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FORMULATION AND DEVELOPMENT OF LIPOSOMAL GEL FOR TOPICAL DRUG DELIVERY SYSTEM

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ABSTRACT

Aim: The aims of this study were to develop liposome enriched Dexibuprofen liposomal hydrogels for topical delivery, perform *in vitro* release studies and *in vivo* permeation studies through mice/rat skin, and evaluate the efficacy of liposomal gels against inflammation induced rats. The purpose was to provide the delivery of the topical drug at a sustained rate across intact skin to improve bioavailability and inflammation control for longer period from liposomal gels.

Method: Phosphatidylcholine, Cholesterol and Dexibuprofen were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred into a pear-shaped flask connected to a Rotavapor (Büchi-type). Rotary evaporation method was used for the formulation of liposomes.

Result: liposome prepared was evaluated for particle size measurement, percent drug entrapment, diffusion study, skin permeation study and in vivo study. F-7 batch found to be optimized batch having particle size 5.40 µm, % drug entrapment 61.70, % CDR 75.35 %. Hence F-7 batch further evaluated for skin permeation study, skin deposition study, in vivo study and stability study.

Conclusion: The present study has been a satisfactory attempt to formulate and evaluate liposome of Dexibuprofen and liposomal gel with a providing sustained delivery of drug. From skin permeation study and in vivo study it was concluded that the prepared liposome of Dexibuprofen may prove to be potential candidate for safe and effective sustained drug delivery over an extended period of time which can reduce dosing frequency.

INTRODUCTION: Liposomes have been receiving a lot of interest as a carrier for advanced drug delivery. Liposomes were first produced in England in1961 by Alec D. Bangham, who was studying phospholipids and blood clotting [1]. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble. Water soluble medications added to the water were trapped inside the aggregation of hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer.

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell.

Liposomes can be composed of naturally derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine [2].

The lipid bilayer can fuse with other bilayers, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs, (which would normally be unable to diffuse through the membrane), they can be dilevered past the lipid bilayer [3].

Usually liposomes composed of cholesterol and phospholipids. The structure, composition and proportion being practically the same as in the host cell membranes [3].

Inflammation is a host defense mechanism in response to various stimuli like microbial invasion, heat, cold, irradiation, trauma, tissue necrosis or immune reactions. The manifestation of inflammation is characterized by heat, redness, swelling, pain or loss of function.

Potent anti-inflammatory drugs available today often have serious shortcomings in that they show a wide variety of side effects, especially when used for a long period of time. Often side effects cause discontinuation of the treatment. Thus, there is an evident therapeutic need for improved anti-inflammatory treatment. Many investigators have proposed that liposomes can be utilized as carriers to deliver drugs and thereby improve the therapeutic effect of different types of drugs [4].

Local application of a liposome-encapsulated drug may also be a useful way of maintaining the drug at the site of application for a longer period of time and thereby improve its therapeutic efficacy [5].

It is generally thought that the main mode of action of NSAID is inhibition of the cyclooxygenase. Such an inhibition would result in inhibition of production of prostaglandins, prostacyclin and thromboxane. These are arachidonate metabolites which can initiate as well as maintain inflammation. The most common side effect of the NSAIDs involves ulceration of gastrointestinal tract. This ulceration can also be explained on the basis of inhibition of prostaglandin generation because these phospholipid metabolites are important for the maintenance of the protective mucus layer of the gut.

NSAIDs are the most widely used anti-inflammatory drugs even though they have to be used with restriction due to the adverse side effects.

Liposomes have been widely used to enhance the efficiency of drug delivery though various routes of administration and have been shown to be significantly superior to conventional dosage forms especially for intravenous and topical administration. However, therapeutic applications of systemically administered liposomes have been limited by their rapid clearance from the bloodstream and their uptake by reticuloendothelial system (RES) in liver and spleen. Furthermore, the use of liposomal formulations in oral administration has been limited due to physiological factors [6].

The three major factors are pH, bile salt, and pancreatic enzymes in the gastrointestinal (GI) tract can destabilize the structure of the vesicles and limit their potential.

Topical liposomal administration might offer an opportunity for developing a novel delivery system that could overcome these limitations experienced with the systemic and oral liposomal formulation as well as conventional products. The major advantages of topical liposomal drug include:

1. Reduction of side effects and incompatibilities that may arise from undesirably high systemic absorption of drug.
2. Markedly increasing the liposomal drug accumulation in the desired tissues.
3. Capability for incorporation of a wide variety of hydrophilic and hydrophobic drugs.

Additionally, their ability to provide a sustained/controlled release and an enhancement of the cellular penetration of the incorporated material could improve their potential for being applied topically [7].

Skin has been considered as a promising route for the administration of drugs because of its accessibility and large surface area. Topical drug delivery system, designed to deliver a variety of drugs to the body through diffusion across the skin layers, is appealing for several reasons including avoidance of the variable

absorption and metabolic breakdown associated with oral treatments, drug administration can be continuous, and minimal intestinal irritation can be avoided [8].

Liposome is has been used in topical drug delivery system because of its much higher diffusivity in skin compared to most bare. Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used to administer drugs by several routes such as the oral, parenteral, and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have to act topically, such as local anaesthetics, but also for enhancing topical delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration.

Due to the fore mentioned advantages, in this study liquid-state liposomes were chosen to serve as the drug delivery system. Although liposomes demonstrated promise for Topical drug delivery, the practical application of these formulations onto the skin is less. However, these can be incorporated into the gels than can be applied onto the skin. It has been found that liposomes incorporate into the gels are stable [9].

However, the major limitation of using liposomes topically is the liquid nature of preparation. That can be overcome by their incorporation in an adequate vehicle where original structure of vesicles is preserved. It has already been shown that liposomes are fairly compatible with gels made from polymers derived from cross linked poly (acrylic acid), such as Carpool® resins.

Moreover, some Carpool® has proved excellent bioadhesive properties on the mucosal surface that would increase residence time and at the same time increase absorption of the drug. Therefore, it seemed logical to choose gels prepared from Carbopol 974P as a vehicle for the incorporation of liposomes destined for topical delivery.

In this study, such an application for Dexibuprofen has been investigated. Hydrogels are clinically acceptable systems that offer many advantages, such as suitable rheological properties, good tissue compatibility and convenience in handling and ease of application. Carbopol gels are approved for pharmaceutical use in several different administration routes. Cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration and they provide higher and sustained skin concentrations of drugs compared to conventional gels and creams. Moreover, carbopol gels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH [9].

