Development and characterization of liposome-enriched ketoprofen liposomal hydrogels

Abstract

The aim of this study was to develop liposome-enriched Ketoprofen liposomal hydrogels and carry out *in vitro* release profile experiment. The aim was to achieve sustained topical drug delivery for extended time interval from liposomal gels. Phosphatidylcholine, Cholesterol and Ketoprofen were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred to a flask attached to rotavapor. Rotavapor method was used to formulate liposomes. The liposomes prepared were assessed for particle size and percent drug entrapment. F-7 and F-8 batches were found to be optimized batches having optimum sizes, drug entrapment efficiencies and cumulative drug releases. F-8 batch was further evaluated for stability study. In the current study, Ketoprofen liposomes and liposomal gel were prepared and characterized *in vitro*. The results show that the prepared liposomes of Ketoprofen might turn out to be potential candidates for effective and safe sustained drug delivery thereby resulting in the reduction of dosing frequency.

Keywords: liposomes, hydrogels, ketoprofen, carbopol, NSAIDs

Introduction

Liposomes were first produced in England in1961 by Alec D. Bangham, while studying blood clotting and phospholipids **1**. Liposomes are spherical vesicles with membranes consisting of a phospholipid bilayer and are used for the delivery of genes and drugs to a cell. Liposomes can be prepared from natural phospholipids with mixed lipid chain-like egg phosphatidylethonalimine **2**.

The liposomal lipid bilayer has the potential to fuse with other bilayers, therefore releasing their content. By preparing liposomes in a solution of drugs or DNA, the delivery past lipid bilayer can be achieved **3**.

Potent anti-inflammatory drugs possess a range of side effects, particularly when being used for prolonged time. It has been observed that these side effects often result in suspension of the therapy. Therefore, there is a dire therapeutic need for the improvement of anti-inflammatory therapy. Researchers have suggested that liposomes could be used as drug delivery agents thus improving the therapeutic effects of various drugs **4**. Liposome encapsulated drug's local application might prove beneficial in sustaining the drug level at application site for longer duration and thus increase its therapeutic efficiency **5**.

For topical and intravenous administration, liposomes have been extensively used in the enhancement of drug delivery efficiency and are being considered better in comparison to conventional dosage forms. Nonetheless, owing to their quick bloodstream clearance and their reticuloendothelial system (RES) uptake in spleen and liver, their therapeutic applications of have been limited. Moreover, due to physiological factors the oral use of liposomal formulations has also been restricted **6**.

Besides, due to their sustained/controlled drug release potential and improvement of the payload cellular penetration improves their prospective for being administered topically **7**.

Because of its approachability and huge surface area, skin has been considered as a likely route for drug administration. Due to factors such as minimal intestinal irritation, continuous drug administration, evasion of oral treatment-associated metabolic breakdown and avoidance of the variable absorption, topical drug delivery systems designed for drug delivery through diffusion across the skin layers is interesting **8**. Researchers have found that liposomes-incorporated gels are stable **9**.

Ketoprofen is a nonsteroidal ant-inflammatory drug with analgesic action which acts by inhibiting cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes reversibly, which decreases production of proinflammatory prostaglandin precursors. It has short biological half-life 2- 2.5 hours requires multiple dosing. It results in the variation of blood levels of drug and dose-related side effects, multiple dosing frequencies has been found to possess variable rates and quantity of drug release thus leading to inefficient therapy and poor patient compliance **10**. Because of associated adverse effects and short biological half-life, alternative routes other than oral are preferable such as topical route for systemic delivery.

The aim of this study is to fabricate liposome-enriched ketoprofen-liposomal hydrogels, perform characterization studies and to perform *in vitro* release studies. The goal is to offer topical drug delivery at a constant rate across integral skin to control inflammation and enhance bioavailability for longer duration from the gels.

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Materials and Methods

Chemicals and Reagents

Ketoprofen, Soya lecithin (Phosphatidylcholine), Cholesterol, phosphate buffered saline, chloroform, triethylamine, glycerol, methanol and Carbopol 974P.

Preparation of liposomes:

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

The final suspension containing multilamellar vesicles were vortexed for two 5-min intervals and were kept for 30 min **11-12**.

Table 1. Liposomal constituents (Ketoprofen=100mg in all formulations)

Formulation Code	Quantity of lecithin (mg)	Quantity of Cholesterol (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

Preparation of 1% Carbopol Gel:

1g carbopol resin was dispersed in 88g distilled water in which 10g glycerol was added previously. The mixture will be stirred until thickening will occur and then neutralized by drop wise addition TEA until transparent gel appears **13**.

