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Formulation and Evaluation of Prednisolone Proliposomal Gel for Effective Topical Pharmacotherapy

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ABSTRACT

The present objective for the study was to prepare proliposomal gel bearing a steroidal anti-inflammatory agent, prednisolone intended for topical application. Various proliposome formulations were prepared using thin film hydration technique by varying the lipid phase composition (lecithin/cholesterol). Proliposome formulations were characterized for drug content, entrapment efficiency, surface morphology, surface charge, FTIR and stability studies. Topical proliposomal gels were prepared by incorporation of proliposome into strusctured vehicle carbopol (2%). Alternatively, hydrogels containing prednisolone were prepared and their drug release properties were investigated. Pharmacodynamic activity was also determined for optimized proliposomal gel and was compared with commercial marketed gel. A spherical shape of reconstituted prednisolone liposome with an average vesicle about 2~6μm was observed in photomicrographs. The percentage entrapment of drug was increased with increase in phospholipid composition in the range of 85-98%. FTIR studies showed no possible drug-excipient interaction. Proliposomal gel showed prolonged release of prednisolone than the hydrogels. Anti-inflammatory activity proliposomal gel showed maximum percentage of inhibition of edema 60% when compared to commercial marketed gel 55%. Stability studies indicated that product is stable and should be stored at low temperatures. Proposed prednisolone proliposomal gel showed sustain release with enhanced anti inflammatory activity implicating its potential in effective topical pharmacotherapy for the treatment of rheumatoid arthritis.

Keywords: Prednisolone, Proliposome, Anti-inflammatory, Rheumatoid arthritis, Transdermal delivery.

INTRODUCTION

Rheumatoid arthritis is a chronic auto immune disease characterized by joint synovial inflammation and progressive cartilage, bone destruction leading to gradual immobility. [1-2] The synovial fibroblast activates macrophages and transcription factor NF-κB which aids in progression of disease as well as mediating inflammation. As a result of inflammation, the synovial thickens, the cartilage begin to disintegrate gradually leads to destruction. [3-5] Recent research suggest that calcifying Nanoparticles (also known as nanobacteria) are present in synovial fluid and are responsible for provocation of inflammation leading to bone and joint destruction. [6] Drugs useful in treatment of rheumatoid arthritis are classified as first line agents having Non-steroidal anti inflammatory drugs and steroidal anti inflammatory drugs.

Prednisolone (11β)-11, 17, 21-trihydroxypregna-1, 4-diene-3, 20-dione is a steroidal drug with predominant glucocorticoid and low mineral corticoid activity. It is mainly used for the treatment of a wide range of inflammatory and auto-immune diseases such sclerosis, rheumatoid arthritis [4], autoimmune hepatitis [7] etc. Prednisolone is also known as 'disease modifying anti-arthritic drugs' because of its anti inflammatory [8] action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase. [9]

When steroidal anti inflammatory drugs such as prednisolone are given orally results in systemic side effects like bone loss, increased susceptibility to infection, osteoporosis, peptic ulcers and buffalo hump. [10] Parental route of administration results in rapid clearance rate of drug which ultimately compels invasive and frequent administration of drug. [11] Despite note worthy advances have been made over recent years for the management of rheumatoid arthritis, the currently available methods, have a dose limiting therapeutic index with compromised safety implications. Attempts will be made in developing and characterizing a specific drug delivery system targeting drugs to synovium or specific tissues which in turn increase drug efficacy with minimum extra synovial toxicity.

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Proliposomes are novel generation of carrier mediated drug delivery system having several advantages over conventional liposomes. [12] It has shows better stability and ease of sterilization on large scale by preventing drug over loading. [11-13] Maximum amount of drug encapsulation helps in more penetration of drug and producing a sustain release effect at the site of administration. [13]

The main objective of the study is to formulate and evaluate prednisolone proliposomal gel formulation for effective topical pharmacotherapy in treatment of rheumatoid arthritis. Detailed literature survey revealed that there are only to ophthalmic formulations of prednisolone 1% for treatment of eye infections but no attempts or formulations are developed till date to evaluate the topical delivery of prednisolone.

