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Development, Optimisation and Evaluation of Ketoprofen Lipospheres

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ABSTRACT

Lipospheres represent a novel type of fat-based encapsulation system produced for the topical drug delivery of bioactive compounds. The goal of this research work was to develop lipospheres, including ketoprofen applied for topical skin drug delivery. Ketoprofen lipospheres were formulated by melt emulsification method using stearic acid and Phospholipon® 90G. The lipospheres were analysed in terms of particle size and morphology, entrapment efficiency, Differential scanning calorimetry, *In-vitro* drug release, *In-vivo* (Anti-inflammatory activity). Outcomes of research revealed that particle size was found to be 9.66 μm and entrapment efficiency 86.21 $\pm$ 5.79 %. *In-vivo*, the study of ketoprofen loaded lipospheres formulation shows a higher plain formulation concentration in plasma (5.61 mg/mL). For dermis, ketoprofen retention was 27.02 $\pm$ 5.4 mg/mL for the lipospheres formulation, in contrast to that of the plain formulation group (10.05 $\pm$ 2.8 mg/mL). The anti-inflammatory effect of liposphere drug delivery systems was assessed by the xylene induced ear oedema technique and compared with marketed products. Finally, it seems that the liposphere drug delivery system possesses superior anti-inflammatory activity as compared to the marketed product gel consistencies. Liposphere may be capable of entrapping the medicament at very high levels and controlling its release over an extended period. Liposphere furnishes a proper size for topical delivery as well as is based on non-irritating and non-toxic lipids; it's a better option for application on damaged or inflamed skin.

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INTRODUCTION

In the present era, it seems to be marked evident that only the new drug development is not at all sufficient to precede a successful drug therapy. Still, it involves the development of a suitable drug carrier system as well. In this regards, the lipid particles which are based on triglycerides, fatty acids or waxes used as lipid matrix are being characterised as a potential carrier drug delivery system, particularly for lipophilic substances (Bunjes and Koch, 2005).

Lipospheres serves as a type of fat-based encapsulation system intended to be used in topical and parental drug delivery of biologically active com-

pounds. Lipospheres were recorded as a particulate dispersion of solid spherical particles having a particle size ranging from 0.2-100 μm including a solid hydrophobic fat core of triglycerides or fatty acid derivatives which is stabilised by a phospholipid monolayer (Domb, 1990).

Unsaturated fatty acids present in soybean phosphatidylcholine may be held responsible for increasing penetration capacity. The unsaturated fatty acids due to their packing nature, rupture the lipid structure of stratum corneum and hence increases the percutaneous penetration of drug moiety; furthermore, they intensively increase the fluidity of stratum corneum as well.

Moreover, lecithin tends to have a greater affinity for epidermal tissue and seems to have the capability to improve skin hydration. The topically administered phospholipids are considered as safe due to their biodegradable nature and presence of natural body constituents (Kirjavainen *et al.*, 1998).

The internal core of lipospheres consists of the drug dispersed or dissolved in a solid fat matrix. Concerning lipospheres, the inconsistently found nomenclatures as nano-sized scale particles are known as solid lipid nanoparticles (SLN). These are being used in several drug deliveries like anti-inflammatory drug compounds, local anaesthetics, antibiotics, anticancer agents, vaccines, adjuvants and insect repellents (Amselem and Domb, 1996).

One of the best and most commonly used carriers of choice for topical drug delivery is lipospheres like stable lipid nanoparticles (SLN) due to their approved lipid components. i.e. their additives are used in commercially available topical cosmetics or pharmaceutical formulations.

The smaller size of lipid particles promises close contact of the drug to the stratum corneum and may improve the fraction of drug penetrating the mucosa or skin. The important considerations like reduced systemic availability and increased drug stability can be achieved by controlled release through these carriers due to their solid lipid matrix (Souto *et al.*, 2004).

Lipospheres carrier system promises various merits over other systems enlisted as physical stability (avoidance of coalescence), high entrapment efficiency for hydrophobic drugs, economic and reasonable cost of ingredients, easy to formulate and scale-up, high dispersion in the water medium, sustained or prolonged release of entrapped drugs, controlled particle size (Domb *et al.*, 1996).

Ketoprofen falls under the broad classification of NSAID, which is most widely, serve as an analgesic, antipyretic and in the treatment of chronic and acute rheumatoid arthritis and osteoarthritis. Due to its adverse effect like gastrointestinal mucosa ulceration, it is avoided for its oral use, and thus better suited for transdermal drug delivery system (Cordero *et al.*, 1997).

