 0.228

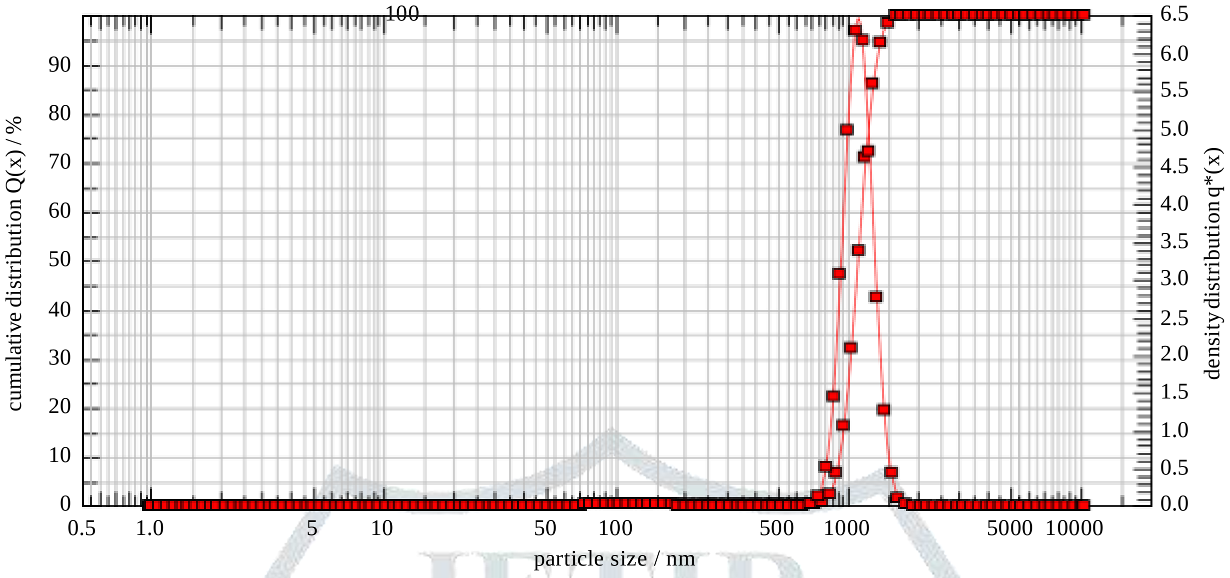

Figure: 3.16. Vesicle size distribution curve of F8

x_{10} = 904.13 nm x_{50} =1088.58 nm x_{90}= 1312.41 nm PDI = 0.375

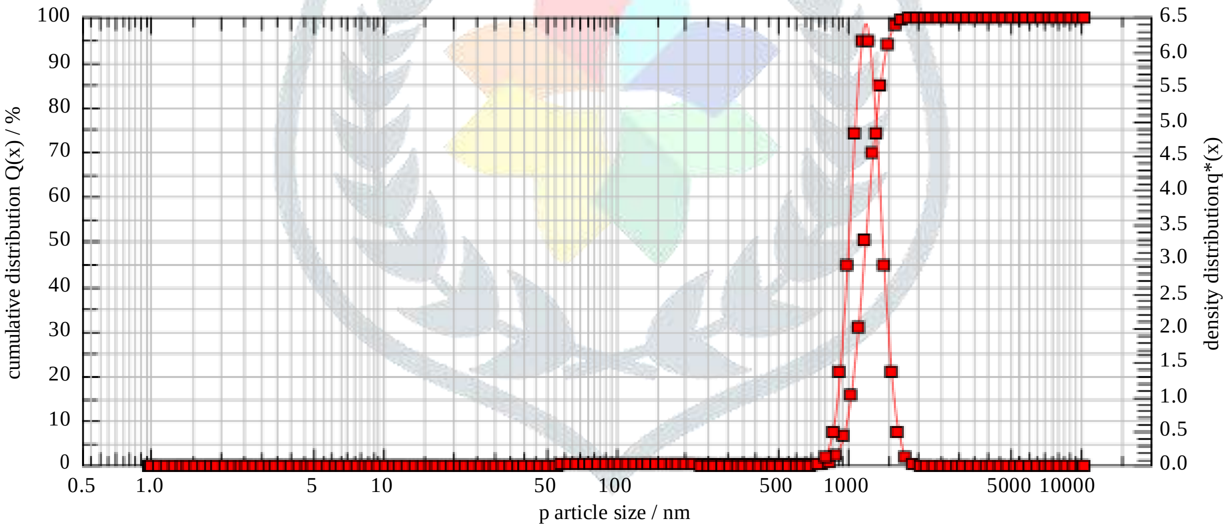

Figure: 3.17. Vesicle size distribution curve of F9

x_{10} = 975.02 nm x_{50} =1176.71 nm x_{90}= 1420.80 nm PDI = 0.378

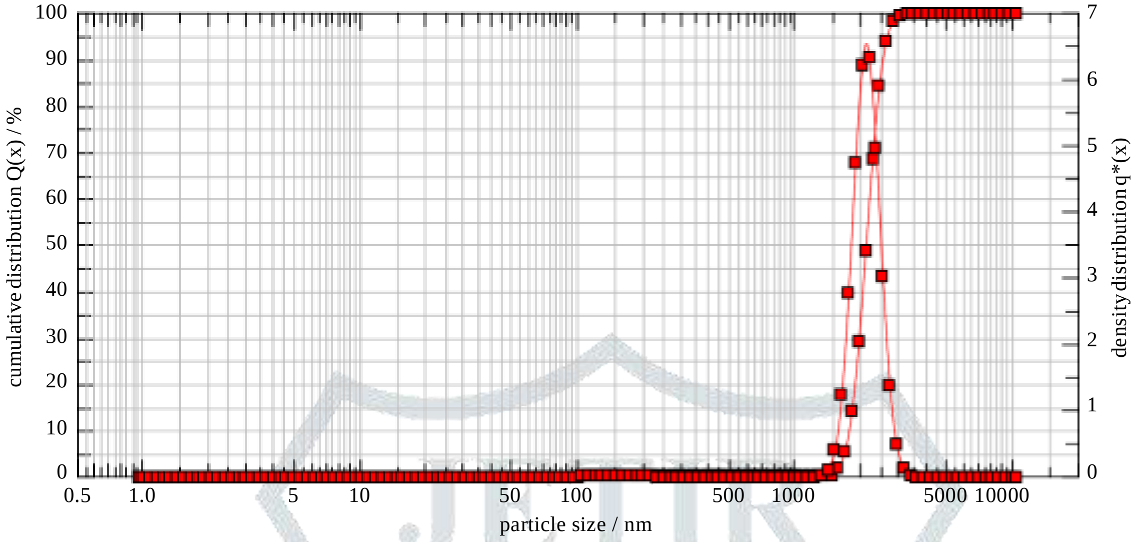

Figure: 3.18. Vesicle size distribution curve of F5 + 5% stearyl amine.