Dexibuprofen[S (+)-Ibuprofen] is considered as pharmacologically active enantiomer of racemic Ibuprofen. It is a nonsteroidal ant-inflammatory drug with analgesic action which acts by inhibiting prostaglandin synthesis and used for the management of pain and inflammation associated with osteoarthritis including dysmenorrhoea and dental pain with dose range 200-400mg 2-3 divided doses, as conventional tablets and in a topical gel dose is 2-5%. It has short biological half-life 2- 4 hrs requires multiple dosing. It leads to fluctuation in the drug blood levels and dose related adverse effects, multiple dosing also fail to release the drug at the desired rate and in the desired amount which often results in poor patient compliance and inefficient therapy [10].

The most common adverse effects of the drug are gastritis, peptic ulceration, local mucosal irritations, and depression of renal functions. Because of the short biological half-life and associated adverse effects, alternate routes other than oral as well as topical route for systemic delivery are preferable [10].

Topical dosage forms are desirable for the chronic use of this drug, especially in the case of rheumatic symptoms and osteoarthritis based inflammation. The efficacy of topical Dexibuprofen depends greatly on the capacity of the preparation to allow the drug penetrates through the skin.

Since the permeability of intact skin for Dexibuprofen is low, in order to increase skin permeability, the promoting effect of ethanol on percutaneous absorption of Dexibuprofen and the combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of Dexibuprofen have been performed.

However, these approaches do not only have limited skin permeability, but also cause skin irritation as well. Stratum corneum (SC) is a main barrier of many compounds passing through the skin. Several approaches have been developed to weaken this skin barrier. One possibility for increasing the penetration of drugs and many cosmetic chemicals is the use of vesicular systems, such as liposomes and niosomes.

The aims of this study were to develop liposome enriched Dexibuprofen liposomal hydrogels for topical delivery, perform *in vitro* release studies and *in vivo* permeation studies through mice/rat skin, and evaluate the efficacy of liposomal gels against inflammation induced rats. The purpose was to provide the delivery of the topical drug at a sustained rate across intact skin to improve bioavailability and inflammation control for longer period from liposomal gels.

MATERIALS AND METHODS: Dexibuprofen was received as a gift sample from Noven lifescience, Hyderabad, Soya lecithin (Phosphatidylcholine) was received as a gift sample from Phospholipid GmbH Nattermannallee, Germany, Cholesterol and Carbopol 974P were purchased from S.D. Fine Chem. Ltd., Mumbai. All other reagents used were of analytical reagent grade.

Preparation of liposomes: Phosphatidylcholine, Cholesterol and Dexibuprofen were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred into a pear-shaped flask connected to a Rotavapor (Büchi- type). Speed was maintained at 150 r/min, vacuum applied and the thin film were formed by slow removal of the solvents at 40^{o}C. The lipid film was maintained under vacuum for 12hr in a desiccator to remove solvent traces and subsequently it was hydrated with a Saline Phosphate Buffer of pH 7.4 solution at 40^{0}C under continuous rotation of the flask until a dispersion was formed (about 1h).

The final suspension consisted of multilamellar vesicles was subjected to vortexing for two 5-min periods and kept for 30 minutes. The formulation plan for the formation of liposome given **table 1** [11, 12].

TABLE 1: FORMULATION PLAN FOR LIPOSOME

Batch Code	Amount of Soya lecithin in mg	Amount of Cholesterol in mg
F-1	100	20
F-2	100	30
F-3	100	40
F-4	150	20
F-5	150	30
F-6	150	40
F-7	200	20
F-8	200	30
F-9	200	40

Dexibuprofen-100mg = Constant

Preparation of Liposomal gel [13]:

Preparation of 1% Carbopol Gel: Carbopol resin 1gm was dispersed in distilled water 88 gm in which glycerol 10 gm was previously added. The mixture was stirred until thickening occurred and then neutralized by drop wise addition TEA until transparent gel appeared.

Incorporation of liposome in 1% Carbopol gel: Liposome containing drug was mixed in to 1% Carbopol gel by an electrical mixer 25rpm/2 min, with the concentration of liposome in hydrogel being 2.5% (w/w liposome suspension / total)

Evaluation of Liposome:

Light Microscopy [14]: Light microscopy has been utilized to examine the gross size distribution of large vesicles. The size of the liposomes can be characterized with a stage micrometer and an eyepiece micrometer. The eyepiece micrometer is calibrated using stage micrometer. The sizes of around 100 particles were measured and their average particle size was determined.

Drug entrapment efficiency [13]: The liposome suspension was ultra-centrifuged at 5000 rpm for 1 hr by using ultra centrifuge to separate the free drug. Supernatant contained liposomes in suspended stage and free drug at the wall of centrifugation tube. The supernatant was collected and again centrifuged at 5000 rpm at for 30 minutes. A clear solution of supernatant and pellets of liposomes were obtained. The pellet containing only liposomes was resuspended in distilled water until further processing.

The liposomes free from unentrapped drug were soaked in 10 ml of methanol and then sonicated for 10 min. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 221.40 nm. The entrapment efficiency was then calculated using following equation.

Amount of drug entrapped = Amount of drug present in supernatant – total amount of drug added.

% Entrapment efficiency

= (Entrapped drug/Total drug added) X 100

Zeta potential (ζ) determination [13]: Charge drug loaded vesicles surface was determined using Zetameter. Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined.

In vitro Release Studies [14]: In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane (HiMedia molecular weight 5000) was placed between receptor and donor compartments. Dexibuprofen liposomal suspension was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (18 ml). The diffusion cells were maintained at 37±0.5^{o}C with stirring at 200rpm throughout the experiment. At fixed time intervals, 1ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible Spectrophotometer at 224nm. Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of Dexibuprofen release from liposomal suspension.

Physicochemical Properties of Dexibuprofen loaded liposomal gel [14]: The liposomes enriched hydrogel were characterized for their physicochemical properties such as color, odor and pH, viscosity and drug content.

Drug content and content uniformity [14]: The gel sample (100mg) was withdrawn and drug (Dexibuprofen) content was determined using UV spectrophotometer at 221.40 nm. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using UV spectrophotometer at 221.40 nm.

Measurement of pH [15]: The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Viscosity study [15]: The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 1.5 rotations per minute and viscosity was measured in Cps.

Skin Permeation Studies [16]: Following procedure is adopted for the study of liposome skin permeation study:-

A. **Selection of Animals:** For the present study, adult male Albino mice (30±5gm) will be selected. During all the work, the Animals will be maintained at a temperature 25±2°C and commercial pellet diet & water ad libitum.