The goal of the work was proposed to formulate Ketoprofen Lipospheres for topical application to overcome the adverse effects resulting from its oral drug administration. The prepared lipospheres was characterised to validate its predefined properties, and anti-inflammatory activity was evaluated and compared with the current standard.

MATERIALS AND METHODS

Ketoprofen was provided as a generous gift sample from BEC Chemical, Mumbai, India (99% purity) stearic acid, xylene were purchased from Loba Chemicals Pvt. Ltd., and Himedia Lab Pvt. Ltd. Mumbai India, Soybean lecithin (Phospholipon® 90G) were purchased from Nattermann, Cologne, Germany (PC content 94–102%).

Diethyl ether, disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride were purchased from Loba Chemicals Pvt. Ltd. and Himedia Lab Pvt. Ltd. Mumbai India. High-Performance Liquid Chromatography grade, Methanol and water purchased from Himedia Lab Pvt. Ltd. Mumbai, India. Dialysis membrane, 12.000 to 14.000 molecular weight cut off was purchased from Spectrum Laboratories Inc., Rancho Dominguez, and Canada. All other excipients were of analytical purity grade and used as received.

Preparation of ketoprofen lipospheres (Melt Method)

Lipid material of variant quantities (stearic acid), i.e. 200, 400 and 600 mg were used as the core material, and quantity weighed 200 mg of soybean phosphatidylcholine was utilised as the coating material. The corresponding core to coat ratio (Cr/Ct) was 1:1, 2:1 and 3:1, respectively. Ketoprofen was also used in various concentrations, i.e. 20 and 40 mg; core material was softening on a water bath.

Then the required quantity of ketoprofen was dispersed into the lipidic melt. Phosphate buffered saline (PBS) 10 ml at 75^0C was mixed with 200 mg of phospholipid. Uniform emulsion was obtained by homogenising a mixture by a magnetic stirrer for 15 minutes. The milky formulated emulsion was then rapidly cooled up to 20^0C with a three-blade rotor continuously for the next 5 min for the formulation of homogeneous dispersion of lipospheres (Nasr *et al.*, 2008).

Determination of Entrapment efficiency

About 40 mg of Lipospheres were accurately weighed and crushed with the help of glass mortar and pestle. Then the powdered Lipospheres were dispersed in 4 ml Phosphate Buffer having pH 7.4 and kept for 24 hrs. After that, the solution was filtered, and the filtrate was analysed for the drug content (Iannuccelli *et al.*, 2006).The formula calculated the entrapment efficiency of the drug:

$$Percentage\ encapsulation\ efficiency\ = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100$$

Characterisation of Ketoprofen Lipospheres

Electron microscopy

By using Scanning Electron Microscopy (SEM) (JELO 5400, Japan), a complete study of surface characteristics of the selected ketoprofen lipospheres formulation were analysed. The samples for SEM were prepared by lightly sprinkling the lipospheres powder on a double adhesive tape which stuck to an aluminium stub. The stubs were then coated with gold to a thickness of about 300Å using a sputter coater.

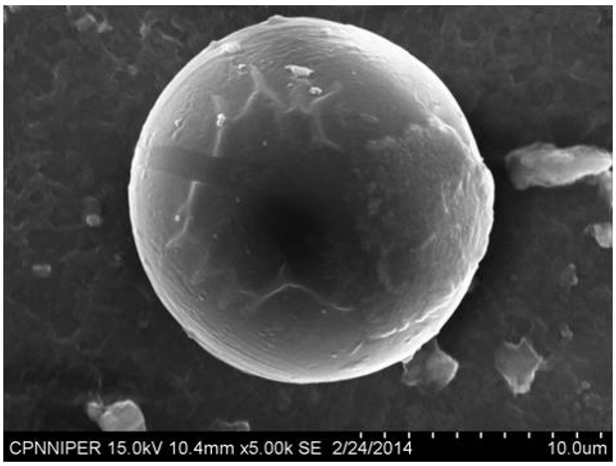

Figure 1: Scanning electron microscope photomicrograph of ketoprofen lipospheres

Particle size analysis

Calibrated stage micrometre method is used to calculate the size of liposphere formulation, 10x lance compound microscope is used for it. Dried lipospheres were firstly re-suspended in distilled water and located in a glass slide using a stage micrometre, the number of division of calibrated eyepiece was counted.