Incorporation of liposomes in 1% Carbopol gel:

Liposome-containing drug was mixed in to 1% carbopol gel by using an electrical mixer at 25rpm/2 min, with the liposomal concentration in hydrogel kept at 2.5% (w/w liposomal suspension / total).

Drug entrapment efficiency:

The liposome suspension was ultra-centrifuged at 5000 rpm for one hour by using ultracentrifuge to isolate the free drug. Supernatant contained suspended liposomes and free drug was found to be gathered on the wall of centrifugation tube. The supernatant was extracted and centrifuged again at 5000 rpm for 30 min. Clear solution of supernatant and liposomal pellets was obtained. The blank liposomal pellet was resuspended in distilled water.

The liposomes without unentrapped drug were soaked in methanol (10 ml) and then subjected to sonication for 10 min. To release the drug, vesicles were broken and the drug content calculated. The drug absorbance was recorded at 221.40 nm. The entrapment efficiency was calculated using the equation given blow.

Quantity of drug entrapped = Quantity of drug present in supernatant – total quantity of drug added.

% Entrapment efficiency = (Entrapped drug/Total quantity of drug added) X 100

Particle size and Zeta potential determination:

Particle size and charge on drug-loaded vesicles' surface was determined using particle analyzer. Analysis time was 60 s and average zeta potential on the liposome was determined.

In vitro Release Studies:

In vitro release studies were performed using dialysis bags. Dialysis membrane (molecular weight cut-off 5000) was placed in the beakers container buffer. Ketoprofen liposomal

suspension was placed beaker containing buffer, pH 7.4. The beakers 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 563nm.

Physicochemical Properties of ketoprofen loaded liposomal gel :

The liposomes-enriched hydrogel was characterized for its physicochemical properties including drug content, viscosity, pH, odor and color.

Drug content and content uniformity:

The gel sample (100mg) will be withdrawn and drug (Ketoprofen) content will be determined using UV spectrophotometer at 563 nm. In case of liposomal gel, it will be shaken with sufficient quantity of methanol to extract the drug and will then be analyzed by using UV spectrophotometer at 563 nm.

Measurement of pH:

The pH of different formulations of gels was determined on digital pH meter. 1g gel was dissolved in 100 ml distilled water and stored for 2 h. The pH measurement of all formulations was done in triplicate and average values were calculated **15**.

Stability Studies:

In this study, stability study was conducted at 2-8 ^{0}C, at room temperature and at 40^{0}C ±2^{0}C / 75 % Relative Humidity ± 5 % for specified time up to 30 days for optimizing the formulation. The liposome suspension was kept in 20 ml sealed vial. The samples were withdrawn intermittently and analyzed for drug content, following the method described in drug entrapment studies section. The liposome suspension was analyzed for entrapment efficiency of drug and liposomes-loaded gel formulations were analyzed for drug content **17-19**.

Results and discussions

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 Ketoprofen gel formulations were white viscous creamy preparations with a smooth and homogeneous appearance and Plane Ketoprofen gel were transparent. The gel was easily spreadable and possessing fair mechanical properties with acceptable bioadhesion.

Particle Size Analysis:

The average particle size of liposomes was determined using zetasizer as described in Table 2 and Figure 1. The particle size of formulation F-8 (5.62 µm) was discovered to be smallest in comparison to other formulations.

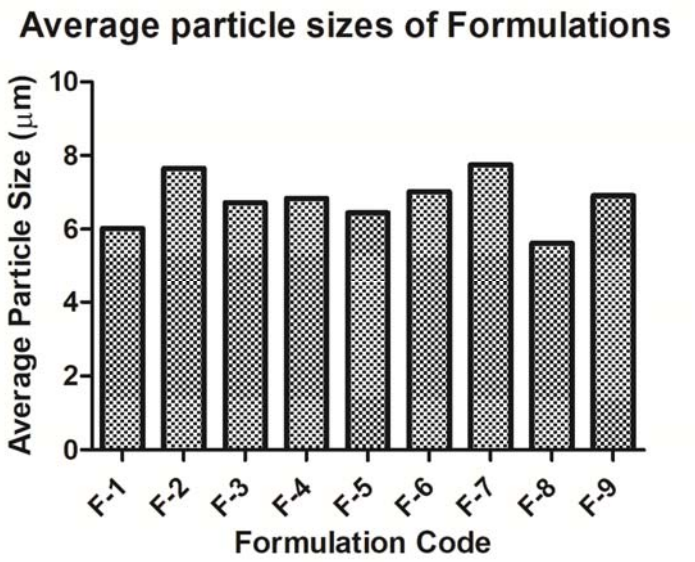

Figure 1. Average Particle sizes of ketoprofen-loaded formulations.