B. Preparation of skin: The abdominal hair of Albino male mice, weighing 30±5 g, was trimmed using trimmer 24hr before treatment. After anesthetizing the mice with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1hr before starting the diffusion experiment. All surgical and experimental procedures were reviewed and approved by the Institutional animal and ethics review committee, Vidya Bharti College of Pharmacy, Amravati, Maharashtra. REG. NO. 1504/PO/11/CPCSEA, dated: 23/09/2011.

C. ***Ex-vivo* Permeation studies:** A system employing improved Franz diffusion cells with a diffusion area of 3.14cm^2 was used for permeation studies. The excised mice skin was set in place with the stratum conium facing the donor compartment and the dermis facing the receptor compartment. Dexibuprofen liposomal gel and plane Dexibuprofen gel was applied to the skin surface in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (18 ml). During the experiments, the diffusion cell was maintained at 37±0.5°C and stirred at

200rpm. After application of the test formulation on the donor side, at fixed time intervals, 1 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible spectrophotometer at 224nm.

D. **Drug deposition studies:** For determination of drug deposited in skin, cell was dismantled after a period of 24 h and skin was carefully removed from the cell. The formulation applied on skin surface was swabbed first with phosphate buffer pH 7.4 and then with methanol. The procedure was repeated twice to ensure no traces of formulation are left onto skin surface. The skin was then cut into small pieces and drug present in skin was extracted in phosphate buffer pH 7.4 using bath sonicator and determined spectrophotometrically after suitable dilution.

***In-vivo* Studies [16]:** The animals used for *in vivo* experiments were adult male Wister rat (150±170gm). The animals were kept under standard laboratory conditions, at 25±1C and 55±5% relative humidity with a 1- 2hr light/dark cycle. The animals were housed in polypropylene cages, with free access to a standard laboratory diet and water.

Carrageenan induced paw edema method was used to study the *in vivo* performance of the prepared drug delivery system. Anti -inflammatory activity was determined by measuring change in the volume of inflamed paw, produced by injection of Carrageenan (0.1ml of 1% w/v) using Plethysmometer. Male Wister rat selected for the study were weighed and marks were made on the right hind paw just behind tibia - tarsal junction on each animal.

Thus, every time the paw was dipped in the Plethysmograph (mercury displacement method) up to the fixed mark to ensure constant paw volume. Rats were divided into 4 groups including one controlled group with each group comprising of 5 animals. The paw volume was noted at 0, 2, 4, 6, 8, 10, 12 and 24hr. The formulations were applied transdermally to Wister rat of respective groups, excluding the animals of controlled group. The controlled group animals were applied with plane gel base containing no drug. After 30min of transdermal application of formulations, 0.1ml of 1%w/v Carrageenan (in 0.9% normal saline) was injected in the sub planter region of the right hind paw of rat. The initial reading just after injection and subsequent paw volumes was measured up to 24hr. The percent inhibition of edema induced by Carrageenan was calculated for each group using the following equation:

$$\% \text{ Inhibition of edema} = 100\left[1-\frac{a-x}{b-y}\right]$$

Where;

a = mean paw volume of treated animals after Carrageenan injection

x = mean paw volume of treated animals before Carrageenan injection

b = mean paw volume of control animals after Carrageenan injection

y = mean paw volume of control animals before Carrageenan injection

Differential scanning calorimetry: The drug-excipients compatibility study was carried by using DSC. DSC spectra help to detect the interaction of the drug with the excipients. DSC spectroscopy of excipients and drug loaded liposome were carried out. The DSC spectra of drug loaded liposome were compared with the DSC spectrum of the pure drug and excipients. DSC measurements were performed on a SIIO 6300 (Japan) differential scanning calorimeter. The accurately weighed sample was placed in an aluminium pan. An empty aluminium pan was used as reference. The experiment was carried out in nitrogen atmosphere at scanning rate of 5^{o}C/min in the range of 0–110^{o}C.

Stability Studies [17, 18, 19]: In the present study, stability studies were carried out at 2-8 ^{0}C, room temperature and at 40^{0}C ±2^{0}C / 75 % RH ± 5 % for a specific time period up to 30 days for the optimized formulation. The liposomal suspensions were kept in sealed vials (20 ml capacity). Samples were withdrawn periodically and analyzed for drug content, in the manner described under drug entrapment studies. The liposome suspension was analyzed for the drug entrapment efficiency and liposome loaded gel formulations were analyzed for the drug content.

RESULTS AND DISCUSSION:

Characterization of Dexibuprofen liposomes and Dexibuprofen loaded liposomal gel:

Physicochemical properties: The liposome suspensions were milky white in color, odorless and fluid in nature. The prepared Liposome Dexibuprofen gel formulations were white viscous creamy preparations with a smooth and homogeneous appearance and Plane Dexibuprofen gel were transparent. They were easily spreadable with acceptable bioadhesion and fair mechanical properties.

Particle Size Analysis: Average particle size of liposome as determined by optical microscopy by using stage micrometer and ocular micrometer are shown in **Table 2** and in **Figure 1** and graphical presentation given in **figure 3**. The particle size of formulation F-7 was found to be 5.40 smaller µm as compared to other formulation.

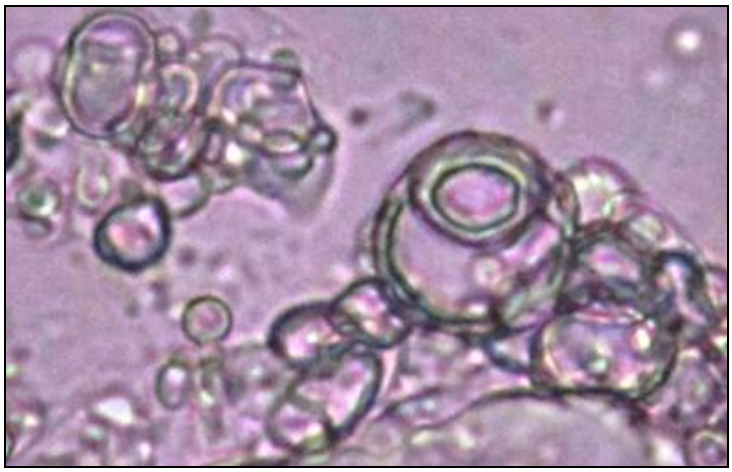

FIGURE 1: MICROSCOPIC VIEW OF DEXIBUPROFEN MULTILAMELLAR VESICLES (A: 10X-15X; B: 45X-15 X)

Scanning Electron Microscopy: The determination of shape and surface morphology was done by scanning electron microscope JEOL-5400, Japan. SEM analysis of the samples revealed that all liposome prepared were spherical in shape.