x_{10} = 1957.01 nm x_{50} =2113.07 nm x_{90} =2541.86 nm PDI = 0.150

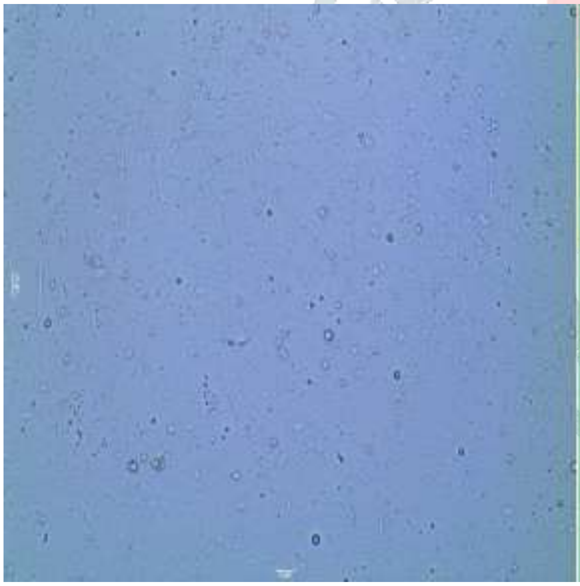

Fig: 3.19.a) Motic image of F5 at 40x

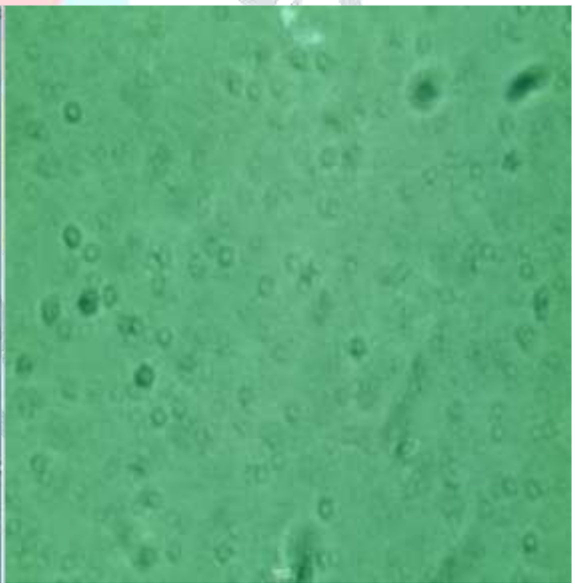

Fig: 3.19.b) Motic image of F5 at 100x

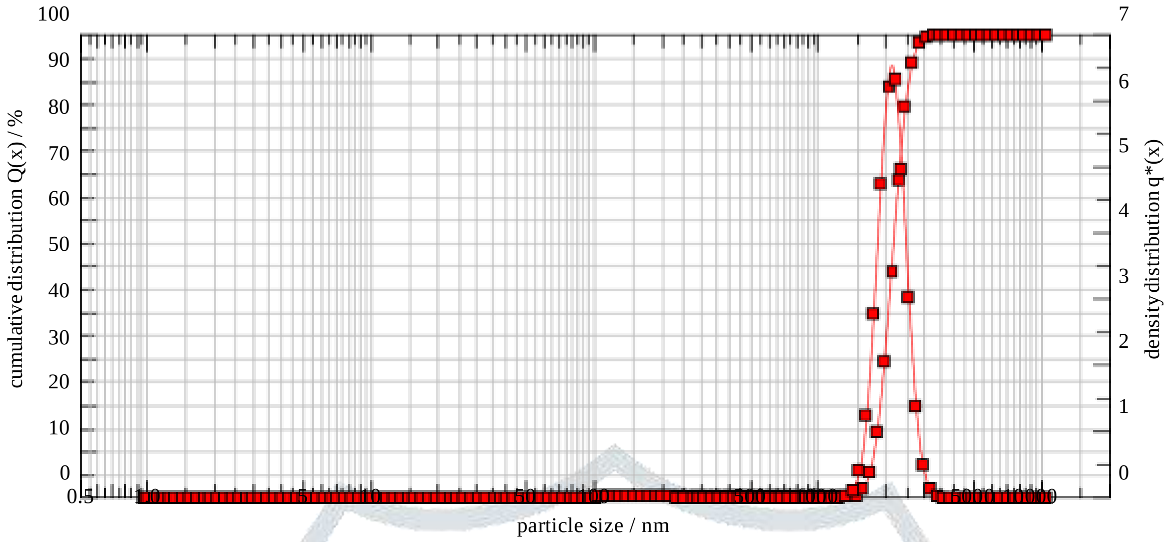

Figure: 3.20. Vesicle size distribution curve of optimized batch

x_{10} = 1957.01 nm x_{50} =2113.07 nm x_{90} =2541.86 nm PDI = 0.150

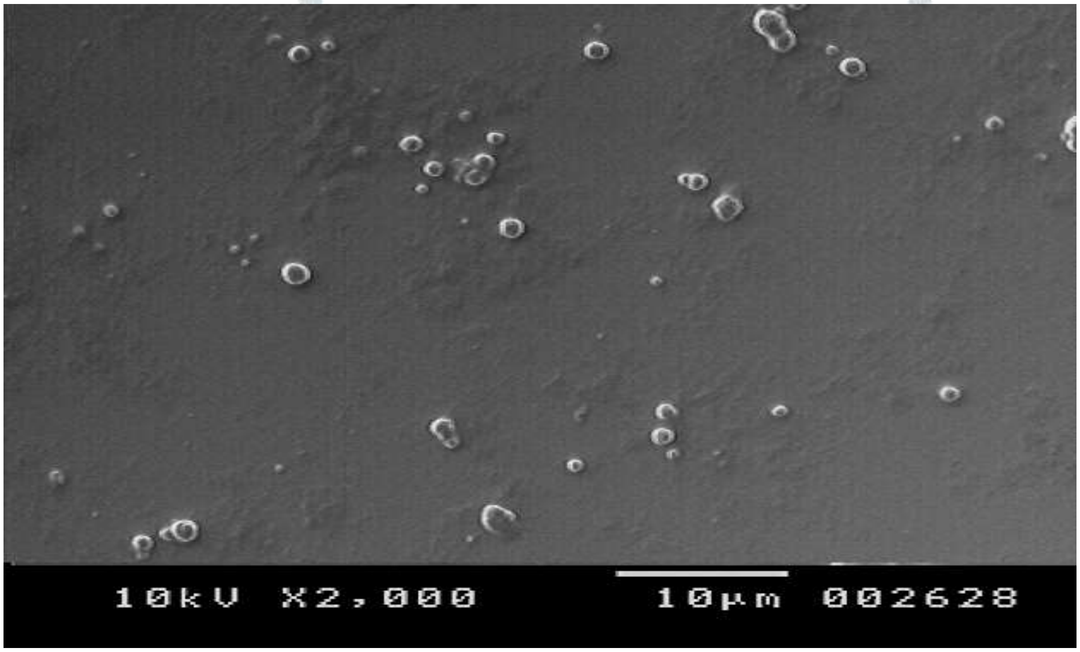

Fig: 3.21. SEM image of optimized formulation.

