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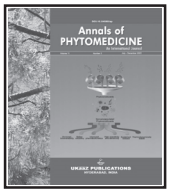

Original Article : Open Access

Design, formulation and evaluation of piperine proliposomal drug delivery system for its anti-inflammatory and antibacterial activity

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Abstract

The main aim of the present investigation was to prepare proliposomes of piperine. Because liposomal suspension has short shelf-life and to overcome the stability concern associated with liposome, a new "pro-liposome" method was developed that can produce liposomes easily when there is a need and without excessive manipulation. Pro-liposomes are the most commonly used film hydration technique, and they are commercially available in powder form, they are very easy to distribute, measure, and store, making them an effective and diverse system. Liposomes can be either produced by *in vivo* method based upon the influence of biological fluids in the body or by an *in vitro* method using a suitable hydrating fluid before administration. Pro-liposomal formulations have been developed to overcome the solubility and bioavailability problems of many drugs.

1. Introduction

All pharmaceutical products formulated for systemic delivery *via* the oral route of administration, irrespective of mode of delivery (immediate, sustained or controlled release) and the design of dosage forms (either solid dispersion or liquid), must be developed within the intrinsic characteristics of GI physiology, pharmacokinetics, pharmacodynamics and formulation design to achieve a systemic approach to the successful development of an oral pharmaceutical dosage form (Chain, 1992; Jukanti *et al.,* 2011; Payne *et al.,* 1986).These agents are formulated to produce maximum stability and bioavailability. For most drugs, conventional methods of drug administration are effective, but some drugs are unstable or toxic and have narrow therapeutic ranges (Duraisami *et al*., 2021; Vijayalakshmi *et al*., 2021). Some drugs also have solubility problems (Payne *et al.,* 1986).

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a phospholipid bilayer molecule. The size of a liposome ranges from 20 nm up to several micrometers. Liposomes are comprised of one or more phospholipids that can entrap both hydrophilic and hydrophobic drugs. Liposomes can be given through various routes. It offers formulations for both controlled and sustained release formulations (Jukanti *et al.,* 2011; Sri Bhuvaneswari *et al*., 2021; Jain *et al.,* 2008).

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Proliposomes are a new type of carrier-mediated drug delivery system that has many advantages over conventional liposomes. Liposomal suspension has a short shelf-life, and a new "pro-liposome" method is developed to overcome the stability concern associated with liposomes. This method can produce liposomes easily when needed and without excessive manipulation. It is composed of porous powders that act as carriers; both phospholipids and drugs are dispersed in an organic solvent. Maltodextrin-based proliposomes are a potentially suitable method that appears to be as good as or better than the conventional liposome preparation (Jain and Jain, 2008; Zylberberg and Mastostevic, 2016).

Piperine is a terpenoid found in the dried, unripe fruit of *Piper nigrum* L. (black pepper), which belongs to the Piperaceae family. It has a strong odour and is used as a stimulant as well as an insecticide. Alongside its use as a traditional medicine, it is insoluble in water but readily soluble in alcohol. Piperine increases oral bioavailability by inhibiting various metabolizing enzymes. Piperine shows anti-inflammatory, antitumor, antioxidant, analgesic, and antibacterial activity. Recent *in silico* studies of piperine show antiviral activity against the CoV-2 virus (Thenmozhi and Yoo, 2017).

2. Materials and Methods

2.1 Materials

Piperine was extracted from of *P. nigrum* (black pepper). Soya lecithin, maltodextrin, and di-sodium hydrogen phosphate were purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Glyceryl monosterate, chloroform, potassium dihydrogen phosphate, and sodium hydroxide were obtained from Loba Chemie Pvt. Ltd. Ethanol was purchased from Dr. Reddy's. Cholesterol, potassium hydroxide,

and HCl were obtained from Nice Chem Pvt. Ltd. Deionized water was delivered by Leo Scientific, Erode.

2.2 Methods

Proliposomes were prepared using the film hydration method, which includes soya lecithin; cholesterol and carriers like maltodextrin are used as stabilizers. Maltodextrin and cholesterol may differ in formulations. Here, maltodextrin was taken in a round bottom flask, and then piperine, soya lecithin, glyceryl monostearate, and cholesterol were added to the flask, and it was dissolved by adding chloroform to it. Some more chloroform was added to the slurry in case the surfactant loading was low. The round bottom flask was inserted, and the solvent was vaporized under reduced pressure using a temperature of 45 ± 2°C at 60 rpm until the final product reached a free-flowing, dry state. The resultant product was kept overnight in desiccators and dried at room temperature under vacuum. The final product obtained was stored in a container and used for further assessment (Rahamathulla *et al.,* 2020; Chu *et al*., 2011).

Table 1: Formulation design of piperine proliposomes

Formulation code	Piperine (mg)	Ratio (µmol) (carrier : cholesterol)	Cholesterol (mg)	Maltodextrin (mg)	Surfactant (mg)
F1	10	1:1	100	100	75
F2	10	1:2	100	200	75
F3	10	2:3	200	300	75

2.3 Lyophilization of proliposomes

The formulated proliposomes were freeze-dried to improve the shelf life and analyse the dissolution behavior. At the time of lyophilization, a cryoprotectant of 1% mannitol was added to each formulation. Proliposomes are lyophilized with a Virtis freeze dryer. After being stored in a Virtis freeze dryer for two days at –50°C at 2 ml, the sample was kept in a deep freezer overnight at –70°C (Ohshima *et al*., 2009).