Scanning electron photographs of the liposome were shown in **Figure 2**.

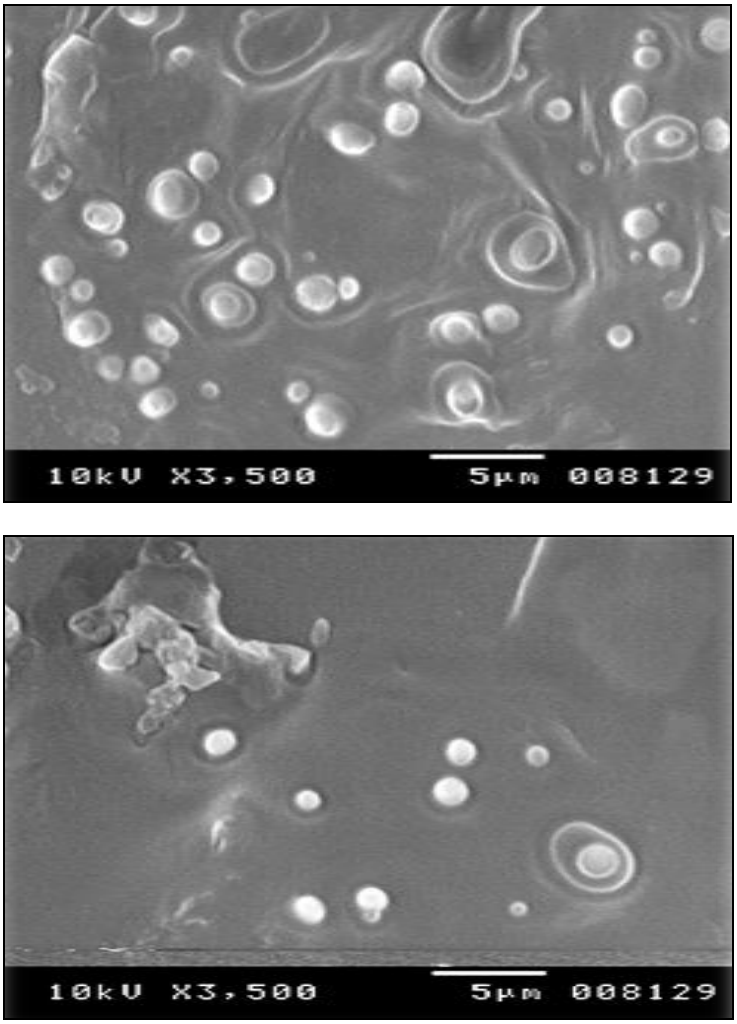

FIGURE 2: SCANNING ELECTRON MICROSCOPE PHOTOGRAPHS OF THE LIPOSOME

Drug Entrapment Efficiency: The results of % drug entrapment efficiency are shown in **Table 2**. The formulation F-1 shows the least entrapment about 46.02% and higher drug entrapment was shown by F-8 formulation. **Figure 4** shows the comparison of % entrapment efficiency of formulations F-1 to F-9.

TABLE 2: RESULTS OF AVERAGE PARTICLE SIZE AND PERCENT DRUG ENTRAPMENT EFFICIENCY

Formulation Batches	Average Particle Size in µm	%Drug Entrapment
F-1	6.12±0.07	46.02±0.510
F-2	7.71±0.13	52.40±0.39
F-3	6.52±0.105	46.11±0.905
F-4	6.96±0.15	48.42±1.33
F-5	6.37±0.12	65.02±0.66
F-6	7.02±0.17	62.73±0.97
F-7	**5.40**±0.14	**61.70**±0.23
F-8	9.18±0.08	68.25±0.720
F-9	7.02±0.095	62.1±0.780

Standard deviation=±

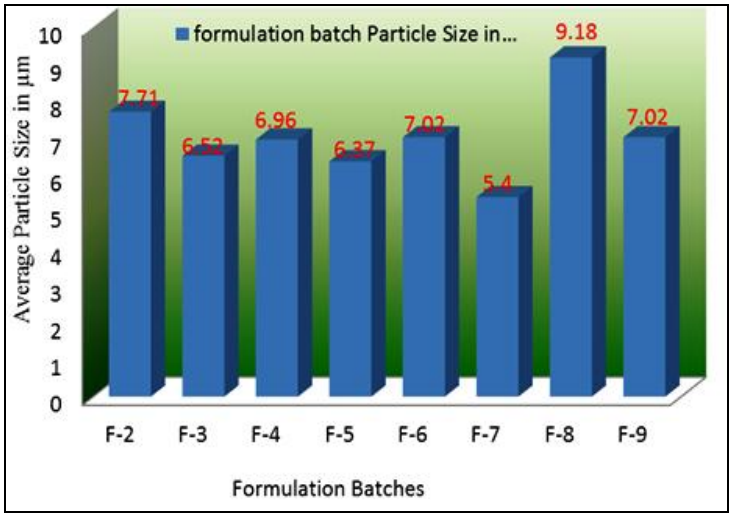

FIGURE 3: COMPARISON OF AVERAGE PARTICLE SIZE OF DEXIBUPROFEN LIPOSOME

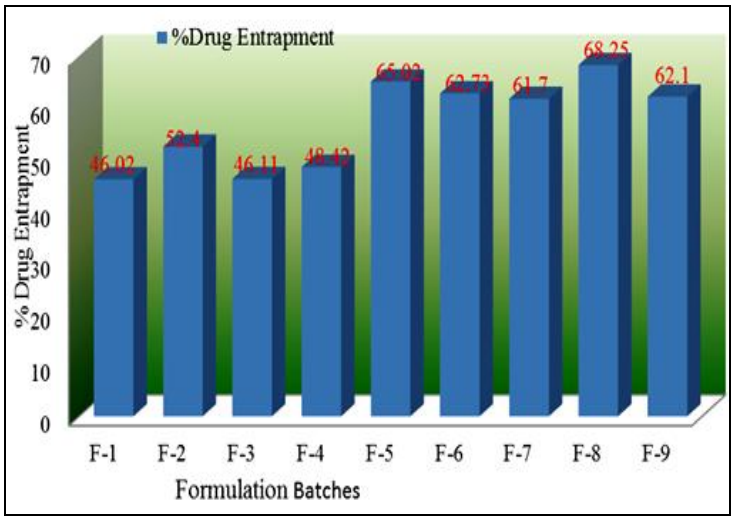

FIGURE 4: COMPARISON OF DRUG ENTRAPMENT EFFICIENCY OF DEXIBUPROFEN LIPOSOME

***In-vitro* Drug Release Study:** *In vitro* release studies were performed using modified Franz diffusion cell. Dialysis membrane (Hi Media molecular weight 5000) was placed between receptor and donor compartments. The *in vitro* diffusion study in saline phosphate buffer pH 7.4 was carried out Using Dialysis membrane. The *in vitro* drug release for F-1 to F-9 shown in **table 3** and the Plot of % Cum. Drug Released Vs. Time For Formulations F-1, F-2, F-3, F-4, F-5 given in **figure 5** and Plot of % Cum. Drug Released Vs. Time For Formulations F-6, F-7, F-8,F-9 given in **figure 6**. *In vitro* drug release of F-7 was found to be highest 75.35% as compared to the other formulation.