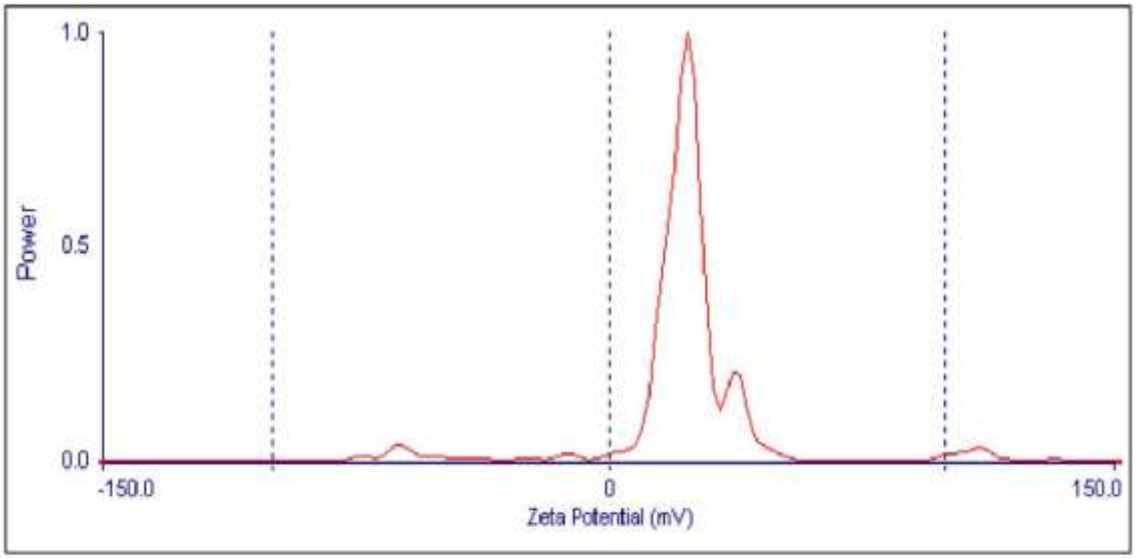

Fig: 3.22. Zeta potential graph of optimized formulation

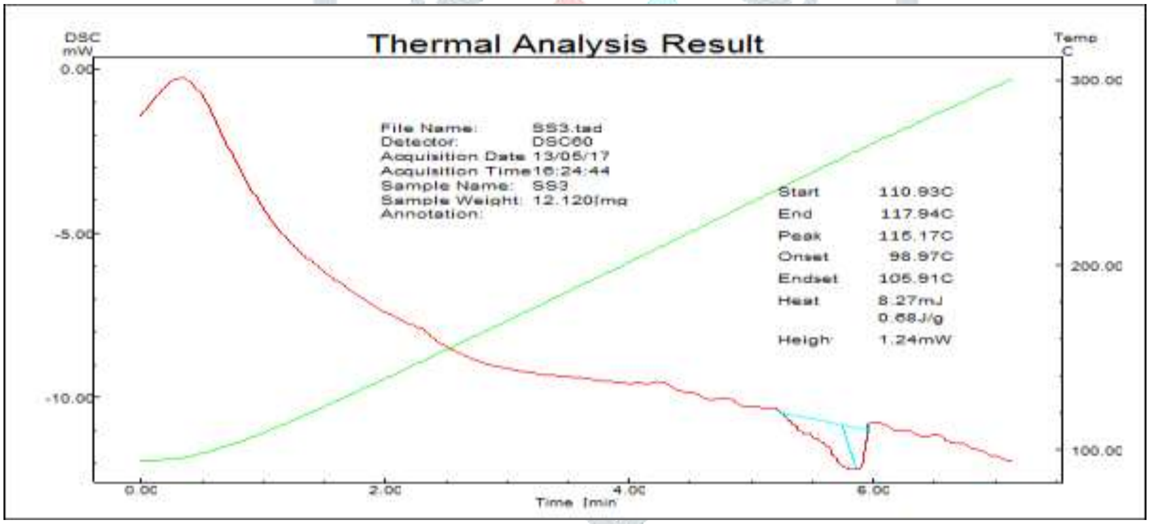

Fig: 3.23. DSC thermogram of freeze dried formulation.