MATERIALS

Prednisolone API was gift sample from AKUMS Laboratories Ltd., Haridwar. Lecithin was gift sample from bright Labs; Hyderabad. Mannitol, cholesterol, stearic acid used were from S.D fine chemicals; Mumbai. Acetonitrile, potassium dihydrogen ortho phosphate and ortho phosphoric acid used in the study were of analytical grade and obtained from Qualigens Ltd., Mumbai. HPLC grade water was obtained from SD-Labostar (3 TWF-UV) water purification system.

Analytical method

The pharmacology of the proliposome formulation of prednisolone largely determined by the extent of which prednisolone is encapsulated inside the proliposome bilayer. Therefore quantification of prednisolone within proliposome using suitable analytical method is important. In the present study, a newer analytical method for determination of prednisolone in proliposome formulation was developed. The RP-HPLC method was developed using phenomenex Luna C18 analytical column comprising 5µm particles with 4.6×250mm of column dimensions. The mobile phase comprising of acetonitrile: 0.025M potassium dihydrogen ortho phosphate buffer [pH-3.0] in the ratio (50:50) v/v. The flow rate was maintained at 1.0 ml/min and the elute was monitored by using U.V detector at 240nm.The retention time of prednisolone was 6.4 minutes. The method was validated for its specificity, accuracy, precision, and linearity, limit of detection, limit of quantification, robustness and stability parameters. LOD and LOQ were 35 and 62.5ng/ml respectively. The linearity of the drug was in the range of 1-5µg/ml with co-efficient of correlation 0.999. The percent recovery of the prednisolone drug was 100.64%. The proposed method can be used for quantitative and entrapment efficiency determination of prednisolone in proliposome formulation as well in other pharmaceutical dosage forms. [14]

Preparation of proliposome Formulation

Proliposome formulation containing prednisolone was prepared by using thin film deposition on carrier method using vacuum rotary evaporator [15] (Heidolph, Germany). Optimization of proliposome formulation was done by preparing varying concentration of mannitol, lecithin and cholesterol. 1g of mannitol powder (sieved with 100 mesh) was placed in round bottomed flask at 60-70°C and 115 rpm under vacuum 30 minutes for complete drying.

Prednisolone 10mg and lecithin, cholesterol were dissolved in mixture of chloroform and methanol in the ratio of 8:2 (v/v) for various formulation as shown in Table 1. Initially 5 ml aliquot of organic solvent was introduced into round bottomed flask at 37°C and rotated. After complete drying remaining 5ml aliquots of solution was used. The flask containing proliposome formulation was kept in vacuum desiccator overnight and then sieved with 100 mesh.

Preparation of Carbopol Gel base

0.5g Carbopol 934 was weighed (shimadzu, Japan), dispersed in water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 1-2 ml of glycerin was added to for gel consistency. Similarly 1 and 2% carbopol gels were prepared. [16-17]

Preparation of proliposomal gels

1g of proliposome formulation was dissolved in 10ml of methanol and centrifuged (REMI, India) at 6000rpm for 20mins to remove the unentrapped drug .The supernant was decanted and sediment was incorporated into the gel vehicle. The incorporation of the proliposomes into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 min. [16-18] All the optimized formulation was incorporated into three different gel concentration 0.5, 1 and 2% w/w.

Table 1: Composition of optimized prednisolone proliposome formulation

Formulation	Mannitol (mg)	Lecithin (mg)	Cholesterol (mg)
PRD 3	1000	200	30
PRD 7	1000	200	50
PRD 14	1000	100	50
PRD 16	1000	100	40

Characterization of proliposomes

Prepared proliposomal formulation was characterized for the following parameters [19]

Vesicle Size and Count: A drop of distilled water was added to few proliposome granules on glass slide without cover slip to observe formation of liposome from proliposome formulation. Vesicle size and count was recorded under Digital optical microscope [Metzer, India] with magnification 4X.

Surface Morphology: After gold coating of proliposome and mannitol particles, their surface morphology was viewed and photographed by scanning electron microscope.