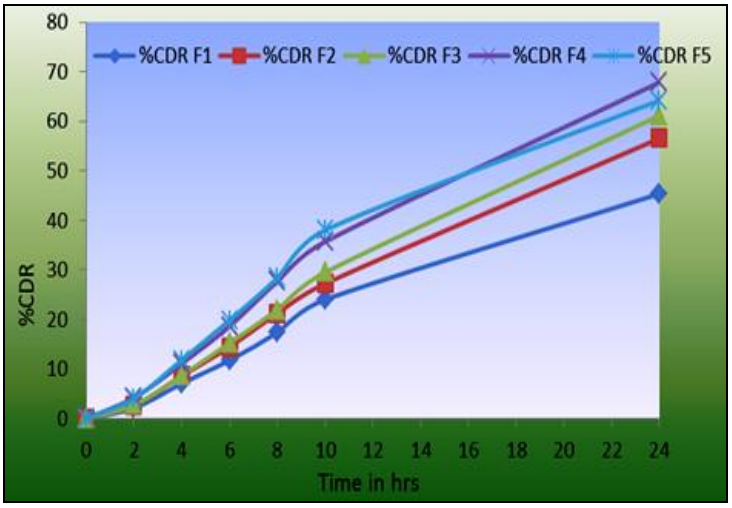

FIGURE 5: PLOT OF % CUM. DRUG RELASED VS. TIME FOR FORMULATIONS F-1, F-2, F-3, F-4, F-5

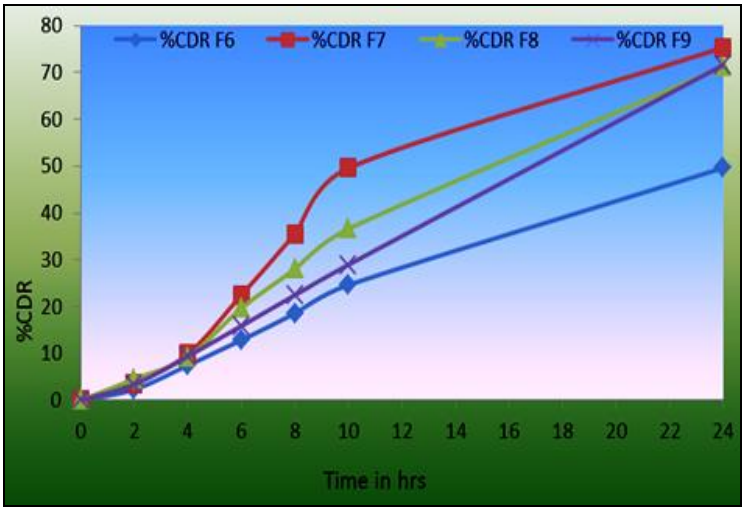

FIGURE 6: PLOT OF % CUM. DRUG RELASED VS. TIME FOR FORMULATIONS F-6, F-7, F-3=8, F-9

TABLE 3: *IN VITRO* DRUG RELEASE FOR F-1TOF-9 FORMULATION

Time in hrs	% Cumulative drug released								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
2	2.1229	2.65	2.73	4.2	4.04	2.32	3.67	4.54	3.37
4	7.0918	8.63	8.74	11.11	11.87	7.47	9.89	9.27	9.42
6	11.7666	14.47	15.25	18.64	19.86	12.87	22.47	19.68	15.89
8	17.3562	21.22	21.97	27.7	28.38	18.56	35.58	28.03	22.47
10	24.0111	27.24	29.52	35.72	38.03	24.63	49.6	36.62	28.966
24	45.3817	56.56	61.14	67.86	64.24	49.65	75.35	71.29	71.61

The cumulative percent drug release for formulation batch F-7 was found to be higher i.e. 75.35% and formulation batch shows smaller %CDR i.e. 45.38 after 24 hours. Form the particle size measurement, percent drug entrapment study and in vitro drug released study i.e., %CDR, it was concluded that formulation batch F-7 having particle size 5.40 µm, percent drug entrapment 61.70 and *in vitro* drug released study i.e., %CDR 75.35% after 24 hours shows good result as compared to other batches.

Though the F-7 batch having drug content was low compared to F-8 batch but the particle size and % CDR was found to be higher. Hence the F-7 batch was considered for the further evaluation study.

pH of gels: The pH values of prepared LDG and PDG were 5.40±0.27 and 5.52±0.15, respectively.

Viscosity of gels: The measured viscosity of prepared LDG and PDG were 11409±185 cPs and 107458±125 cPs, respectively.

Drug content and content uniformity: Drug content of prepared LDG and PDG were 98.40±0.71% and 99.01±0.23%, respectively.

Skin Permeation Studies: The *in-vitro* release profile is an important tool that predicts in advance how a drug will behave *in-vivo.* The *in-vitro* permeation of Dexibuprofen using albino mice skin from LDG (liposome Dexibuprofen gel) was compared with that of PDG (plane Dexibuprofen gel) containing 2.5% w/w of Dexibuprofen. The permeation of Dexibuprofen was calculated in terms of the % cumulative drug released and flux (n=3) at each sampling time points during 24 hours study and results shown in **table 4** and the Cumulative percentage drug release from different formulations i.e. from liposomal gel, plain gel shown in **figure 7**.