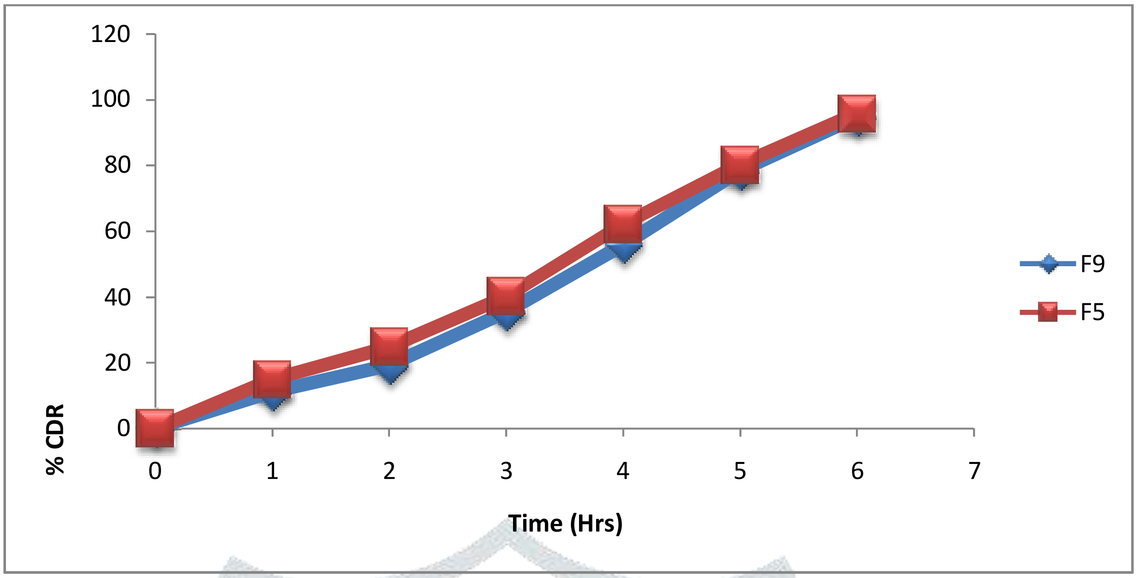

Fig:3.24. In vitro drug release kinetic studies

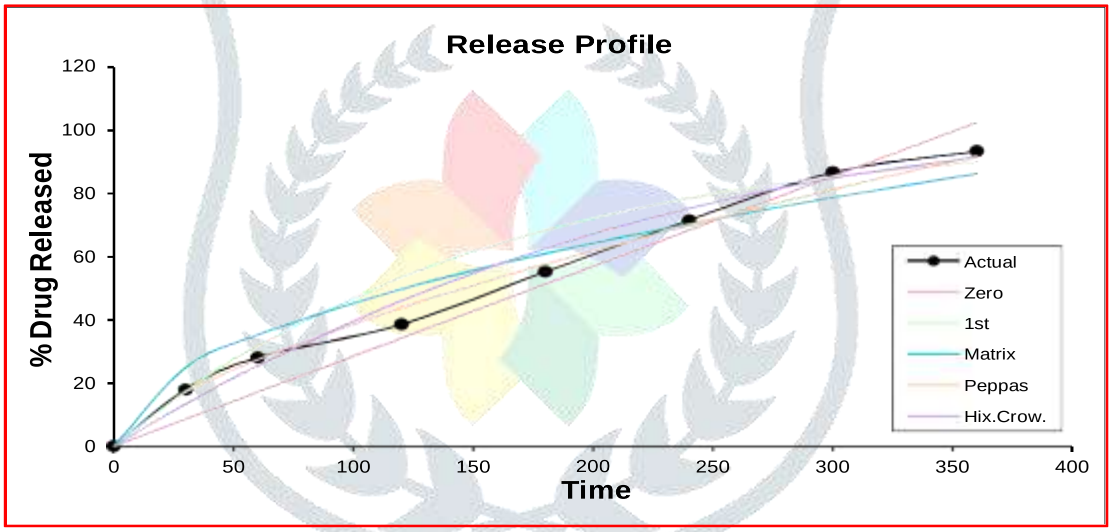

Fig: 3.25. Drug release profile of optimized formulation.

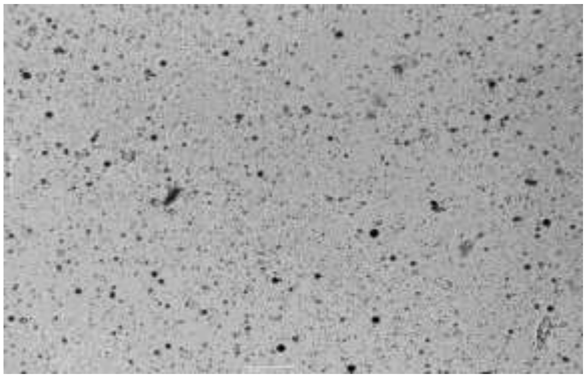
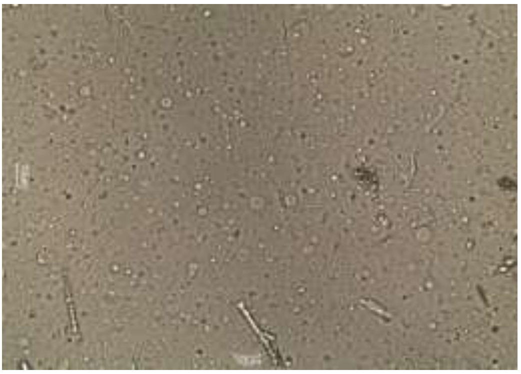