2.4 Determination of λmax for piperine

10 mg of piperine were dissolved in 100 ml of 0.1 N HCl (pH 1.2) to prepare a 0.1 mg/ml stock solution. From the stock solution, dilute (10 µg/ml) the solution and make up the volume. The λmax was measured using a UV spectrophotometer in the 200-400 nm range, and the same procedure was repeated with phosphate buffer (pH 6.6) to determine the maximum concentration of piperine in the medium (Elnaggar *et al*., 2009).

2.5 FTIR analysis

FTIR spectroscopy was used to investigate the possibility of an interaction between a pure drug and polymers. The FTIR spectrum of pure piperine, soya lecithin, cholesterol, maltodextrin, and the physical nature of the drug were observed (Rahamathulla *et al*., 2020).

2.6 Angle of repose

The angle of repose of the proliposome formulation was determined by the fixed funnel method. The proliposome powder was poured into the funnel, which was fixed at 5 cm above the surface. The powder flowed down the surface, and the angle of repose was calculated by measuring the height and radius of its base (Thakkar *et al*., 2011). The angle of repose was computed using the given formula:

$$\text{Tan } \theta = h / r$$

where, 'h' and 'r' are the height and radius of the powder cone.

2.7 Particle size analysis

The particle size assessment of the proliposome was determined using a Microtac blue wave particle analyzer. Before measuring the sample, it should be diluted with deionized water to a suitable concentration. The result produced confirms the findings of nano-sized particles (Thakkar *et al*., 2011).

2.8 Zeta potential

The zeta potential of prepared proliposomes was analysed using a Malvern zetasizer. Prior to analysis, the samples were diluted with de-ionized water, and conductivity was adjusted by the addition of sodium chloride. The large number of particles was equally charged, increasing the electrostatic repulsion between the particles and thereby increasing the physical stability of the formulation. Particle charge in a colloidal system was traditionally measured as zeta potential, which was determined by electrophoretic mobility of particles in an electrical field (Thakkar *et al*., 2011).

2.9 Saturation solubility study

The saturation solubility studies were carried out for both unprocessed drug and different batches of lyophilized proliposomes. 10 mg of pure drug and proliposome equivalent to 10 mg of piperine was weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks are sealed and placed in rotary shaker for 24 h at 37^0C and equivalent for 2 days. The samples were collected at different time interval and filtered. The samples were analyzed spectrophotometrically at 340 nm (Dixit *et al*., 2012).

2.10 Drug content

When proliposomes are hydrated, there is a possibility that some of the free drugs will present outside liposomes, and it is important to know the amount of free drug. This can be calculated by determining whether the drug is encapsulated or free of encapsulation. Separation of free drugs is achieved by using ultra-filtration. The drug content of the developed proliposomes was determined using a UV-Visible spectrophotometer. Weighed batches of proliposomes containing 5

mg piperine were dissolved in 10 ml of ethanol. The stock solutions were diluted with distilled water and analysed by UV absorbance at 340 nm (Ahn *et al.,* 2009).

2.11 Entrapment efficiency

The efficiency of entrapment is determined by hydrating the proliposome to form liposome dispersion and then separating the unentrapped drug. Separation by ultracentrifugation followed by ultra-filtration can be used to determine unentrapped drug (Gupta *et al*., 2011).

Percentage drug entrapped is given by,

EE % = Entrapped drug / Total amount of drug added × 100

2.12 *In vitro* drug release studies

The *in vitro* release of piperine and its formulated proliposomes was carried out in a USP-Type II dissolution apparatus using the paddle method at a rotation speed of 50 rpm. The dissolution study was carried out in freshly prepared acidic buffer (pH 1.2) and also in phosphate buffer (pH 6.6). 10 mg of pure drug and a proliposome containing 10 mg of piperine were taken and kept in dissolution medium. The volume of dissolution medium was 900 ml, and the temperature was maintained at 37.0 ± 0.2^{0}C, respectively. Samples were withdrawn at various time intervals and then filtered. The filtered samples were analysed at 340 nm using a UV-Visible spectrophotometer. The results obtained for different proliposome formulations are compared with the dissolution profile of an unprocessed drug (Ezawa *et al*., 2015).

2.13 Drug release kinetic data analysis

The release data obtained from the best formulation was further investigated for fitness in various kinetic models such as Zero-order, Higuchi's, and Peppa's 'r^2' and 'k' values were calculated for the linear curve obtained by regression analysis of the plot (Ezawa *et al*., 2015).

2.14 Scanning electron microscopy

The surface morphology and size distribution of proliposomes were studied by scanning electron microscopy. Piperine proliposomes were subjected to scanning electron microscopy to confirm their nano-sized formulation and morphological structure. The sample was lightly sprinkled on a double-sided adhesive tape stuck to an aluminum stub, and the stubs were coated with platinum of a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The stubs containing coated samples were placed in a scanning electron microscopy chamber to analyse the surface morphology (Niehaus and Samuelsson, 1968).