Differential scanning calorimetry (DSC)

Ketoprofen sample, soya lecithin and drug-loaded lipospheres of the selected formulation were analysed by to differential scanning calorimeter (DSC) which was carried out by heating the sample from

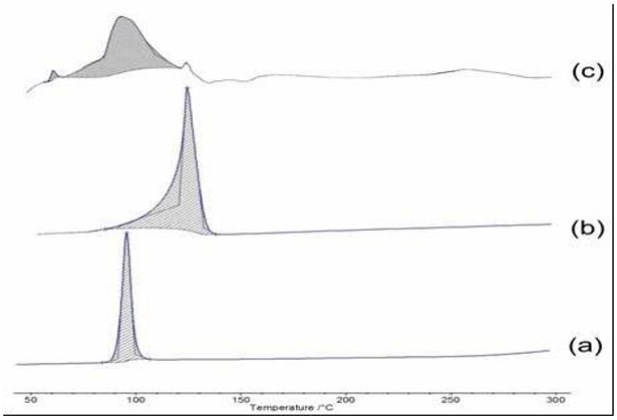

Figure 2: Differential scanning calorimetry thermogram of (a) ketoprofen (b) Soya lecithin (c) Drug loaded lipospheres

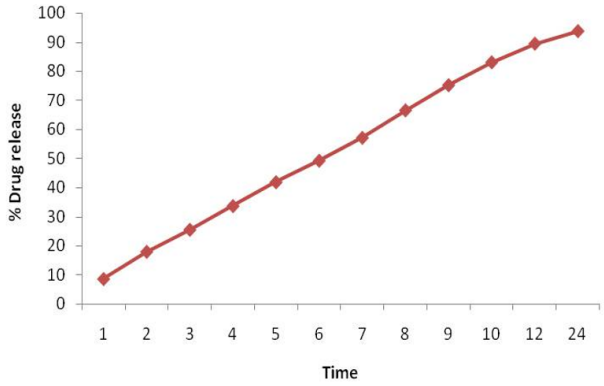

Figure 3: *In-vitro* drug release

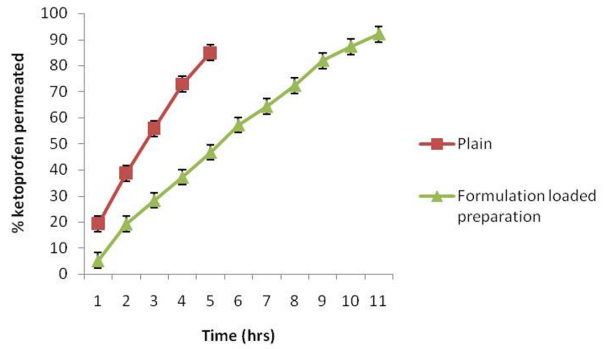

Figure 4: Percentage drug release profile through percutaneous from the excised rat skins

30^0C to 400^0C at the heating rate of 10^0C/min, in a nitrogen environment (nitrogen gas flow rate of 60.0ml/min). The differential scanning calorimetry was conducted on NETZSCH DSC 200F3 240-20-427-L Proteus Software.

High Performace Liquid Chromatography analysis of ketoprofen lipospheres

High Performace Liquid Chromatography (HPLC) system for ketoprofen lipospheres consists of an

Table 1: The Optimisation of Different Lipospheres Preparation, Entrapment Efficiency Percentages, Particle Size of Different Lipospheres Preparations (Mean ± Sd, N = 3)

Batch code	Core / coat ratio	Amount of drug (mg)	Stirring rate (rpm)	Entrapment efficiency per cent ± SD	Particle size (μm)
F1	1:1	20	650	81.12±0.21	12.39
F2	1:1	20	700	82.02±1.23	11.13
F3	1:1	20	750	83.98±2.04	9.08
F4	1:1	40	650	82.50±3.01	13.21
F5	1:1	40	700	83.76±1.23	11.01
F6	1:1	40	750	84.01±5.12	9.05
F7	2:1	20	650	80.01±3.41	13.02
F8	2:1	20	700	81.21±3.08	12.78
F9	2:1	20	750	83.65±0.78	10.04
F10	2:1	40	650	84.23±3.01	12.65
F11	2:1	40	700	86.21±5.79	9.66
F12	2:1	40	750	86.09±1.65	9.42
F13	3:1	20	650	79.45±2.85	17.08
F14	3:1	20	700	81.78±1.04	15.47
F15	3:1	20	750	82.09±2.38	14.04
F16	3:1	40	650	81.92±0.69	19.12
F17	3:1	40	700	82.57±1.49	16.09
F18	3:1	40	750	83.36±3.21	15.01