Fig: 3.26.a) Marketed gel under 40xFig: 3.26.b) Niosomal gel (1%) under 40x

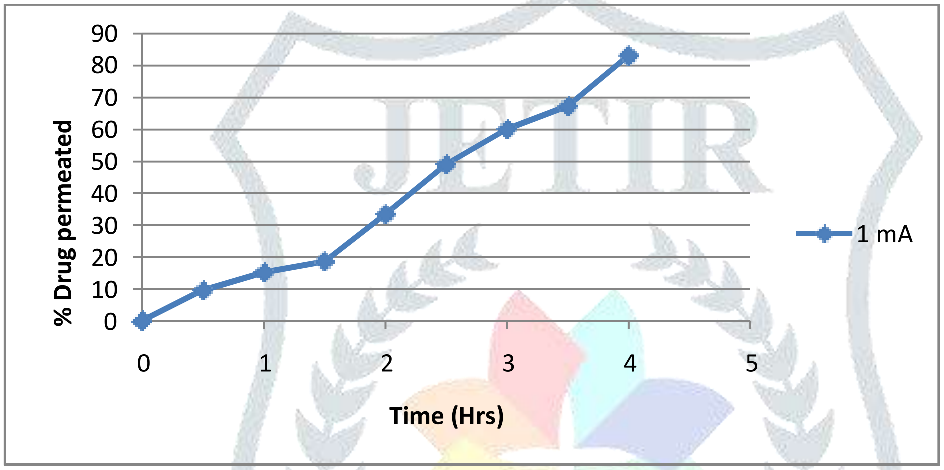

Fig: 3.27. In-vitro Anodal trans-dermal Iontophoretic permeation study of continuous

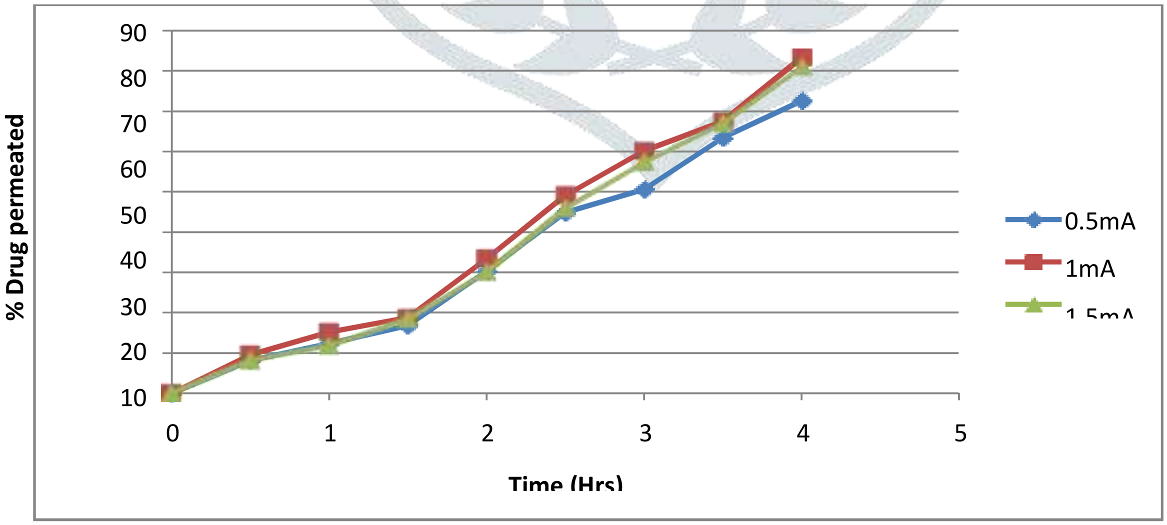

Fig: 3.28. In vitroAnodal iontophoretic permeation studies to optimize current density current.