2.15 Powder X-ray diffractometry

Piperine and piperine proliposome X-ray diffraction patterns were studied using an X-ray diffractometer with copper as the target filter and a voltage/current of 40 KV/Ma at a scan speed of 1°/min. The samples are analysed at a 2θ-angle range of 5-70° (Chu *et al*., 2011).

2.16 *In vitro* anti-inflammatory activity

2.16.1 Denaturation of protein albumin

The protein denaturation method is used to evaluate the *in vitro* anti-inflammatory activity of piperine and F3 (Punit *et al*., 2019**)**. Aceclofenac is used as a reference drug. Different concentrations of piperine and F3 were prepared in doses of 10 g/ml, 20 g/ml, 40 g/ml, 80 g/ml, and 160 g/ml, respectively, and the standard drug was prepared in doses of 10 g/ml, 20 g/ml, 40 g/ml, 80 g/ml, and 160 g/ml, respectively. The reaction mixture contains various concentrations of the standard drug, piperine (2 ml), pH 6.4 phosphate buffered saline (2.8 ml), and fresh hen's egg albumin (2 ml). It is incubated for 15 min at 27°C. After incubation, denaturation was produced by boiling the reaction mixture at 70°C for 10 min. The reaction mixture was cooled, and absorbance at 660 nm was measured with distilled water as a blank. The experiment is performed thrice. The formula shown below was used to get the percentage inhibition for protein denaturation (Dharmadeva *et al*., 2018).

% inhibition = $A_c - A_t / A_c \times 100$

where, A_t = Absorbance of text, A_c = Absorbance of control.

2.17 *In vitro* antimicrobial study

2.17.1 Agar diffusion method

The piperine extract was tested for its inhibitory effects on *S. aureus* and *E. coli* using the diffusion method. Normal saline was diluted using the bacterial strains. Using sterile cotton swabs, 100 ml of bacterial suspension was poured onto the surface of the agar medium in 100 ml increments on each plate, and the mixture was left at room temperature for 15 min. On the agar surface, 6 mm diameter holes were drilled using a cork borer. Each hole was loaded with 50 l of each concentration (0.25, 50, and 100 g/l). As a control, two wells are used, one of which contains 95% methanol (a negative control) and the other a solution of the antibiotic amoxicillin (a positive control). The dishes were incubated for 24 h at 37°C. Measurement of the inhibition zones around the holes and comparison with the control coefficient made up using 95% methanol (Eve *et al*., 2020).

2.18 *In vivo* anti-inflammatory activity

2.18.1 Carrageenan induced paw edema

The piperine and F3 were chosen from an *in vitro* anti-inflammatory study using the protein denaturation method because they have the highest significant value. They were subjected to *in vivo* anti-inflammatory activity by the carrageenan-induced paw edema method. A wister rat was divided into 4 groups; each group consists of 3 animals.

Group I serve as negative control (Distilled water)

Group II serve as positive control (Diclofenac 5 mg/kg p.o)

Groups III and IV received Piperine and F3, respectively, at a dose of 100 mg/kg p.o)

After 1 h, paw edema was induced in the left hind paw of rats in sub-plantar tissues by injecting 1% w/v of carrageen (1 ml) in saline solution. After carrageenan induction, the paw perimeter of rats was measured at hourly intervals for 4 h using Vernier calipers. The right hind paw of rats serves as "normal," which is not inflamed; the paw perimeters were compared with the standard group (Diclofenac) for evaluation of anti-inflammatory activity (Amdekar *et al*., 2012). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines. The percentage inhibition of anti-inflammatory activity was calculated by using the formula:

% inhibition = $T_c - T_t / T_c \times 100$

where,

T_t = thickness of paw perimeter in test;

T_c = thickness of paw perimeter in control.

3. Results

3.1 Ultra-violet (UV) absorption spectra

The piperine was analyzed by spectrophotometrically in between 200 nm-400 nm. The maximum absorbance (λmax) was found at 340 nm which was used for quantitative analysis.

3.2 FTIR analysis

The potential interaction between pure drugs and polymers was examined using FTIR spectroscopy. In the FTIR investigation, all characteristic peaks due to pure piperine were observed in maltodextrin-based proliposome spectra, which show no any remarkable change in their position following successful preparation. The FTIR spectra summarized in Table 2.