Table 2. Average Particle Size (µm) and Drug Entrapment efficiencies (%)

Formulation Code	Average Particle Size (µm)	Drug Entrapment (%)
F-1	6.02±0.06	43.12±0.410
F-2	7.65±0.06	47.37±0.33
F-3	6.72±0.74	51.46±0.66

F-4	6.84±0.11	53.11±1.09
F-5	6.45±0.66	67.07±0.34
F-6	7.02±0.17	62.73±0.97
F-7	7.75±0.10	64.30±0.24
F-8	**5.62±0.81**	**61.25±0.65**
F-9	6.91±0.06	66.1±0.44

Drug Entrapment Efficiency:

The results of % drug entrapment efficiency are shown in Table 2. The formulation F-1 shows the least entrapment about 43.12% and higher drug entrapment was shown by F-5 formulation. Figure 2 shows the comparison of % entrapment efficiency of formulations F-1 to F-9.

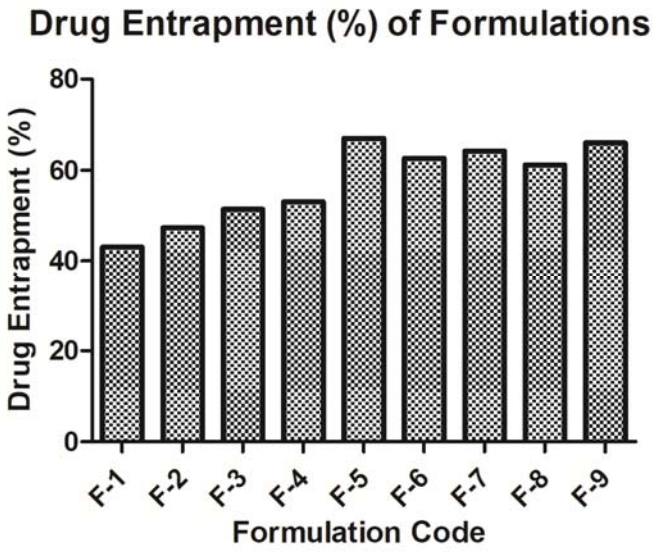

Figure 2. Drug Entrapment (%) of ketoprofen-loaded formulations.

In vitro Release Studies:

In vitro release studies were performed using dialysis bags. Dialysis membrane (molecular weight cut-off 5000) was placed in the beakers container buffer. Ketoprofen liposomal suspension was placed beaker containing buffer, pH 7.4. The beakers were maintained at 37±0.5oC 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 563nm. The *in vitro* drug release from formulation F-7 was discovered to be highest 75.35% in comparison to other formulations.

From the *in vitro* drug release study, percent (%) drug entrapment study and particle size analysis, it was concluded that formulation batch F-7 and F-8 showed promising results in comparison to other batches. Although the batch F-7 had low drug content in comparison to F-8 batch however the % CDR and particle size were found to be higher. Henceforth the batch F-8 was considered for the further studies.