Table 2: Skin Permeation Parameters of Plain Formulation and Ketoprofen Loaded Formulation

Sr. no.	Formulation	Transdermal flux	Enhancement ratio	Permeation coefficient
1	plain formulation	4.95	1.00	4.95×10^{-4}
2	ketoprofen loaded formulation.	8.053	1.62	8.053×10^{-4}

Table 3: Pharmacokinetic Parameters For In Vivo Permeation Study

Pharmacokinetic parameter	Unit	Ketoprofen loaded lipospheres	Plain formulation
AUC_{O-12}	h mg/l	22.878	4.267
$AUC_{O-\infty}$	h mg/l	23.667	5.743
T_{max}	h	5	8.321
C_{max}	mg/l	5.612	1.32
MRT_{O-12}	h	6.213	8.562
$MRT_{O-\infty}$	h	6.542	9.351

Table 4: Anti Inflammatory Activity of Placebo And Ketoprofen Loaded Lipospheres Compared To The Marketed Gel (Mean ± Sd, N = 3)

Group	Dose	Ear thickness (μm) Normal thickness	2 hrs after treated inflammation	% inflammation inhibition
Control saline (Group – 1)	(Saline) water 10 ml	148±3.6	180±3.6	0
Plain drug (Group – 2)	Cream 20 mg	147±3.1	171±3.1	25
Ketoprofen lipospheres (Group -3)	Cream 40 mg	148±1.7	158±2.1	68
Marketed product (Group -4)	Gel 100 mg	149±2.3	160±1.2	65

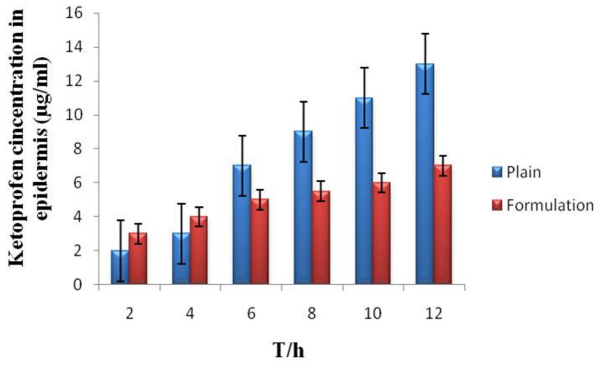

Figure 5: The Amount of Ketoprofen in Epidermis

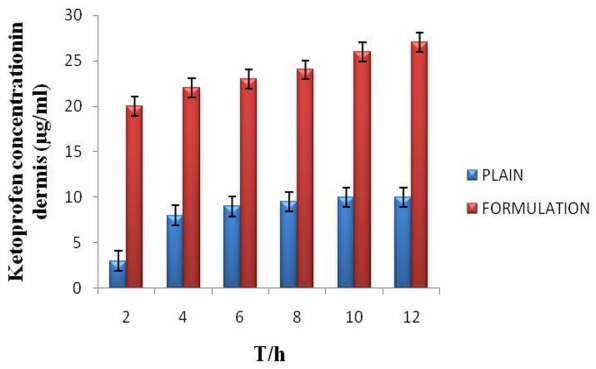

Figure 6: The Amount of Ketoprofen in Dermis

ultraviolet lamp (190-800), and a diode array detector. A 4.6 mm ID × 250 mm (5μm) Zorbax SB-C18 column (Agilent Carry 1220, USA) was utilised. Methanol and water (45:44 *v/v*) are used as a mobile phase at a flow rate of 1.1 ml/min. The analysis was carried out by absorbance at 259 nm.

In-vitro release of ketoprofen from lipospheres

Dialysis tube (MWCO 2000 Da) was used for the study of the release of drug equilibrated at temperature 37 ± 0.5 ^{0}C, 100 rpm speed. Ten milligrams of liposphere were placed into the beaker containing 2 ml phosphate buffer (PH 7.4) and kept in a dialysis tube. For complete separation of the drug in phosphate-buffered saline, the dialysis tube was kept in 50 ml of aqueous recipient phosphate-buffered saline (PBS) medium having pH 7.4 with continuous agitation at 100 rpm and 37±2 ^{0}C. At a suitable time interval, the complete PBS medium was replaced with the required volume of fresh PBS medium and samples were characterised by high performances liquid chromatography (Agilent Cary 1220).