Table 2: Interpretation of FTIR spectra

Transition	IR absorption wave number (cm^{-1})					
	Range (cm^{-1})	Piperine	Soya lecithin	Cholesterol	Maltodextrin	Proliposomes
O – H stretching alcohols, phenols	3500 – 3200	3363.51, 3421.13	3417.63, 3363.62	3419.56	3357.84, 3344.34	3357.84, 3344.34
O – H stretching carboxylic acid	3300 – 2500	-	3066.61 – 2732.94	3099.39 – 2653.87	3330.84	-
C – H stretching alkane	3000 – 2850	2765.67, 2845.76	2956.67 – 2854.45	2937.38 – 2867.95	2929.67	2929.97, 2916.17, 2848.67
C = O stretching carbonyl	1760 – 1665	1765.87, 1623	1735.81	1714.60 – 1672.17	-	1731, 1718.46
-C = C- stretching alkanes	1680 – 1640	1657, 1648.67	1649.02	1672.17	1650.95	1627.87
C – H bending alkanes	1470 – 1450	1457.13	1458.08	1467.73	1458.08	-
C – O stretching alcohol, carboxylic acid	1320 – 1000	1324.12, 1221.23, 1234, 1312.23	1234.36, 1066.56	1132.14 – 1022.20	1242.07, 1157.21, 1080.06, 1022.20	1161.19, 1332, 1220.86, 1182.28
= C – H bending alkenes	1000 – 650	913.34, 786.76, 675.98	860.19 – 659.61	985.56 – 738.69	929.63, 763.76, 707.83, 607.54, 576.68, 443.95	939.27, 848.62, 769.54, 717.47

3.3 Angle of repose

Angle of repose of proliposomes formulation was measured by fixed funnel method. The flow property of proliposome powder was summarized in Table 3. which shows that proliposome powder F3 has an angle of repose was found to be 29.54 among than other formulation. It indicates good flow property of formulated formulations.

Table 3: Angle of repose of proliposome formulations

S. No.	Formulations	Angle of repose
1	F1	33.21 ± 0.15
2	F2	31.88 ± 0.12
3	F3	29.54 ± 0.71

3.4 Particle size and polydispersity index

The particle size distribution has most important characteristics affecting the *in vivo* fate of proliposomes. The particle size was found to range between 100.61 d nm and 80.52 d nm (Figure 1). The proliposomes mean particle size range of F3 was 80.52 d nm among all formulations. Proliposomes are performed physically more stable by the polydispersity index, which measures the degree of particle size distribution. The polydispersity index of formulation F3 shows 0.284.

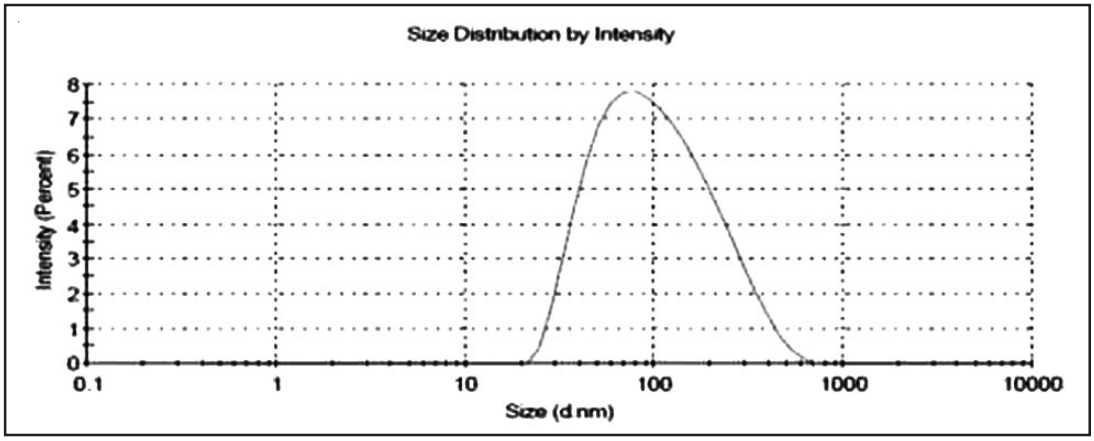

Figure 1: Particle size and polydispersity index of F3 formulation.

3.5 Zeta potential of proliposomes

The determination of zeta potential of proliposomes formulation was essential as it provides an indication about the physical stability of proliposomes. Repulsion between these particles with similar electric charges prevents particle aggregation and ensures easy redispersion, although larger repulsive forces can be produced by positive or negative zeta potential values. Proliposomes formulation F3 was found to be in the range of – 4.75 mV which indicates good physical stability of proliposomes (Figure 2).

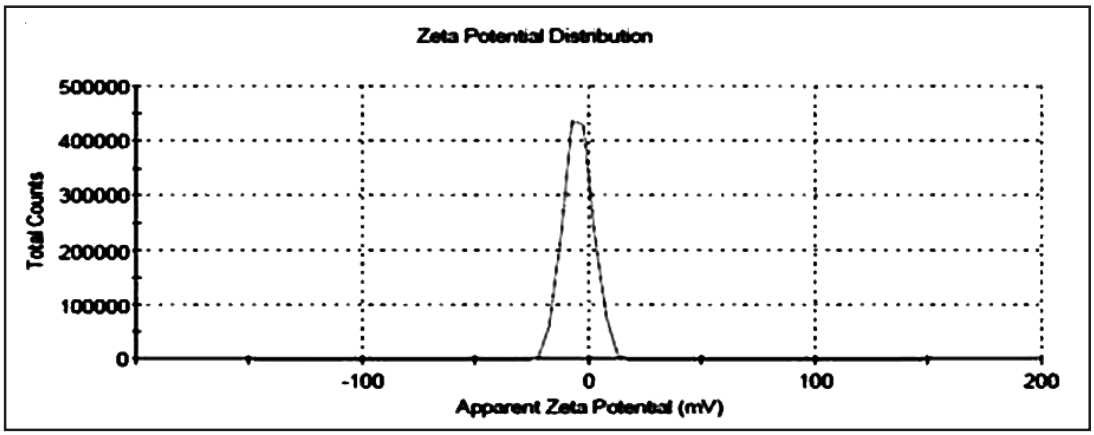

Figure 2: Zeta potential of F3 formulation.