Surface charge: Proliposome formulation of the optimized batches was dissolved in phosphate buffer pH 7.5 and made a higher serial dilution 1000 X until a clear solution is obtained. Sample was analyzed using Zeta Analyzer [Haroba, Japan] for determination of surface charge.

Drug content: 1g of proliposome formulation was weighed [shimadzu, Japan] and vesicles were lysed with 25 ml of methanol by sonication [citizen, India] for 15 min. The clear solution was diluted to 100 ml with methanol. Then 10 ml of solution was diluted to 100 ml with saline phosphate buffer pH 7.4. Aliquots were withdrawn and drug content was calculated for prednisolone using RP HPLC developed method. [14]

Entrapment efficiency: 1g of proliposome formulation was weighed [shimadzu, Japan] equivalent to 10mg of drug and was transferred to a 100 ml volumetric flask containing 25 ml of mobile phase, then sonicated and filtered through 0.45µm membrane filter. The filtrate was finally diluted to 10X with mobile phase and appropriate dilutions were made to obtain concentration in the range of 1-5µg/ml. The steady base line was recorded by using the optimized chromatographic conditions. The assay was subjected for calculating regression equation. The procedure was repeated for 6 times

and the percentage of drug in the formulation was calculated for optimized batches of proliposomal formulations.

$$\text{Encapsulation Percentage (\%)} = [(C_t - C_f)/ C_t] \times 100$$

*C_t is the concentration of total drug content, and C_f is the concentration of free drug.

Yield of Proliposomes: After complete drying, the drug loaded proliposomes was collected and weighed accurately. The yield of proliposomes was calculated by

$$\% \text{ yeild} = \frac{\text{Total weight of proliposmes (g)}}{\text{Total weight of drug +Total weight of excipients}} \text{ X 100}$$

Drug-Excipient Interactions by FTIR studies: The compatibility between pure drug, mannitol, cholesterol and lecithin was detected by using FTIR studies (Bruker ALPHA-T, Germany). Then IR spectrum was recorded individually over the wave number of 8000^{-1} to 500 cm^{-1}.

Characterization of Gels: Optimized gel base was evaluated for following parameters for both plain gel and gel loaded with proliposomes. [20]

Physical examination: Macroscopic examination for visual (aspect, consistency, homogeneity, color), olfactory (smell), tactile (touch and thermal sensation) features was done for proliposomal gel formulation.

pH optimization: Triethylamine was used to neutralize the gel and to prepare gels with different pH values, 5.5; 6.5 and 7.5 using pH meter [Lab India, India].

Viscosity and Rheological properties: The rheological analysis of the experimental gels was performed using a Brookfield viscometer pro D II + apparatus, equipped with standard spindle LV1 with 61 marking. Viscosity of 1, 1.5 and 2% carbopol gel was determined and optimized concentration was selected.

Drug content and content uniformity: The gel sample (1g) was withdrawn and drug (prednisolone) content was determined using RP-HPLC developed method. Similarly, the content uniformity was determined by analyzing drug concentration in gel taken from 3 to 4 different points from the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using RP-HPLC method. [14]

Stability studies as per ICH guidelines: For stability studies, formulations were stored in tight sealed, ambered colored glass containers at various temperatures 8°C, room temperature and at 40°C for a period of three months using stability chambers [Cintex, India]. Regular tested for changes in surface morphology, color, residual drug content.

In-vitro studies

Percentage cumulative amount of Drug Release from Dialysis Membrane: An *in vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, molecular weight 5000 Daltons) was placed between receptor and donor compartments. Proliposomal gel equivalent to 1g was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 500rpm [Remi, India] throughout the experiment. At fixed time interval, 5ml of aliquots was withdrawn for every 1, 2, 3, 4, 6, 8, 12, 14 hours from receiver compartment through side tube and analyzed by RP-HPLC method. [14] Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism and order of drug release from proliposomal gel [21]

Percentage cumulative amount of Drug Release from Rat skin

Preparation of skin: The abdominal hair of Wistar male rats, weighing 150±25 g, was trimmed using trimmer [rexino, India] 24 h before treatment. After anesthetizing the rat 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 1 h before starting the diffusion experiment.