In-vitro permeation study

In-vitro permeation of ketoprofen lipospheres was determined by using full-thickness dorsal skin of albino rat (male, 120 ± 20 g), which was approved by the Institutional Ethical Committee was adopted for this research. Ethical Committee registration no. is 147/PO/a/11/CPCSEA.

All albino rats were made fasted before they are to be used. With the utilisation of the razor, the upper hairs of the dorsal skins were removed. The remaining fur of albino rat is taken away by using 8% sodium sulfide solution. After 24 hrs, when the skin of the rats was harvested, they were sacrificed. Carefully and attentively the subcutaneous connective tissues and fat are removed (Chen *et al.*, 2013). The skin was placed on Franz diffusion cells with the stratum corneum side facing in the upward direction into the donor compartment.

Physiological saline (20 ml) solution containing 1% Tween 80 is filled in a receptor compartment, and the temperature kept at 32 ± 0.5 ºC using a water bath. Nearly 250 ml of ketoprofen lipospheres is applied on the stratum corneum of the skin surface. To maintain an equivalent volume of 0.5 ml of the acceptor fluid is collected at the proper interval and replaced by an equivalent quantity of fresh

medium. The samples were characterised by high-performance liquid chromatography, and the cumulative quantity of ketoprofen permeated through excised skin was plotted as a function of time.

In addition to that ketoprofen fluxes (Js, μg cm^{-2} h^{-1}) through the skin, from the ketoprofen loaded formulation and plain formulation is estimated from the slope of the linear portion of the cumulative quantity permeated within the skin per unit surface area Vs time plot.

Permeation availability examination of BUD

As it has been mentioned above the conditions feeding management of the rat is the same. Before performing a study, the dorsal skins of albino rat is cleaned with a physiological saline solution after taking away the hairs, approximately before 24 hours. Firstly 0.6 ml of ketoprofen loaded lipospheres is applied on the dorsal surface of albino rat and after that simple formulation is used on the dorsal surface (3.14 cm2) on another rat. One ml of blood sample is taken at fixed intervals of time (0.5, 1, 2, 3, 6, 9 and 12 hrs) by retro-orbital puncture, the blood samples were centrifuged at 4000 rpm for 5 minutes, and plasma is obtained from the supernatant. After performing the above procedure, the rat is to be made sacrificed and the administrated skin so collected is appropriately stripped.

To eliminate the ketoprofen remains on the surface, the excised skin is appropriately and thoroughly washed with alcohol and water. Further separate epidermis and dermis a heat separation membrane is to be performed (Kligman, 1963).

After, samples of the skin are applied on a large watch glass (60 $\pm$ 1ºC) for 2 minutes, and by using a dull scalpel blade, the epidermis is scraped from the skin. The sample pieces of the dermis or epidermis are correctly mixed with physiological saline solution (1 ml) and allowed for homogenisation for 3 minutes. The quantity of ketoprofen in the dermis, epidermis or blood samples is studied by using high-performance liquid chromatography, respectively.

***In-vivo* study**

Anti-inflammatory study of lipospheres

Animal handling

The National Institute of Health Guide for the use and care of Laboratory Animals approved by the Institutional Ethical Committee was adopted for this research. Ethical Committee registration no. is 147/PO/a/11/CPCSEA.

120 to 200-gram albino rats of both sexes which are acclimatised within the animal facility of Guru Ramdas Khalsa Institute of Pharmacy, Jabalpur, Madhya Pradesh were kept in standard cages where they are feed and taken care on standard animal pellets (obtained from Nuvita Feed Ltd., Kampala) and water ad libitum.

An albino rat for antipyretic activity measurements, CTO 12667 electronic probe thermometers are used for measurement of basal anal temperature, which should be exceeding 37°C. For the study of anti-inflammatory activity, albino rats were randomly classified into four groups and made free with access to water and food overnight.

Xylene induced ear oedema

Before inducing inflammation, i.e. oedema, the thickness of the right ear of every albino rat is measured using vernier callipers. Inflammation was induced 30 minutes after dose administration by applying 0.05 ml of xylene by employing a microliter pipette (Transferpette®, Germany) through the ear veins until the inner and outer surface of the ear was complete moistened (Kou *et al.*, 2005).