3.6 Saturation solubility

The saturation solubility study shows F1 - F3 in the range of 80.14% ± 0.51, 89% ± 0.68, and 96.32% ± 0.35, respectively. Piperine indicates 25.45% ± 0.23 (Figure 3). Formulation F3 has very fine particle size and large surface area, therefore when the proportion of carrier increases, a large surface is placed for adsorption of drug. The solubility profile of proliposomes increases saturation solubility, dissolution velocity and reduction of particle size which leads to increase the dissolution rate. The saturation solubility of formulation F3 indicates maximum solubility compared to unprocessed drug and among all other formulation.

3.7 Drug content and drug entrapment

The drug content of piperine proliposome was represented in the Table 4.The formulation F3 was found to be in the range of 96.72%. The result indicates the presence of high drug content and loss of drug was lower during preparation process. The formulations F3

shows entrapment efficiency of 85.35% upon hydration with phosphate buffer and a slow release rate over the duration of 2 h. It shows optimum surfactant cholesterol ratio to provide high entrapment of piperine.

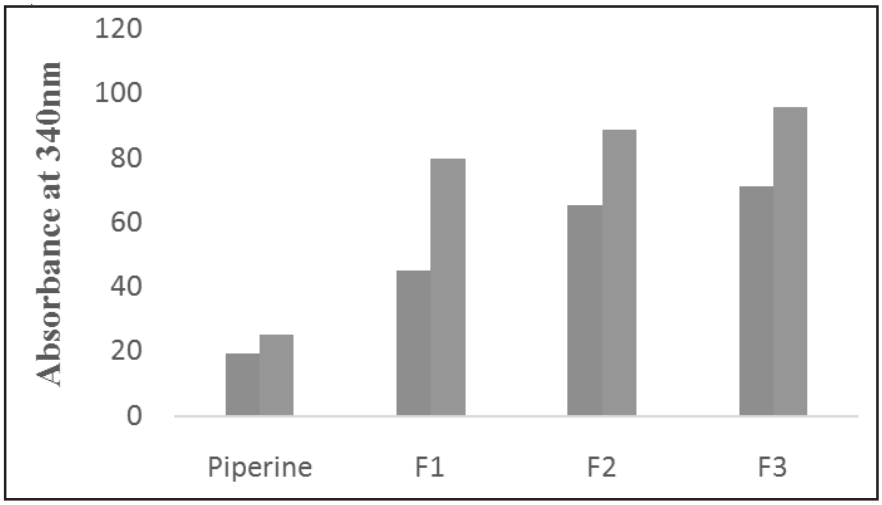

Figure 3: Saturation solubility studies of pure drug and formulation.

Table 4: Percentage drug content and entrapment efficiency of proliposome formulation

S.No	Formulation code	% Drug content	% Drug entrapment efficiency
1	F1	91.35 ± 0.31	69.46 ± 0.53
2	F2	94.38 ± 0.13	54.30 ± 1.62
3	F3	96.72 ± 0.13	85.35 ± 0.26

3.8 *In vitro* drug release study

The release data of the comparative *in vitro* release study was carried out for 12 h and graphically represented as percentage drug release shown in the Figure 4. The dissolution rate of pure drug is less, only 24.8% of the drug was released in 0.1N HCl and 34.42% of drugs were released in phosphate buffer at the end of 12 h. The cumulative percentage release was found to be in the range of 75.94% ± 0.05, 84.62% ± 0.09, and 89.56% ± 0.34, respectively. The release study was significantly increased from the all formulated proliposome. The dissolution rate of proliposomes formulation was increased than pure drug. Based on the data formulation, F3 shows maximum *in vitro* release in phosphate buffer at the end of 12 h.

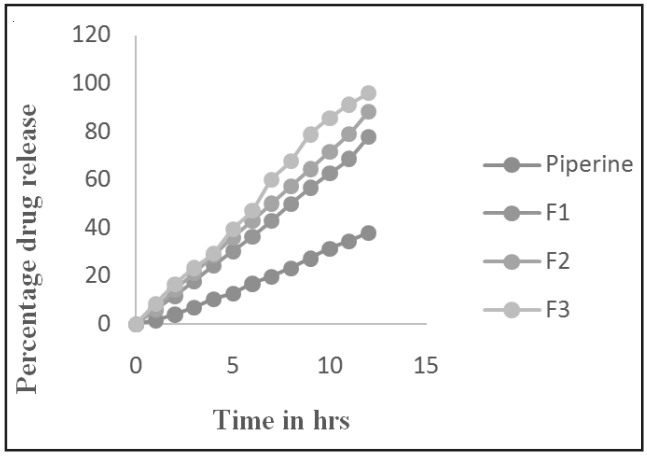

Figure 4: *In vitro* release study of phosphate buffer.