TABLE 4: PERCENTAGE CUMULATIVE DRUG RELEASE AND FLUX FROM DIFFERENT FORMULATIONS

Time in Hr	LDG		PDG	
	Avg. %CDR	Avg Flux (µg $cm^{-2}hr^{-1}$)	Avg. %CDR	Avg. Flux (µg $cm^{-2}hr^{-1}$)
0	0.000	0.00	0	0
1	4.379	174.33	5.46	236.06
2	6.423	81.35	13.52	306.86
3	12.291	233.62	22.03	323.88
4	19.874	301.89	30.9	337.4
5	26.293	255.49	39.24	317.42
6	31.584	210.64	49.81	401.85
7	36.988	215.13	59.96	385.89
24	50.662	32.02	78.75	41.93

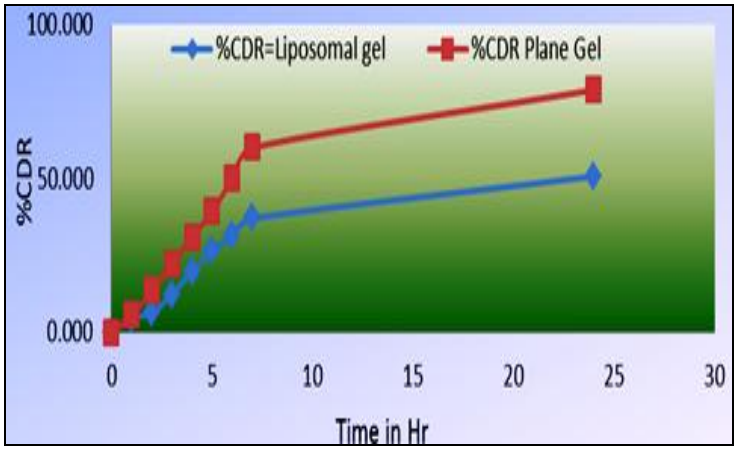

FIGURE 7: CUMULATIVE PERCENTAGE DRUG RELEASE FROM DIFFERENT FORMULATIONS I.E. FROM LIPOSOMAL GEL, PLAIN GEL.

From the results shown in table 4 it can be concluded that cumulative permeation of Dexibuprofen was significantly greater from PDG than from LDG. Plot of % cumulative drug release vs. time (hours) is shown in figure 7. The release of Dexibuprofen from LDG is much slower than from non-liposomal formulations. Dexibuprofen from liposomes showed release of about 50.662% after 24 hours.

The lower flux value of liposomal gel is suggestive of prolong drug release. Multilamellar liposomal formulation produced sustained release of drug because of the presence of several lipid bilayers that release the drug slowly over prolonged period of time.

Skin Retention study: Liposomal encapsulation of Dexibuprofen shows drug reservoir effect in skin so in-vitro skin deposition of Dexibuprofen was also calculated. Results of in-vitro skin deposition are recorded in **table 5**. From results shown in table 5 it can be concluded that liposomal encapsulation showed more drug retention compared with plain drug gel. The higher drug skin retention in case of liposomal gel may be due to, creation of reservoir effect for drug in skin due to deposition of other components of liposomes with drug into the skin and thereby increasing the drug retention capacity into the skin.

TABLE 5: PERCENTAGE OF DRUG RETAINED IN SKIN AFTER 24 HR

Test Formulation	% Drug Retained
Plane Dexibuprofen Gel	15.5%
Liposomal Gel	31.57%

***In vivo* Studies:** The *in vivo* performance of selected Dexibuprofen liposome loaded gel were carried out using Carrageenan- induced rat paw edema method. Results are given in **table 6** and graphical presentation for Percent of inhibition of edema given in **figure 8**.

TABLE 6: PERCENT OF INHIBITION OF EDEMA

Sr. No.	Time in Hrs	Percent of inhibition of edema		
		Teat liposome gel	Test plane gel	Std. Diclofenac Diethylamine gel
1	0	0	0	0
2	2	25.63±0.093	30.31±0.101	18.14±0.0434
3	4	31.74±0.1208	34.1 ±0.0424	25.36±0.1343
4	6	40.28±0.0357	37.46±0.05585	31.6±0.1378
5	8	47.88±0.1572	41.35±0.1364	38.77±0.0497
6	10	56.25±0.1028	46.35±0.0974	45.2±0.101
7	12	68.04±0.0518	50.55±0.067	52.21±0.076
8	24	90.83±0.1648	64.58±0.0738	69.51±0.1545

± SEM

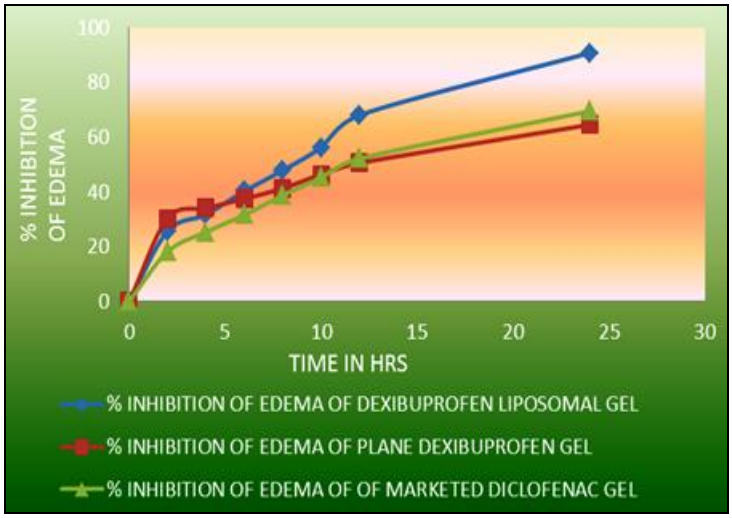

FIGURE 8: PERCENT OF INHIBITION OF EDEMA

Formulations Dexibuprofen liposome loaded gel under study not only decreased the inflammation to the larger magnitude, but also sustained this magnitude. In Dexibuprofen liposome loaded gel formulation the maximum inhibition was observed at 12th hr with higher value 68.04%, and even after 24hr, 90.83% inhibition was observed as compared to plane Dexibuprofen gel and standard Diclofenac Diethylamine gel at 12 hr was 50.55%, 52.21 and at 24 hrs 64.58, 69.51 respectively. The possible reason could be the drug concentration in the blood, which was maintained for longer duration in case of formulation Dexibuprofen liposome loaded gel in comparison to the plane Dexibuprofen gel. In comparison to Dexibuprofen gel, the formulations Dexibuprofen liposome loaded gel which was applied transdermally gave good results. The anti-inflammatory activity of the formulation (Dexibuprofen Liposome Loaded Gel) was maintained for longer period of time due to slow release of the drug. This was attributed to gel structure and the surface active properties of the gel.

The Dexibuprofen liposomal gel showed better pharmacological activity when compared with the plane Dexibuprofen gel this due to the penetration capability of liposome into the deeper layers of the skin and shown better results than the plane Dexibuprofen gel.