Jss= Steady state flux, Kp= Permeability coefficient, Er= Enhancement ratio, Q_4 = Cumulative amount permeated in 4 hr.

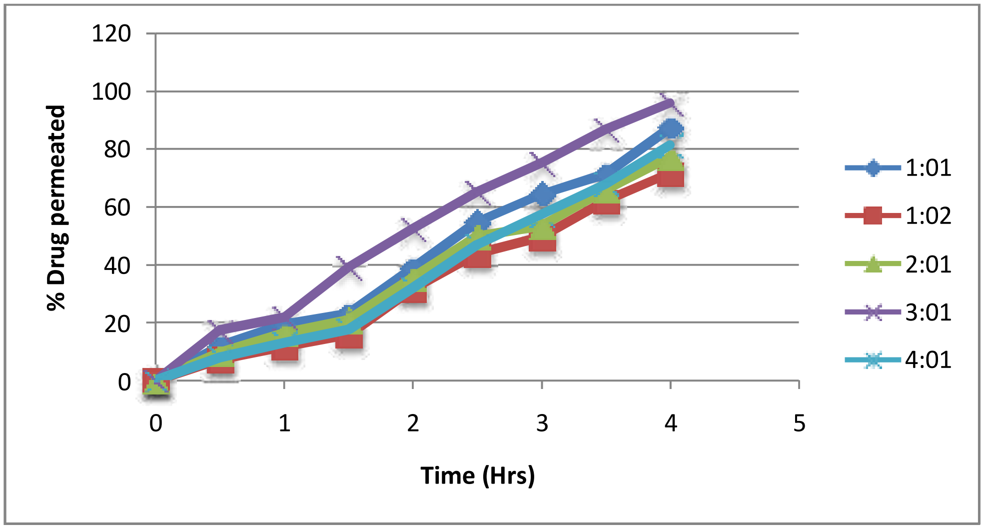

Fig: 3.29. In vitropermeation studies to study the effect of pulsatile current.

Iontophoretic permeation comparison study

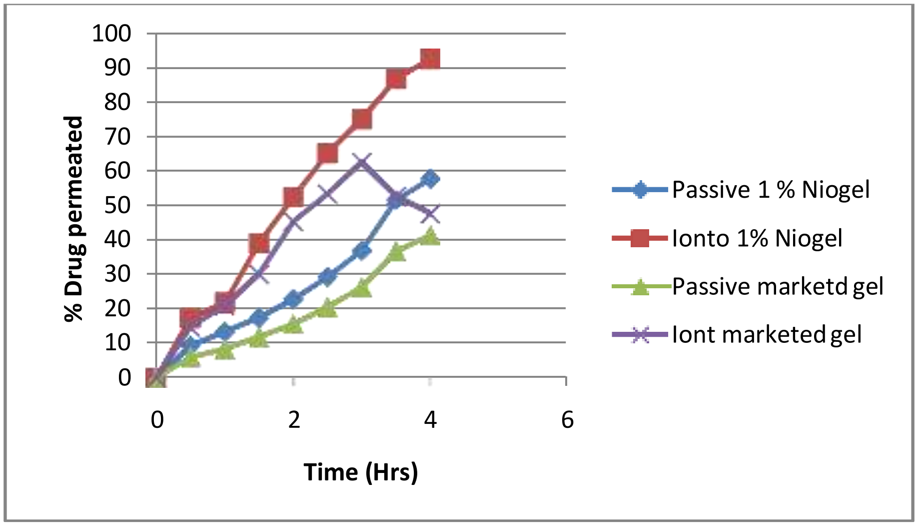

Fig:3.30.Comparative permeation study of niosomal gel and marketed gel with or without iontophoresis.

Table 2.1: Three different formulations by Rotary Evaporation and Ether Injection method

Surfactant-Span 60 (milimoles)	**Cholesterol (milimoles)**	**Rotary Evaporation method**		**Ether Injection method**	
		Entrapment efficiency (%)	Vesicle size (µm)	Entrapment efficiency (%)	Vesicle size (µm)

3	1	64.76	1.2-2	58.2	3.2-4
2	1	72.60	1-1.5	62.31	3.1-3.4
1	1	65.16	1.3-1.8	56.98	2.8-3.8

Table: 2.2. Coded formulations

Variables	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
X_1 [Milimoles]	1 [3]	1[3]	1[3]	0 [2]	0 [2]	0 [2]	-1 [1]	-1 [1]	-1[1]
X_2 [Milimoles]	-1[1.25]	0[1]	1[.75]	-1[1.25]	0 [1]	1[.75]	-1[1.25]	0 [1]	1[.75]