3.9 Drug release kinetic data

The various kinetics studies used to describe the release kinetics shown in Table 5. Zero order plots shows the corresponding characteristic release of formulation, Higuchi kinetics, the plots were found to be fairly linear as indicated by their highest regression value. The coefficient of correlation values (r^2) was in the range of 0.897, 0.918, and 0.912, which was identified by correlation factor. Kosmeyar peppa's model formulation F3, 'n' value is 0.8917, revealing that high linearity as followed by zero order kinetics.

Table 5: Drug release kinetic data

Formulation	Phosphate buffer(pH 6.6)				
	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Peppa's	
				n	r^2
F1	0.9989	0.9462	0.8974	1.031	0.9997
F2	0.9952	0.9108	0.9181	1.0422	0.9989
F3	0.9928	0.8834	0.9122	0.8917	0.9959

3.10 Scanning electron microscopy

Shape and surface morphology of piperine loaded maltodextrin Proliposome formulations was estimated by scanning electron microscopy. It shows a smooth surface. It was to identify the structure of carrier followed by coating of lipid molecule on its surface. If high amount of surfactant molecule is present in the given particles, it seems to be uneven and rough (Figures 7 and 8).

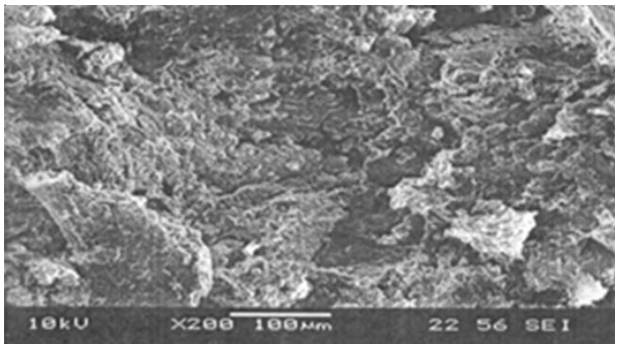

Figure 7: SEM image of pure piperine.

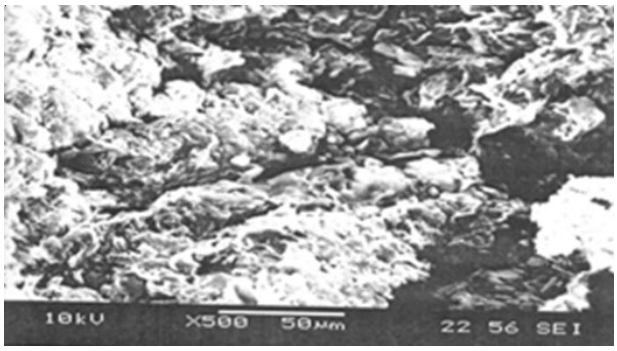

Figure 8: SEM image of F3 formulation.

3.11 Powder x-ray diffraction analysis

The diffraction pattern of pure piperine shows highly crystalline nature which indicates numerous characteristics diffraction peaks at 2θ angle at 19.76°. The diffraction pattern of F3 formulation shows maximum reduction of diffraction peaks, which express the decrease in degree of crystallinity as reduction of sharp peaks (Figures 9 and 10). It helps in the conversion of crystallized piperine to amorphous nature of nanoparticle.

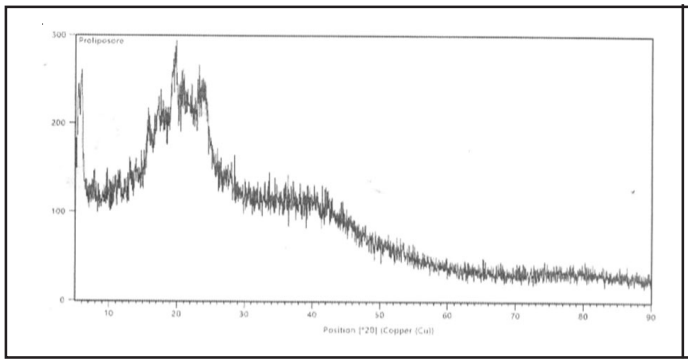

Figure 9: P-XRD of pure piperine.

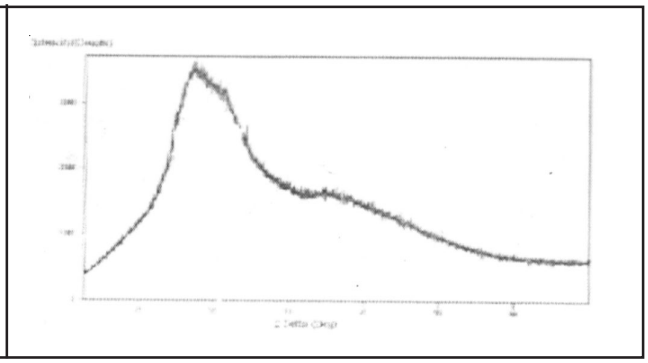

Figure 10: P-XRD of F3 formulation.