Every albino rat was etherised 2 hours later for anaesthesia by using diethyl ether and therefore, the analysis of the inflamed ear thickness repeated. The thickness of the ear as the reference of anti-inflammation was referred to calculate the extent of inhibition (Akindele and Adeyemi, 2007). Group one saline, group second direct drug, third group formulation on ketoprofen loaded lipospheres, and group fourth marketed product. The experimental procedure was similar in the analysis of the formulated product; Percentage anti-inflammatory inhibition was measured as:

$$(\Delta Tp - \Delta Ts/t)/\ \Delta Tp\ \times\ 100$$

ΔTp: Mean change in ear thickness of the placebo group.

ΔTs/t: Mean change in ear thickness of standards or test group.

RESULTS AND DISCUSSION

Lipospheres shows a promising lipid-based topical drug carrier system, including a lipid core stabilised by a layer of phospholipid coat encapsulated in their surface. Various formulation variables have been found to affect the drug embedment inside the lipospheres as well as its drug release profile. As the concentration of lipid particle size was increased, then drug enclosing efficiency of lipospheres was decreased.

Moreover, the enhanced concentration of lipid affects the size of the particles and hardly may decrease the encapsulated capacity but not signif-

icantly. The lipid concentration, increase in particle size due to increase viscosity of emulsion bigger droplets were formed. But increasing the concentration of lipid, drug entrapment capacity of lipospheres was decreased (Quintanar-Guerrero *et al.*, 1996).

The amount of drug is considered to be held responsible for altering the entrapment efficiency of its own and particle size of the lipospheres. As increased in the size of the particle of ketoprofen, liposphere increases the quantity of the drug. The entrapment effectiveness of medication basically in increment partly with a progressive increase in the quantity of drug but, appreciably decreases to, further because of the way that the proportion of medication to the lipid network at some degree increments and as a consequence to be had area for the medication to be entrapped is reduced. It is viewed as that the bad aqueous solubility of medication is pretty helping accountable for its excessive entanglement performance in hydrophobic centres (Barakat and Yassin, 2006).

Stirring velocity negatively affect the particle length (i.e., as the mixing speed expanded, the molecule size diminished). As the stirring velocity turned into increased, the length of the microdroplet of the emulsion turned into reduced ensuing with inside the formation of smaller length microparticles. These findings are much like the ones reported previously (Aberturas *et al.*, 2002). As the agitation rate increases from 650 to 750 rpm, the particle size of the ketoprofen lipospheres was decreased. So it turned into covered that the particle size of lipospheres turned into managed through agitation rate. This turned into because of the smaller emulsion globules created through a better agitation speed ended in unpredictable formed lipospheres, which furnished greater energy to dispense the oil phase in water, however better stirring rate.

But latter enhancing agitation speed, lump formation due to aggregates of lipospheres. The drug entrapment efficiency increased. The drug entanglement effectiveness enhances because of increment in useful surface area on the decrease of the lipospheres size. The stirring rate of 750 rpm F11 was found to be the optimum parameter for ketoprofen lipospheres, as the drug entrapment efficiency were good high, i.e. 86.21 $\pm$ 5.79 % at this stirring rate. After optimisation study, it was found that the formulation F11 has higher entrapment efficiency and small particle size. Thus formulation F11 was selected for further study.

Electron microscopy

Figure 1 illustrates the SEM of freshly prepared ketoprofen lipospheres. It is revealed that the drug-loaded lipospheres are round in shape. Lipospheres formulated including an excessive quantity of the lipid (drug: lipid ratio) displayed smoother surfaces than those formulated taking a lesser quantity of the lipid. However, irregular surfaces and a small length of the lipospheres have been found for the ones formulated with a decrease quantity of the lipid. When phosphatidylcholine is utilised as a coat, then the resultant particle obtained as a round in shape with the irregular surface (Tursilli *et al.*, 2006).

Analysis of particle size

Results shown in Table 1 reveal that the mean size of the particle for lipospheres extended from 9.65 μm F11 which allow them, appropriate applicants, for a topical route. Lipospheres formulated the usage of Cr/Ct proportion. In any case, by expanding Cr/Ct from 1:1, 2:1 and 3:1 increment with inside the mean particle size. Domb et al. acquired comparable outcomes. They expressed that the common particles size increments with expanding fat to phospholipid molar proportion (Domb *et al.*, 1996) which might be attributed to the expanded consistency of the emulsion formed because of higher measure of lipid utilised in the formulation. By further review of the information screen that was increasing the number of drug services for the formulation of lipospheres, from 20 and 40 mg was went with an expansion in the size of the particle. Comparable outcomes were additionally acquired with ketoprofen lipospheres (Bekerman *et al.*, 2004).