Differential Scanning Calorimetry: DSC thermogram of drug, Soya Lecithin, and drug loaded liposome were shown in **figures 9, 10, 11** respectively.

DSC thermogram of Dexibuprofen exhibited single sharp endothermic peak at 53.5°C, corresponding to the melting point of crystalline drug. The DSC thermogram of Soya lecithin was as shown in figure 10. The thermogram of the drug loaded liposome showed no such characteristic peak, indicating that the drug was uniformly dispersed at the molecular level (i.e. amorphous) in liposome. The loss of appearance of sharp peak that indicated a significant reduction of drug crystallinity. It indicated the absence of any strong chemical interaction between drug and other excipients.

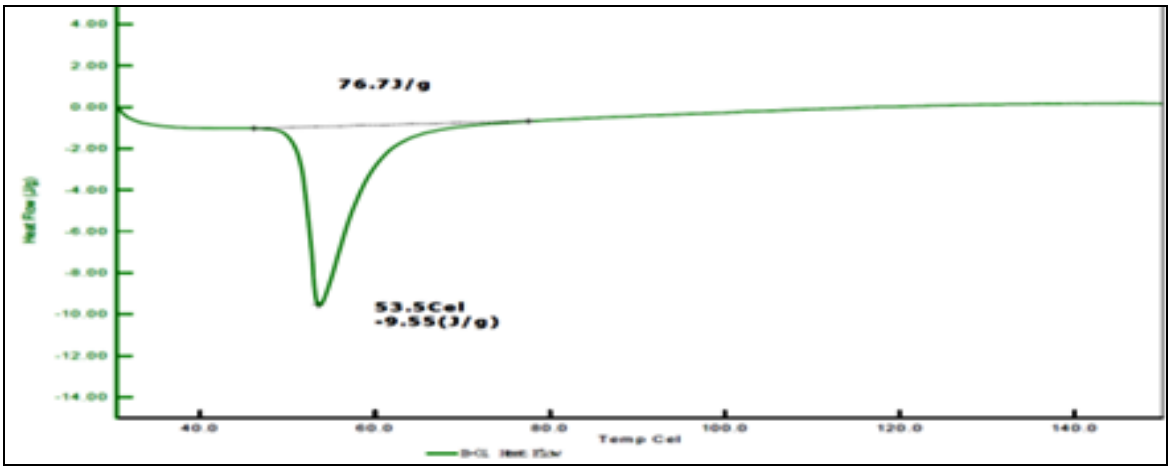

FIGURE 9: DSC OF PURE DEXIBUPROFEN

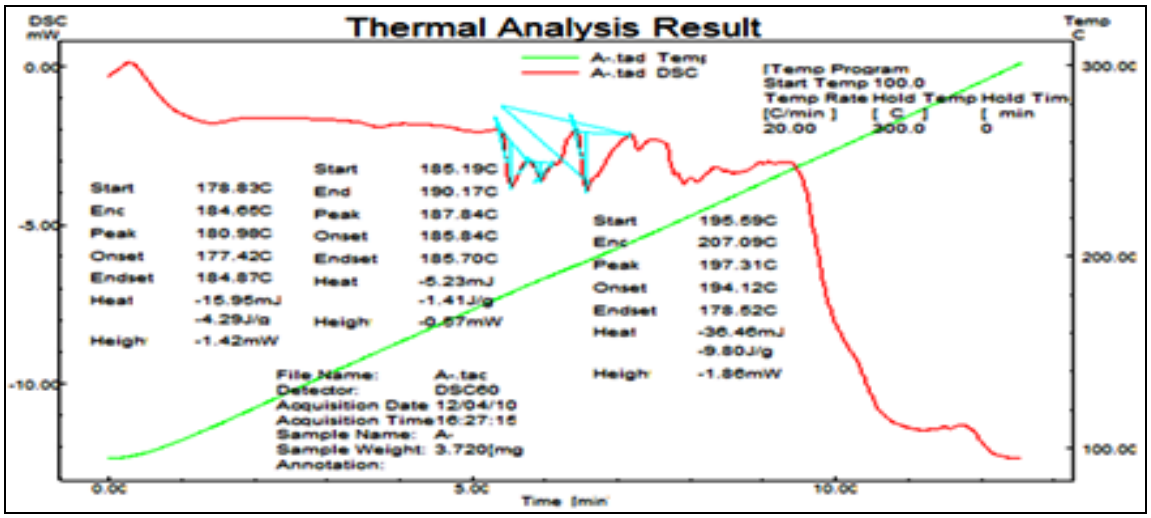

FIGURE 10: DSC OF SOYA LECITHIN

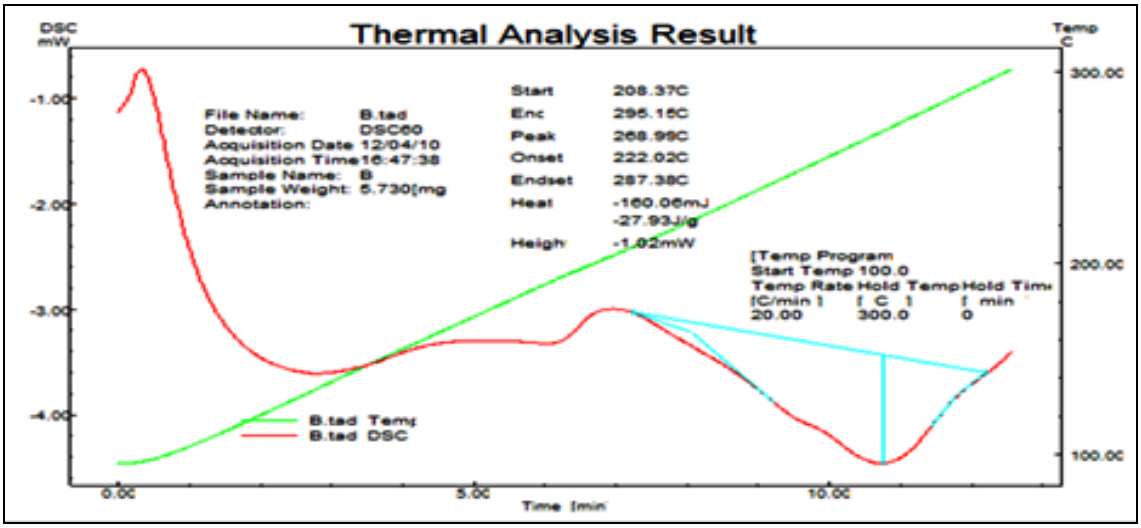

FIGURE 11: DSC OF DEXIBUPROFEN LOADED LIPOSOME

Stability Study: The stability studies of optimum formulation revealed that there is no significant reduction in drug entrapment efficiency and drug content was observed over period of 30 days. The results are shown in **Table 7, 8 and 9** and the Percent drug entrapment Vs days at 2-8°C, room temperature, and 40°C, of liposomal suspension shown in **figure 12** and the Percent drug content Vs days at 2-8°C, room temperature, and 40°C, of liposomal gel in **figure 13**.