3.12 Stability study

Stability testing was carried out to analyze the effect of degradation on piperine proliposome formulations at various storage conditions such as refrigeration, room and elevated temperature, *etc*. The formulation F3 shows good stability (Figure 11). It was identified that there were no significant changes involved in this study. It results that the proliposomes formulations are stable to store under refrigeration conditions with less leakage.

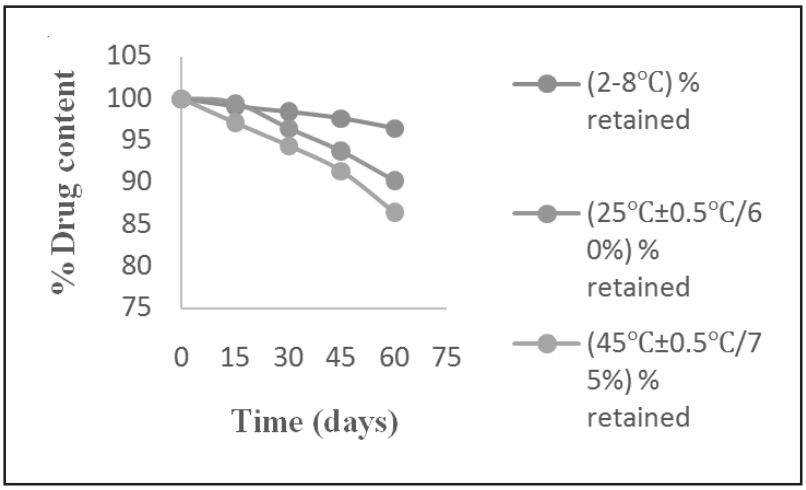

Figure 11: Stability study of F3 formulation.

3.13 *In vitro* anti-inflammatory activity

The denaturation of tissue protein was one of the well reported causes of inflammatory and arthritis diseases. The result obtained indicates the significant difference with piperine and formulation F3. The IC_{50} value for F3 formulation was found to be 35.90 µg/ml was represented in Figure 12. It proves that F3 formulation was more active when compared to piperine. These data clearly indicate that the above mentioned formulation was notably showed their ability to inhibit the denaturation of protein by heat.

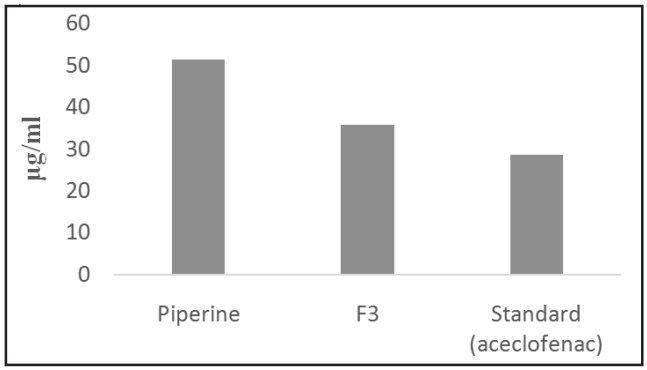

Figure 12: IC_{50} values.

3.14 *In vitro* antibacterial activity

Piperine shows antibacterial activity in the present study against gram positive bacteria (*S. aureus*) with a zone of inhibition of 5 to 7 mm, while formulation F3 shows antibacterial activity against gram negative bacteria (*E. coli*) with a zone of inhibition of 1.9 to 6 mm. The result obtained indicates when the concentration of piperine increases the inhibition zone was also increased (Figure 13).

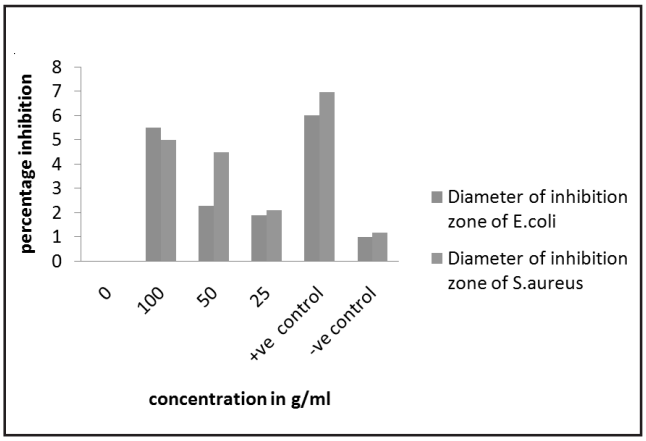

Figure 13: Diametric inhibition zones of *E.coli and S.aureus.*

Table 6: Carrageenan induced paw edema on piperine and F3 formulation

Groups	Paw thickness in (mm)				
	Before	1H	2H	3H	4H
Carragennan (1% w/v)	2.15 ± 0.10	4.33 ± 0.42	4.53 ± 0.54	4.92 ± 0.22	5.26 ± 0.67
Carragennan + Diclofenac (5 mg/kg)	2.81 ± 0.26	4.18 ± 1.52	4.01 ± 0.32	3.55 ± 0.34**	2.91 ± 0.72**
Carragennan + Piperine (100 mg/kg)	2.84 ± 0.32	4.68 ± 0.67	4.38 ± 0.12	3.95 ± 0.56*	3.38 ± 0.54**
Carragennan + F3 (50 mg/kg)	2.65 ± 0.54	4.45 ± 0.72	4.21 ± 0.38	3.61 ± 0.47**	2.95 ± 0.24***

All values are presented as mean ± SEM, n = 3. One way ANOVA followed by dunnet's test was performed as the test of significance. *p<0.05, **p<0.01, ***p<0.001 when all treated group compared with negative control.