Differential scanning calorimetry

Formula F11 was picked as a delegate of ketoprofen lipospheres because it possessed the best entanglement effectiveness. Pure ketoprofen exothermic peak was found 98°C (Figure 2a). The exothermic peak of the pure soy lecithin was observed at 124.8°C (Figure 2b).

The exothermic peak of lipospheres was found between 65 to 97°C and maximum at 122°C (Figure 2c). In this way, it may be inferred that ketoprofen in the lipospheres was in the crystalline section of a molecular dispersion or a solid solution state in the matrix of lipid. Differential scanning calorimetry i taken into consideration as a device to research the melting actions of crystalline substances such as solid lipid nanoparticles (Hou *et al.*, 2003).

***In-vitro* release of ketoprofen from lipospheres**

The release of ketoprofen from the liposphere sown in Figure 3 shows sustain release characteristics in formulation F11 than different formulations, and the entanglement effectiveness is greater in this formulation on account of diminished particle size. The

lipospheres produced via melt method constituting more quantity of drug in their lipid core results in a phospholipid bilayer. Solid cores rich in the drug are regarded to expose the sustained-release character of medication (Lippacher *et al.*, 2001).

Stearic acid provides the highest T-24 hrs value. It may justify the fact that the hydroxyl group present in stearic acid is accountable for making the matrix more susceptible for hydration in dissolution medium and subsequently giving a hydrophilic pathway to a water molecule to arrive at the medication and change the disintegration rate (El-Gibaly and Abdel-Ghaffar, 2005).

Permeation availability examination of BUD

To study *in-vitro* skin permeation on Franz diffusion cells with an effective diffusional area of 3.12 cm^2 is used. Skin Permeation Parameters of Plain Formulation and Ketoprofen Loaded Formulation are shown in Table 2. An initial amount of ketoprofen in the donor rooms is the same, and flux rates of ketoprofen resulted from permeation experiments is shown in Figure 4. It has been observed that the flux rate of ketoprofen liposphere formulation is significantly higher in quantity than that from the simple formulation. There was no lag time shown in the permeation of ketoprofen loaded liposphere, but that of the control has shown a lag time of 1 hrs. Therefore it indicates that permeation followed the zero-order release kinetics and the steady-state flux (Js) of the simple formulation was 4.95 mg cm^{-2} h^{-1}.

In contrast, that of ketoprofen loaded liposphere formulation was 8.053 mg cm^{-2} h^{-1}, respectively. The difference in the significant permeation may also depend on the property and nature of the carrier. After application, the skin surface is covered by a film of ketoprofen loaded liposphere. The oppressed cover makes drug carrier instantly accessible to the skin, which facilitates drug permeation across the stratum corneum.

Moreover, the lecithin in the formulation may also play a role since it can interact with the skin, joining lipid bilayer and loosening intercellular regions between corneocytes (Sinico *et al.*, 2005). This can be adequately and clearly explained by the ability of plain formulation in increasing skin hydration, which well alimentally results in swelling and opening stratum corneum with time (Zhai and Maibach, 2001).

***In-vivo* permeation study**

The intention of *In-vivo* permeation investigation of ketoprofen loaded liposphere is to verify the plausibility of delivery ketoprofen into the blood via the skin. It is evident from the figure that the dispensing of the liposome formulation has contributed are remarkable ketoprofen concentration were as ketoprofen has shown a much lower concentration for the plain. In Table 3, the pharmacokinetic parameter for in-vivo penetration observe is being shown. As it has been shown that the ketoprofen loaded lipospheres formulation has given better concentration of plasma (5.61 mg/l) when contrasted with the plain formulation (1.32 mg/l). The plasma AUC (0 – t) of ketoprofen loaded lipospheres formulation was 22.87 h mg/l, which is altogether larger in amount than that of the plain formulation (4.267 h mg/l) ($P < 0.05$). This outcome has been gotten because of the nearby contact between stratum corneum and liposphere formulation. By framing an occlusive film on pores and skin surface, the drawn-out hydration of skin is kept up by lipospheres formulation, which gives the stratum corneum a compact structure opens up and assisting in most excellent penetration of ketoprofen into the blood via the pores and skin (Kawadkar *et al.*, 2013).