TABLE 7: STABILITY STUDY OF LIPOSOME SUSPENSION

Sr. No	Days	Drug entrapment efficiency (%)		
		2-8°C	Room Temp	40±2° C, 75±5% RH
1	0	61.70	61.70	61.70
2	5	61.63	59.56	58.51
3	10	61.58	57.51	55.47
4	15	61.47	53.29	51.23
5	30	61.26	46.11	41.86

TABLE 8: STABILITY STUDY OF LIPOSOME SUSPENSION

Sr. No	Days	Physical Appearance		
		2-8°C	Room Temp	40±2° C, 75±5% RH
1	0	Milky White	Milky White	Milky White
2	5	Milky White	Milky White	Milky White
3	10	Milky White	Milky White	Faint Yellow
4	15	Milky White	Milky White	Faint Yellow
5	30	Milky White	Milky White	Faint Yellow

TABLE 9: STABILITY STUDY OF LIPOSOME GEL

Sr. No	Days	Drug content (%)		
		2-8°C	Room Temp	40±2° C, 75±5% RH
1	0	98.40	98.40	98.40
2	5	98.38	97.71	96.27
3	10	98.33	97.01	94.18
4	15	98.29	96.66	91.09
5	30	98.23	95.07	85.98

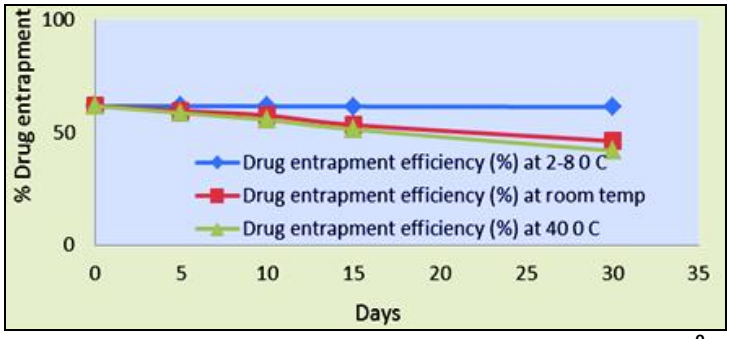

FIGURE 12 PERCENT DRUG ENTRAPMENT VS DAYS AT 2-8°C, ROOM TEMPERATURE, AND 40°C, OF LIPOSOMAL SUSPENSION

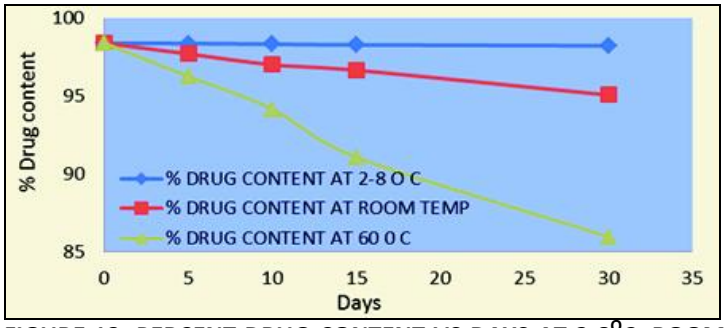

FIGURE 13: PERCENT DRUG CONTENT VS DAYS AT 2-8°C, ROOM TEMPERATURE, AND 40°C, OF LIPOSOMAL GEL

Here, one month stability study of liposomal suspension and liposomal gel were conducted with respect to the liposomes ability to retain an entrapped drug during a defined time period, storage conditions, i.e. at refrigeration condition (2-8°C), at room temperature (25±2°C) and at 40°C. Figure 12, 13 shows liposomes were relatively stable at refrigerated storage condition. The drug leakage percent amounts of original entrapped in liposomes are very small (< 5%) at 2-8°C and have no significant difference after 1 month compared with immediately after preparation.

The results of drug retention studies show higher drug leakage at higher temperature. This may be due to the higher fluidity of lipid bilayers at higher temperature, resulting in higher drug leakage. Loss of drug from the vesicles stored at elevated temperatures may be attributed to the effect of temperature on the gel to liquid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in membrane packing.

Acceleration in drug leakage at higher temperatures, as observed in storage stability studies, suggested keeping the liposomal product in the refrigeration condition.

CONCLUSIONS: The present study has been a satisfactory attempt to formulate and evaluate liposome of Dexibuprofen and liposomal gel with a providing sustained delivery of drug.

From the experimental results it can be concluded that, prior to formulation, preformulation studies were carried out in order to establish compatibility between drug and excipients by DSC spectroscopy. The results of DSC study revealed that there is no physical or chemical interaction between drug and excipients. For the formulation soya lecithin and cholesterol were chosen in varying proportions with the drug.

Film hydration method was used to prepare liposome employing Chloroform and methanol as solvents to dissolve the drug and the excipients. The prepared formulations were characterized for their percentage particle size, morphology, drug entrapment, and in-vitro drug release studies. Almost all the formulations showed fairly acceptable values for all the parameters evaluated. Liposome of different sizes and improved drug entrapment efficiency could be obtained by varying the soya lecithin and cholesterol ratio. The formulations showed good drug entrapment and in vitro released.

The surface morphology of the prepared liposome was studied using scanning electron microscopy. From the SEM study it was conclude that prepared liposomes were spherical in shape.

From the DSC, it was conclude that the physical state drug changes from crystalline to amorphous state. The drug is molecularly dispersed in lipids.

Formulated liposomes were stable at the 2-8^{o}C temperature and humidity in storage for 30 days. From the stability studies it was found that there was no significant change in the drug entrapment and concluded liposome were stable at refrigerator temperature. Incorporation of liposome in Carbopol gel shows better stability for topical drug delivery system.

Hence, finally from skin permeation study and in vivo study it was concluded that the prepared liposome of Dexibuprofen may prove to be potential candidate for safe and effective sustained drug delivery over an extended period of time which can reduce dosing frequency. This study reveals that Liposomal gels were not rapid and fast, but the effect was maintained for prolonged periods. So we conclude that liposomal gels can be successfully utilized for the sustained delivery of Dexibuprofen via the Transdermal route.

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