A system having modified Franz's diffusion cells with a diffusional area of 2.15cm was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment. Drug encapsulated proliposomal gel equivalent to 1g was applied to the skin surface in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24ml). During the experiments, the diffusion cell was maintained at 37±0.5°C and stirred at 500rpm [Remi, India]. After application of the test formulation on the donor side, at fixed time intervals, 5 ml of aliquots were withdrawn from receiver compartment through side tube for every 1, 2, 3, 4, 6, 8, 12, 14 hrs and analyzed by RP-HPLC method for determining the cumulative amount of drug permeated through skin. [14]

Drug Retention Study: The skin was removed from the diffusion cells after completion of experiments. The surface of skin specimens was washed 10 times with 1ml distilled water and dried on filter paper. The effective surface area of the skin was separated and minced with a surgical sterile scalpel then finally homogenized in a vial filled with methanol by using Homogenizer (REMI RQT-124A) at 16,000 rpm for 5 min (REMI Cooling Centrifuge TR-01). The tissue suspension was centrifuged for 15min at 9000rpm and then the supernatant was filtered. Then filtered supernatant tissue suspension was further extracted with methanol and filtered. The filtrate was assayed for cumulative amount of drug retained on the skin by using RP-HPLC method. [14]

In-vivo studies

Pharmacodynamic Studies

Animals used for *in vivo* experiments were adult male Wister rats (150±170g), 3-4weeks, purchased from the Mahaveer Enterprises, Hyderabad. The animals were kept under standard laboratory conditions, at 25±10°C and 55±5% relative humidity with a 12 h light/dark cycle. The animals were housed in polypropylene cages, with free access to a standard laboratory diet and water. All surgical and experimental procedures was reviewed and approved by the Institutional Animal and Ethics Committee [Reg.No-1412/a/11/CPCSEA]**.** Evaluation of Anti-Inflammatory activity of proliposomal gel loaded Anti arthritic drug prednisolone was done using Carragenan induced paw edema method. [22-24]

Anti-inflammatory activity was determined by measuring change in the volume of inflamed paw, produced by injection of Carragenan (0.1ml of 1% w/v) using plethysmometer [VJ plethysmometer, India]. Male Wister rats 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. Wistar rats were divided into three groups including one controlled group with each group comprising of 6 animals. The paw volume was noted at 0, 3 and 6 h.

The formulations were applied topically to Wistar rats of respective groups, excluding the animals of controlled group. After 3 h, 0.1ml of 1% carragenan (in 0.9% normal saline) was injected in the sub planter region. Edema volume was measured 3hr after the carragenan injection; the extent of % swelling was calculated from difference between immediate volume and paw volume 3 hr after the carragenan injection.

$$\text{Swelling [\%]} = \frac{V - V_i}{V_i} \times 100$$

*V - volume 3 h after the carragenan injection and V_i - volume immediately after carragenan injection.

Percentage inhibition is calculated by

$$\text{Inhibition [\%]} = 1 - \frac{\text{\% Swelling of group treated}}{\text{\% Swelling of control}} \times 100$$

Table 2: Characterization results for prednisolone proliposome formulations

Prednisolone Formulation [PRD]	Y_1 % Entrapment efficiency	Y_2 Vesicle size (µm)	Polydispersity index
PRD 3	97.6	6.66	0.412
PRD 7	96.9	6.46	0.239
PRD 14	89.8	5.84	0.331
PRD 16	85.1	5.45	0.249

Table 3: Zeta potential and Percentage yield for optimized proliposome formulations

Formulation	Prednisolone Proliposomes[mv]	Prednisolone proliposome
PRD 3	-49.3	89.61
PRD 7	-54.8	90.95
PRD 14	-64.2	91.65
PRD 16	-63.6	95.82

Table 4: Cumulative drug release of prednisolone formulations for 14 hrs over dialysis membrane

Formulations	% Cumulative Release
PRD 3	62.14
PRD 7	60.12
PRD 14	72.34
PRD 16	85.67
Free Drug Gel	95.12

Table 5: Cumulative amount drug released of proliposomal gel over rat skin for 14 hours