The total quantities of ketoprofen in dermis and epidermis are being studied the residual ketoprofen inside the skin (Figures 5 and 6). As it has been very clearly shown that ketoprofen content which has been deposited in the epidermis for liposphere formulation ranged 4 to 6 mg/ml, with no appropriate substantial fluctuation; however at the other ketoprofen level form the plain formulation group expanded radically, even as much as13.81 mg/ml at 12 hrs. For dermis, the ketoprofen held was 27.02 $\pm$ 5.4 mg/ml for the lipospheres formulation, manifestly notable than that of the plain formulation set (10.05 $\pm$ 2.8 mg/ml).

Although elevated level in the epidermis is being achieved the generally low level in the dermis for plain formulation solution has just demonstrated the restriction in transferring ketoprofen into deep skin, not to mention blood course. It will also be stated that for distinction in permeability between tests set the distinctive function mode will also be responsible. Closed interaction between lipospheres formulation and the stratum corneum and the small size may be the possible motive why lipospheres formulation can boom ketoprofen infiltrating into the feasible skin, even into the bloodstream. It has been stated in a preceding examine that fluorescence-categorised nanoparticles at a length of 70 nm passed the skin hindrance an is likewise noticeable in the deeper skin layer, which is beneath the stratum corneum layer, wherein as bigger size particles of 700 nm in diameter measurement as proven no penetration (Lademann *et al.*, 2011).

Hence ketoprofen lipospheres formulation acquired in examination with small diameter good very easily and closely contacts to the skin surface and form attachment of meagre films on the skin, improving the entrance of ketoprofen. Thus, it's been certainly proved that so that you can yield fluxes, ketoprofen in the benchmark group ought to permeate freely.

In-vivo anti-inflammatory study

Xylene induced ear oedema

Formulation F11 of lipospheres was given in forms, cream textures as it was affirmed that the presence of a matrix of solid particles with a size in the range of micrometre forms a semi-solid dispersion having the suitable consistency for a topical utility which represents an alternative medication carrier system to emulsions and polymeric nanoparticles (Lippacher *et al.*, 2002). As evident in Table 4, variations among the anti-inflammatory activity of the saline control and plain medication had been additives that possess no *in-vivo* anti-inflammatory activity.

The thickness of the ear as the index of anti-inflammation was used to measure the extent of inhibition. Group one saline, group second plain drug, group thread formulation on ketoprofen loaded lipospheres cream, and group fourth marketed product. Percentage of anti-inflammatory inhibition was calculated. Anti-inflammatory % inhibition of plain drug, ketoprofen loaded lipospheres cream and Marketed Rhofenid Sodium Gel was found to be 25%, 68% and 65%. Ketoprofen lipospheres possess good anti-inflammatory activity as compared to marketed preparation.

The lipospheres scattering stayed more prominent to the promoted item in its capacity to suppress oedema beginning from the second hour and all through the entire investigation time proposing the supported calming action of ketoprofen lipospheres. The occlusive impact of the lipospheres can also advance the entry of actives into the pores and skin (Souto *et al.*, 2005). It is significant for a medication that it ought to sustained release for a prolonged duration of time (Mei, 2003).

As a rule, the high explicit surface territory of the submicron-sized lipospheres helps contact of encapsulated drugs with the stratum corneum ensuring in sustained arrival of the drug for an extensive-time period. It has been recently detailed that the *in-vivo* lipospheres distribution shows an excessive affinity to infected tissue wherein they're properly appropriate for being primarily based totally on non-toxic and non-irritant lipids (Cortesi, 2002). Mixing of phospholipid particles with the pores and skin lipids may be held reliable for altering the characteristics of the keratinised layer. It may have served in addition to maintain the drug molecules inside the skin, consequently main to the extended presence of drug molecules (Bhatia *et al.*, 2004) . The drug in its lipo-solubilised state may have determined facilitated entry into the extreme barrier comprising of stratum corneum.

CONCLUSION

From the above data, it is concluded that ketoprofen drugs are suitable in lipospheres, and it can also be considered a promising delivery system for this drug. Liposphere can be able to entrap the drug at very high levels and sustain its release over an extended time. Liposphere possess a suitable size for topical route and is based on non-irritative and non-toxic lipids, and it is perfectly suited on damaged or inflamed skin. As compared to the market product, lipospheres have high stability as well as superior anti-inflammatory.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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