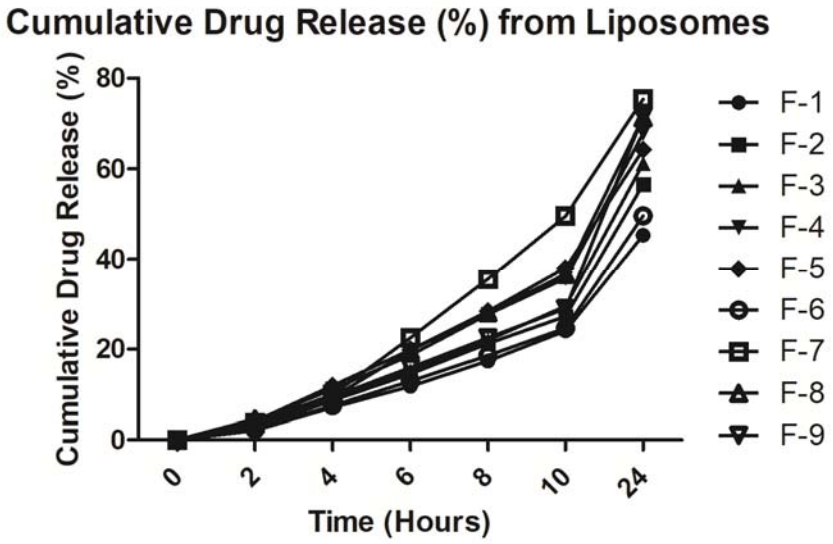

Figure 3. Cumulative Drug Release (%) from Liposomes

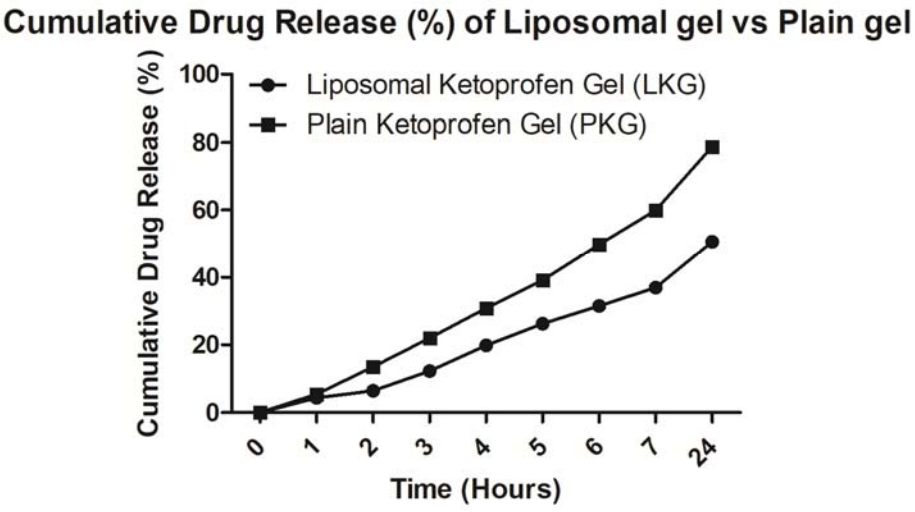

Figure 4. Cumulative drug release (%) from liposomal gel (LKG), plain gel (PKG)

From the results shown in Figure 4 it can be stated that cumulative permeation of Ketoprofen was significantly greater from PKG than from LKG. Plot of % cumulative drug release vs. time (hours). The release of Ketoprofen from LDG is much slower than from non-liposomal formulations. Ketoprofen from liposomes showed release of about 50.662% after 24 hours.

pH of gels:

The pH values of prepared LKG (liposomal ketoprofen gel) and PKG (plain ketoprofen gel) were 5.440±0.11 and 5.62±0.33, respectively.

Drug content and content uniformity:

Drug content of prepared LKG and PKG were 97.40±0.71% and 96.01±0.23%, respectively.

Stability Study:

The stability study showed that there was no significant reduction in drug content and drug entrapment efficiency over a time period of 30 days. The results are shown in Figure 5 and the drug entrapment (%) Vs days at 2-8^{o}C, room temperature, and 40^{o}C, of liposomal suspension shown in figure and the Percent drug content Vs days at 2-8^{o}C, room temperature, and 40^{o}C, of liposomal gel in Figure 6.

One month stability study of liposomal gel and liposomal suspension was conducted to observe the liposomal potential to keep entrapped drug over the time period at various conditions such as refrigerator condition (2-8°C), at room temperature (25±2°C) and at 40°C. Figure 5, 6 shows that liposomes remained comparatively stable at 2-8°C. The drug leakage (%) entrapped in liposomes was found to be very small (< 5%) at refrigerator condition and no significant difference was observed after 1 month in comparison to the freshly prepared formulation.