Formulations	% Cumulative permeation
PRD 3	58.15
PRD 7	51.56
PRD 14	70.43
PRD 16	82.46
Free Drug Gel	89.19

Table 6: Percent proliposomal gel retained over rat skin after 14hrs

Formulations	Percentage drug retained
PRD 3	30.87
PRD 7	35.15
PRD 14	24.67
PRD 16	28.12
Free Drug Gel	12.66

RESULTS AND DISCUSSION

In a preformulation study the optimum concentrations of mannitol, phospholipid and cholesterol was determined to obtain stable liposomes devoid of aggregation, fusion and sedimentation. Prednisolone proliposomes was prepared using thin film hydration technique and method was found to be well suited for the production of liposomes without aggregation. Amount of mannitol, phospholipid and cholesterol was found to be very critical in the preparation and stabilization of proliposomes.

Effect of variables on vesicle size: In the present study, film hydration technique was effective to produce polydispersity index of less than 0.893 which indicates obtained liposome population have narrow size distribution when compared to sonication method. [16] Results showed that with increase in the concentration of phospholipid and cholesterol vesicle size was found to be increased as shown in Table 2.

Effect of variables on entrapment efficiency: Results show that with increase in the concentration of phospholipid and entrapment efficiency found to be increased. From the factorial design experiment PRD 3, PRD 7, PRD14, PRD16 which had maximum vesicle size and percentage entrapment efficiency, selected for the further study of gel formulation (Table 2).

Determination of vesicle count and size: Results of average vesicular size and distribution were calculated for count and distribution as shown in Table 2 and Fig. 1.

Determination of Zeta ζ potential: Surface charge was determined and the proliposomal formulations showed potentials ranging from -49.1 to -63.6 mv which was sufficient to avoid aggregation of vesicles (Table 3).

Surface morphology: The surface morphology of mannitol was different when compared to proliposome formulations. From SEM photographs it is clear that the surface of the mannitol becomes illegible due to deposition of phospholipid on the mannitol surface. Observation under Digital microscope [Metzer, India] revealed that proliposome particles were progressively and rapidly converted into liposomes when hydrated shown in Fig. 2.

Percentage yield of proliposomes: The percentage yield for optimized batches of prednisolone proliposome formulations was found to be increased with increase in the phospholipid concentration shown in Table 3.

Drug-Excipient interaction studies by FTIR: On comparison of IR spectra of proliposomes, pure prednisolone drug, mannitol, phospholipid and cholesterol it was clear that, there was no significant interaction of the encapsulated drug with the phospholipid and water soluble solid support [mannitol] within various formulations shown in Fig. 3, 4, 5 and 6 respectively.

Table 7: Anti inflammatory activity results of selected proliposomal gel PRD 7

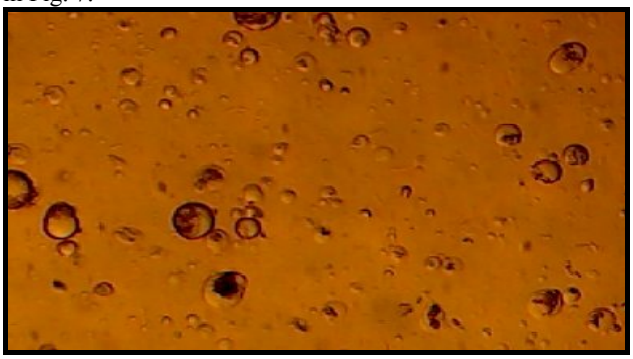
Group	Treatment	Paw volume (ml) 0 h	Paw volume (ml) 3 h	% Swelling	% Inhibition
1	Control	0.85	2.17	160	-
2	Standard Diclofenac	0.85	1.48	73	55
3	Prednisolone	0.92	1.50	65	60

Characterization of proliposomal gels: Prepared proliposomal gel formulation was evaluated for the following parameters:

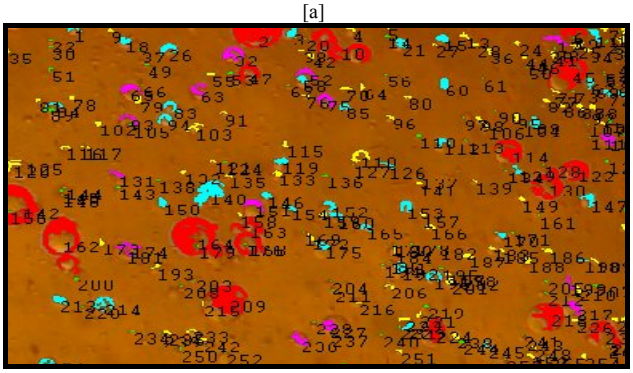
Viscosity measurements: Viscosity measured for optimized plain gel and proliposomal gel showed 10700 and 11309 cps respectively.

Drug content uniformity and pH measurement: There was no significant difference observed in the percent drug at

various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory. The pH values of the prepared proliposome gels were within the limits of 5.5-5.75. Microscopic observations of proliposome gels confirmed the formation of spherical vesicles as shown in Fig. 7.

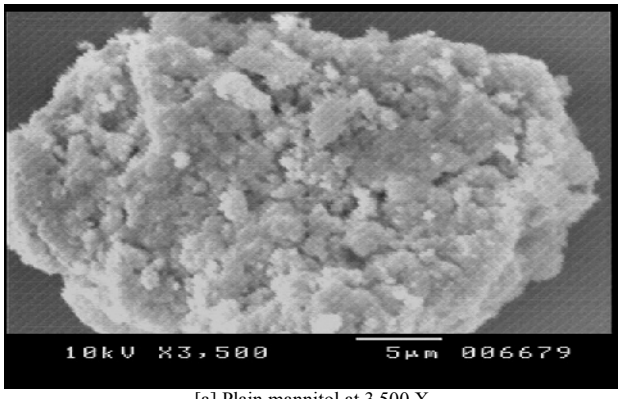


[a]

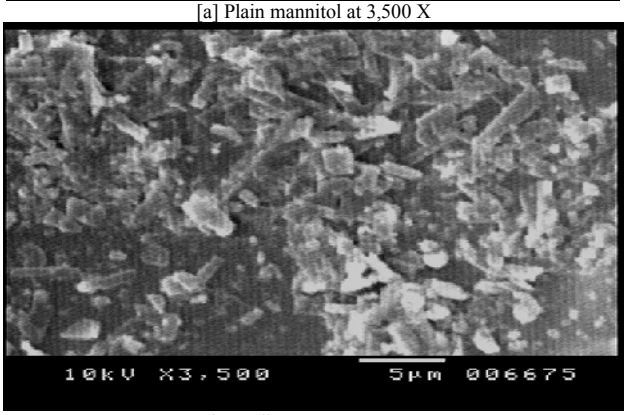


[b]

Fig. 1: [a] Photomicrographs of proliposome formulation, [b] Vesicle count and distribution.



[a] Plain mannitol at 3,500 X



[b] proliposome at 3,500 X

Fig. 2: Scanning Electron micrographs

***In-vitro* Studies:** The release rate of prednisolone proliposomal formulation over dialysis membrane was significantly higher than its flux across skin, indicating the barrier properties of skin for drugs. *In vitro* permeation rate studies such as steady state transdermal flux (SSTF or J_{ss}) for transport of prednisolone across skin was estimated for different formulations.

Calculations for the *in-vitro* rate permeation are as follows:

J_{ss} / SSTF = Q/TA = Amount of drug permeated/ time × area of skin exposed

*Jss is steady state flux measured as the slope of the profile after regression analysis.

The cumulative amount of drug release of various proliposomal gel formulations are shown in Table 4 and 5. The amount of drug retained on the skin was also estimated Table 6.

Skin permeation and drug deposition studies: Results obtained from *in-vitro* drug permeation studies for prednisolone proliposomal gel formulations are shown in Fig. 8 & 9. Results clearly indicate that the amount of drug retained in the skin was considerably higher in case of proliposomal gels when compared to non-liposomal formulation (free drug gel). This shows that liposomes not only enhance the penetration of drug molecules but also helps to localize the drug within the skin Fig. 10.