Table: 2.3. Coded levels

Coded levels	**1**	**0**	**-1**
X_1 [Milimoles] (Non-ionic surfactant in mg)	129 [3]	86 [2]	43 [1]
X_2 (Cholesterol in mg)	48.32 [1.25]	38.66 [1]	28.99 [0.75]

Table: 2.4. Formulations of Factorial dsign

Ingredients (mg)	**Formulations**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Lidocaine HCl	100	100	100	100	20	100	100	100	100
Non-ionic surfactant	129	129	129	86	86	86	43	43	43
Cholesterol	28.9	38.6	48.3	28.9	38.6	48.3	28.9	38.6	48.3

Table: 2.5. Interpretation of diffusion release mechanisms from dosage forms

Release exponent (n)	**Drug transport mechanism**
0.5	Fickian diffusion
$0.5 < n < 1.0$	Anomalous transport (non-Fickian)
1.0	Case-II
> 1.0	Super case- II transport

Table: 3.1. FTIR spectrum ranges of LidocaineHCl

Frequency (cm^{-1})	**Interpretation**
1710-1665	C=0 stretching
1600-1620	C-C stretching

3400-3250	NH aromatic stretching
3330-3270	CH2 stretching
1250-1020	C-N stretching

Table: 3.2. Method selection for preparation of niosomes

Surfactant-Span 60 (milimoles)	**Cholesterol (milimoles)**	**Rotary evaporation method**		**Ether injection method**	
		Entrapment efficiency (%)	Vesicle size (µm)	Entrapment efficiency (%)	Vesicle size (µm)
1	1	**65.16**	1.3-1.8	56.98	2.8-3.8
2	1	**72.60**	1-1.5	62.31	3.1-3.4
3	1	**64.76**	1.2-2	58.2	3.2-4

Table: 3.3. Optimization of speed of rotation

Method	**Speed**	**Result**	**Selected speed**
Thin film Hydration method	75 rpm	Irregular film	100 rpm
	100 rpm	Uniform film	
	125 rpm	Irregular film	

Table: 3.4. Optimization of drug quantity

Method	**Amount of drug added (mg)**	**Result**	**Selected amount of drug (mg)**
Thin film Hydration method	80	Low entrapment efficiency i.e. up to 30-35%	100
	100	Optimum entrapment efficiency	
	120	No change in entrapment efficiency	

Table: 3.5. Screening of Tweens

Surfactant	**Code**	**Surfactant : Cholesterol (milimoles) (milimoles)**		**Entrapment Efficiency(%)**	**Vesicle size (µm)**
Tween 20	NF1	3	1	52.1	1.2-2.8
	NF2	2	1	58	1.5-3
	NF3	1	1	48	1.6-3.2

Tween 40	NF4	3	1	54.2	1.7-3.2
	NF5	2	1	57.3	1.8-3.4
	NF6	1	1	51.2	1.9-3.4
Tween 60	NF7	3	1	48.1	1.2-3.2
	NF8	2	1	52.2	2.2-3
	NF9	1	1	43	3.1-4.2

Table: 3.6. Screening of spans

	Code	**Surfactant : Cholesterol (milimoles) (milimoles)**		**Entrapment Efficiency (%)**	**Vesicle size (μm)**
Span 20	NF10	3	1	62.8	1.2-2
	NF11	2	1	64.2	1.2-2.3
	NF12	1	1	60.8	1.6-2
Span 40	NF13	3	1	61.3	1.8-2.3
	NF14	2	1	65.8	1.7-2.4
	NF15	1	1	58.3	1.7-2.4
Span 60	NF16	3	1	64.76	1.2-2
	NF17	2	1	72.60	1-1.5
	NF18	1	1	65.16	1.3-1.8

Table: 3.7. Optimization batches of formulation F1-F9 by 3^2 full factorial design.

Formu-lation (codes)	**Coded levels**		**% Entrapment Efficiency±S.D**	**% Drug permeated**	**Vesicle Size(nm)**	**Polydispersity index (PDI)**
	X_1(Surfactant in mg)	X_2(Cholesterol in mg)				
F1	1 (129)	-1 (28.99)	52.200±0.124	62.5	1194.4	0.268
F2	1 (129)	0 (38.66)	55.754±0.132	65.46	1447.5	0.360
F3	1 (129)	+1(48.32)	59.179±0.107	61.33	1484.6	0.305
F4	0 (86)	-1 (28.99)	54.029±0.074	61.1	1214.5	0.281

F5	0 (86)	0 (38.66)	60.173±0.085	63.89	1400.9	0.201
F6	0 (86)	+1(48.32)	63.983±0.094	59.1	1407.9	0.283
F7	-1 (43)	-1 (28.99)	62.899±0.194	45.93	985.15	0.228
F8	-1 (43)	0 (38.66)	66.124±0.132	50.09	1088.6	0.375
F9	-1 (43)	+1(48.32)	68.255±0.136	43.97	1176.7	0.378

Table: 3.8. Entrapment efficiency of F1 - F9 batch

Formulation code	**Entrapment efficiency (%)± S.D**
F1	52.200±0.124
F2	55.754±0.132
F3	59.179±0.107
F4	54.029±0.074
F5	60.173±0.085
F6	63.983±0.094
F7	62.899±0.194
F8	66.124±0.132
F9	68.255±0.136