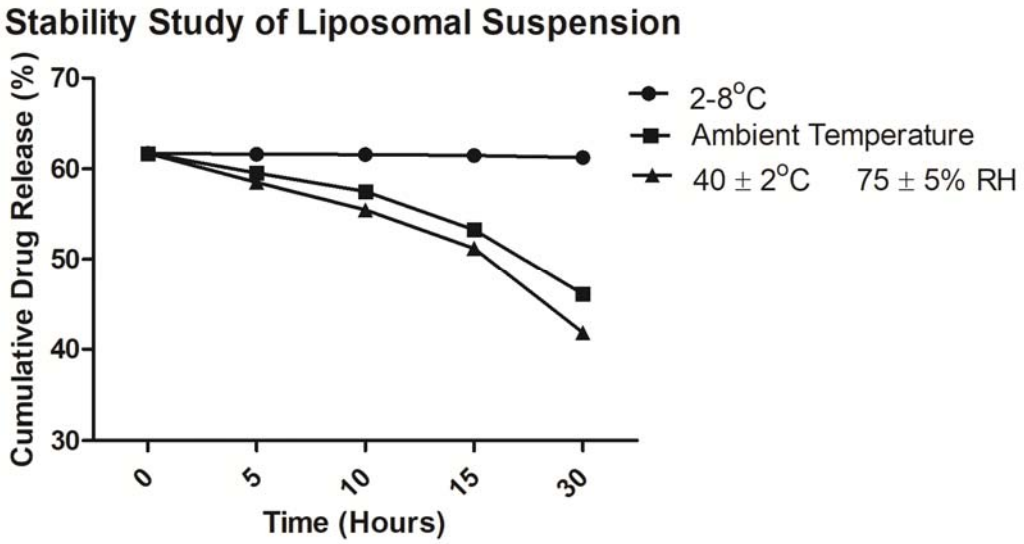

Figure 5. Stability study of Liposomal Suspension

The findings of drug retention study showed high drug leakage (%) at elevated temperature. This might be because of higher fluidity of lipid bilayers at elevated temperatures, leading to high drug leakage. The drug loss from the vesicles kept at high temperature might be because of the effect of temperature on gel to liquid transition of lipid bilayers in addition to likely phospholipid chemical degradation, resulting in membrane packing defects.

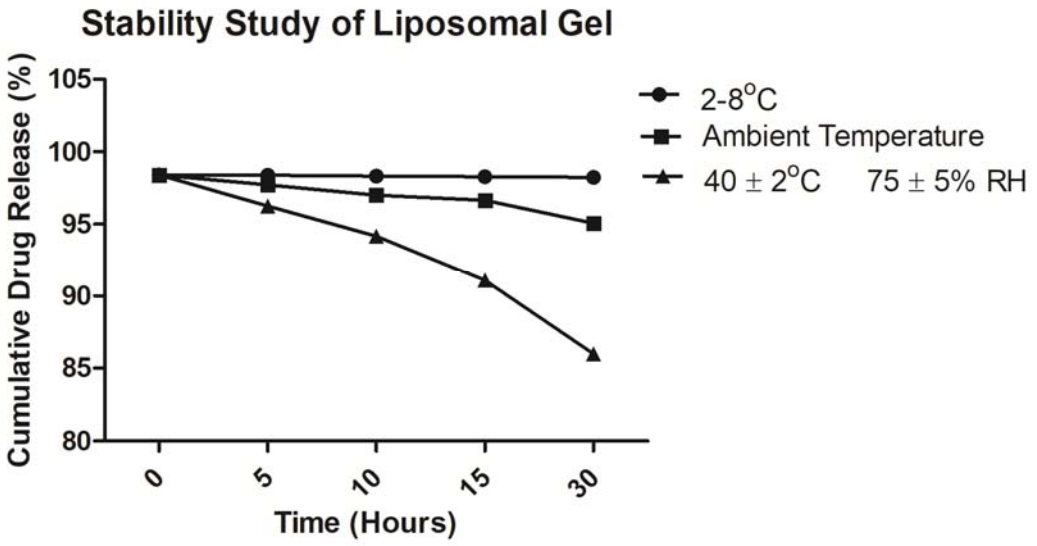

Figure 6. Stability Study of Liposomal Gel

Higher drug leakage at elevated temperature, as found in storage stability study, recommended storing the liposomal product in refrigerator.

CONCLUSIONS:

In the current study, liposomes of Ketoprofen and liposomal gel were formulated and evaluated with an aim to provide sustained drug delivery. Before the fabrication of gel, preformulation study was conducted to know the compatibility between the drug and excipients. Soya lecithin and cholesterol were used at different proportions for the formulation with the drug. Film hydration method was employed to fabricate liposomes and to dissolve the excipients and drug methanol and chloroform were used. The prepared formulations were characterized for in-vitro drug release studies, drug entrapment and particle size analysis. All prepared formulations exhibited satisfactory values for the evaluated parameters. Liposomes of various sizes and enhanced drug entrapment efficiencies can be prepared by changing the cholesterol and soya lecithin ratio. The drug was found to be molecularly dispersed in lipids. The formulated liposomes were stable at refrigerated temperature and humidity for 30 days. No significant alteration in the drug entrapment was found after 30 day study. Liposomal incorporation in carbopol gel showed better stability.

This study revealed that liposomal gels did not release the drug quickly; rather the effect was sustained for longer time. Hence we state that liposomal gels can be used for the sustained delivery of ketoprofen via the Transdermal route.

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