3.15 *In vivo* anti-inflammatory activity

Carrageenan induced paw edema was the experimental model for inducing acute inflammation. Carrageenan is an agent used for testing anti-inflammatory drugs as it is non-antigenic and lack of systemic effects. Inflammation induced by carrageenan was acute, non-immunogenic and highly reproducible. Group I animals paw thickness found at (t=0) was 2.15 mm and this remains increased at the end of 4 h. Group II animals have showed a decrease in paw thickness at each hour which was significant compared to Group I. The paw thickness of Group III animals was found at (t=0) 2.65 mm which showed a mild increase at the end of 2nd h, that is 4.45 mm. after the 4th h it decreased to 2.95 mm. Group IV animals showed a decrease up to the 2nd h, 4.68 mm thickness was found at the end of fourth h, which decreased to 3.38 mm. so, Groups III and IV indicated a statistically significant decreases in paw thickness (p<0.05 and p< 0.01) which was mentioned in Table 6. It is represented by releasing proinflammatory mediators like cytokines, chemokines and oxygen derived free radicals and thereby increasing the vascular permeability and cell migration. The results obtained shows better anti-inflammatory activity of proliposome F3 formulation compared to piperine.

4. Discussion

Maximum absorbance (λmax) was found at 340 nm within the limit. The FT IR results indicate good drug-polymer compatibility and also revealed that there is no chemical interaction and stability issue of drug during the preparation. The distinctive bands of free piperine present in piperine proliposomes indicates the chemical stability of piperine proliposomes

Angle of repose shows good flow property because the composition of surfactant in the formulation decreases, and dry proliposome powder also slightly decreased. The proliposomes mean particle size range of proliposome (F3) was 80.52 d. nm by increasing the carrier concentration proportion, the particle size was decreased. The particle size of the proliposome was homogeneous in size and size distribution. The prepared pro-liposomes zeta potential has been proven to be charged enough to prevent the vesicular aggregation, which indicates good physical stability of proliposomes.

Formulation F3 has very fine particle size and large surface area, therefore when the proportion of carrier increases, a large surface is placed for adsorption of drug. The drug content study the results showed the proliposomes have shown the presence of high drug

content it indicates the drug was uniformly dispersed in the powder formulation. The saturation solubility increases with increase in carrier composition of all carriers. This was due to better wettability in combination with soya lecithin, cholesterol, maltodextrin of proliposomes. With carrier proliposome (F3) a very fine particle size and hence large surface area, so as a proportion of carrier increases; a large surface area is presented for adsoption of the drug. The solubility profile of proliposomes increases saturation solubility, dissolution velocity and reduction of particle size which leads to increase the dissolution rate.

The dissolution study of proliposome formulation was carried out to increase the surface area of drug and better contact possible between proliposomes and dissolution medium. In order to access the goal of improving the dissolution rate of lyophilized piperine in proliposomes was achieved. The kinetic release data indicates that the drug release was independent of concentration and follows zero order kinetics. (Verma *et al.,* 2019) reported that piperine liposome was following sustained release thus they reduce metabolism, good absorption rate which improve bioavailability of drug. From *in vivo* study, it is clear that piperine liposomal formulation

The image of the SEM shows smooth surface. This shows the porous surface character of carrier before and after deposition of Proliposomal formulation the nature of the thermogram is completely change, it shows a broadened peak which may be due to dehydration of piperine and change in particle size showing more amorphous nature. The XRD technique indicates the difference detected between the coarse powder and formulated proliposome. The proliposome was found to be stable when stored under different storage condition and it was confirmed by stability studies.

The prepared proliposome exhibited more excellent activity of anti-inflammatory and antibacterial activity clear that as the concentration was increased the activity also increased compared to pure and standard drug. (Hikal, 2018) reported that piperine is excellent antibacterial agent The agents which prevent the denaturation of protein was more effective for anti-inflammatory drug development. The increase in absorbance of test samples compared to control shows protein stabilization.

5. Conclusion

Piperine loaded maltodextrin based proliposomes were prepared using film hydration method where two different carrier ratios had been used. All the above studies confirmed that formulation F3 is the best formulation. This is due to the decreases in the particle size by increasing the carrier proportion. The dissolution rate of proliposomes formulation was increased better than pure drug. This was due to increased surface area of drug and better contact possible between proliposomes and dissolution medium. *In vitro* anti-inflammatory was investigated and it was clear that the activity increased as the concentration increased. The *in vivo* anti-inflammatory test shows a better anti-inflammatory activity of proliposomes as compared to pure piperine.

The above results provide us the conclusion that the proliposomes, which provide effective drug formulation with low aqueous solubility and poor bioavailability. Proliposomes exhibit higher shelf life than liposomes.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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