Table: 3.9. Model Summary Statistics

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**PRESS**	
Linear	5.16	0.0801	-0.2265	-0.9166	332.25	
2FI	5.34	0.0841	-0.4654	-3.5423	787.42	
Quadratic	1.46	0.9630	0.9014	0.6094	67.71	Suggested
Cubic	1.48	0.9873	0.8985	-1.3132	401.00	Aliased

Table: 3.10. ANOVA for response surface quadratic model

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p value prob > F**	
Model	166.94	5	33.39	15.62	0.0234	Significant
A-conc. Of surfactant	5.49	1	5.49	2.57	0.2073	

B-conc. Of cholesterol	8.40	1	8.40	3.93	0.1417	
AB	0.69	1	0.69	0.32	0.6099	
A^2	75.81	1	75.81	35.47	0.0095	
B^2	76.55	1	76.55	35.82	0.0093	
Residual	6.41	3	2.14			
Cor Total	173.35	8				

Table: 3.11. % of Drug permeated of F1 - F9 batch

Formulation Code	% Drug permeated
F1	62.5
F2	65.46
F3	61.33
F4	61.1
F5	63.89
F6	59.1
F7	45.93
F8	50.09
F9	43.97

Table: 3.12. Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	3.39	0.5756	0.4342	0.1297	141.32	Suggested
2FI	3.71	0.5756	0.3210	-0.5426	250.49	
Quadratic	4.45	0.6343	0.0248	-3.0034	650.06	
Cubic	3.94	0.9043	0.2345	-16.4383	2831.57	Aliased

Table: 3.13. ANOVA for response surface linear model

Source	Sum of Squares	df	Mean Square	F Value	p value prob > F	
Model	93.47	2	46.73	4.07	0.0764	Significant

A-conc. Of surfactant	90.02	1	90.02	7.84	0.0312	
B-conc. Of cholesterol	3.45	1	3.45	0.30	0.6034	
Residual	68.91	6	11.48			
Cor Total	162.38	8				

Table: 3.14. Zeta potential measurement parameters

Zeta potential measurement parameters	
Avg. Zeta potential	28.77mV
Avg. Mobility	225/s
Conductance	713 µS
Temperature	25 ^{0}C
Viscosity	0.890 cps
Refractive Index	1.330
Dielectric constant	78.54

Table: 3.15. Drug release kinetics from LidocaineHClniosomes

Model	**R**	**k**
Zero order	0.9683	0.287
T-test	11.539	(Passes)
1st order	0.9547	-0.0063
T-test	9.682	(Passes)
Matrix	0.9599	5.5242
T-test	9.745	(Passes)
Peppas	**0.9925**	**1.7920**
T-test	23.239	(Passes)

Table: 3.16. Appearance and colour of different gel formulations

Formulation	**Appearance and colour**
Marketed gel	Transparent, homogeneous gel and colourless
Niosomal gel- 0.5%	Transparent,homogeneous gel and fluidy gel

Niosomal gel- 1%	Opaque, homogeneous gel and Off-white
Niosomal gel- 2%	Opaque, homogeneous gel and stiffy mass

Table: 3.17. Viscosity of different gel formulations

Formulation	**Viscosity (cps)**
Marketed gel	12550
Niosomal gel- 0.5%	4500
Niosomal gel- 1%	18200
Niosomal gel- 2%	40850

Table: 3.18. pH of different gel formulations

Formulation	**pH value**
Marketed gel	6.2
Niosomal gel (0.5%)	6.1
Niosomal gel (1%)	5.9
Niosomal gel (2%)	5.9

Table: 3.19. Drug content of different gel formulations

Formulation	**Drug content (%)**
Marketed gel	98.13
Niosomal gel (0.5%)	97.85
Niosomal gel (1%)	97.95
Niosomal gel (2%)	97.52

Table: 3.20. Permeation parameters for optimization of current density

Current density (mA/cm2)	**Q_4 (µg/cm^2)**	**Jss (µg/ cm^2/hr)**	**Kp**	**Er**
0.5 mA/cm^2	8639.2	2159.8	0.230	1.07

1 mA/cm^2	9916.8	2488.12	0.318	1.236
1.5 mA/cm^2	9673.12	2418.37	0.2586	1.195

Table: 3.21. Effect of pulse current on the Permeation parameters

On : Off time (in sec)	**Q_4 (µg/cm^2)**	**Jss (µg/ cm^2/hr)**	**Kp**	**Er**
01:01	10414.23	2603.11	0.2704	1.29
01:02	8541.26	2135.15	0.2283	1.06
02:01	9210.54	2302.55	0.2461	1.19
03:01	11424.4	2856.85	0.305	1.44
04:01	9707.5	2426.8	0.2594	1.23

Table: 3.22. Comparative permeation study of niosomal gel and marketed gel with or without iontophoresis.

Formulations (Gel)	**Q_4 (µg/cm^2)**	**Jss (µg/ cm^2/hr)**	**Kp**	**Er**
Passive diffusion				
Marketed gel	11217.11	1898.24	0.132	--
Niosomal gel 1%	14131.81	2012.41	0.184	--
Iontophoretic diffusion				
Marketed gel	15171.11	3792.9	0.151	1.99
Niosomal gel 1%	22080.18	5520.04	0.2950	2.74

Table: 3.23. Results of stability study

Temperature	**% Entrapment Efficiency**	**Vesicle size (nm)**	**Appearance**
2-8^{o}C	71.98	550.63	White dispersion
R.T.	64.53	-	White dispersion with sedimentation
45^{O}C	46.28	-	White dispersion with sedimentation

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