Pharmacodynamic Evaluations for Anti inflammatory activity: *In-vivo* performance of selected Prednisolone proliposomal gel was carried out using carragenan induced paw edema model. Formulation under this study not only decreased the inflammation to larger but also sustained the magnitude. The optimized prednisolone showed a greater percentage of inhibition at 6 h of 60% when compared to standard marketed diclofenac gel that showed 55% of inhibition Fig. 11. The possible reason could be the drug localization at applied site for longer duration thereby showing a sustain release at site when compared to standard diclofenac gel.

Results of percentage swelling and percentage inhibition was calculated and given in Table 7.

Stability studies: The results indicated that at elevated temperature and freeze temperature there was slightly but insignificantly decrease in % entrapment efficiency and particle size for proliposome batch. Deposition of proliposome on skin from liposomal gel was found insignificantly decreased during stability period. Result suggests that keeping the proliposomal product in refrigeration conditions minimizes stability problems of liposomes.

Preparation of proliposomes using factorial design was found to be well suited and sound approach to obtain stable proliposome formulation for anti-arthritic drugs. Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Rheological studies of all proliposomal gels prepared with 0.5, 1 and 2% w/w carbopol gave clear idea of concentration of carbopol (2%) is required for preparation of stable proliposomal gel formulation. *In vitro* studies of proliposomal gels encapsulating anti –arthritic drug, prednisolone were found to increase the skin permeation and deposition showing a sustain effect when compared to marketed gel. The aim to reduce the dosing frequency and to maintain drug concentration at site for longer time is

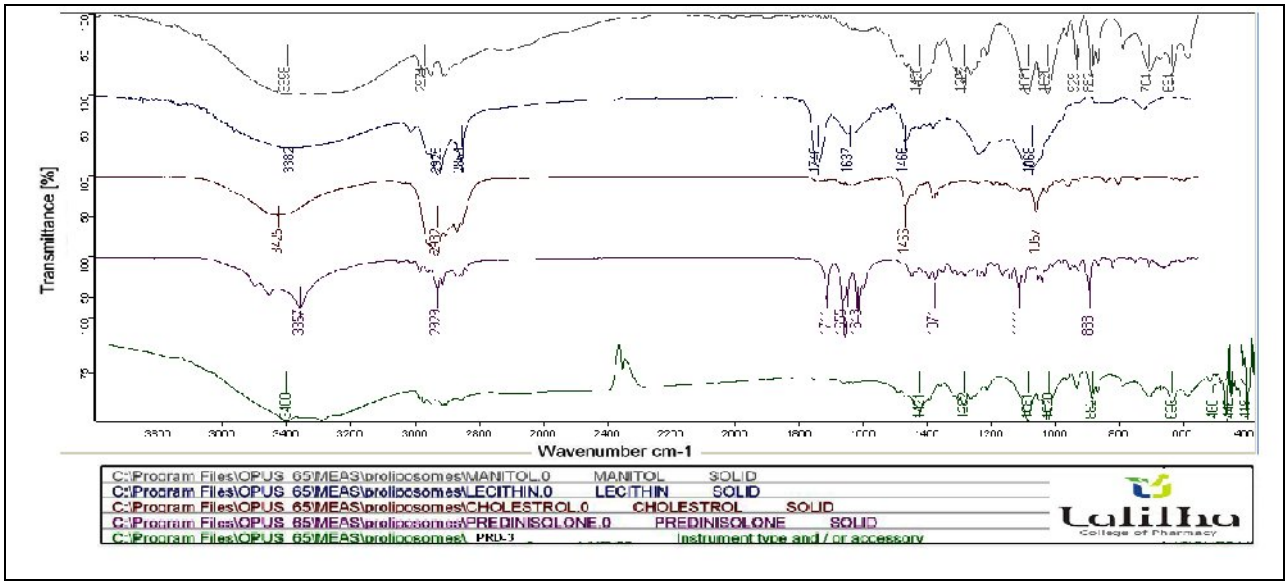


Fig. 3: FTIR Spectra of PRD 3

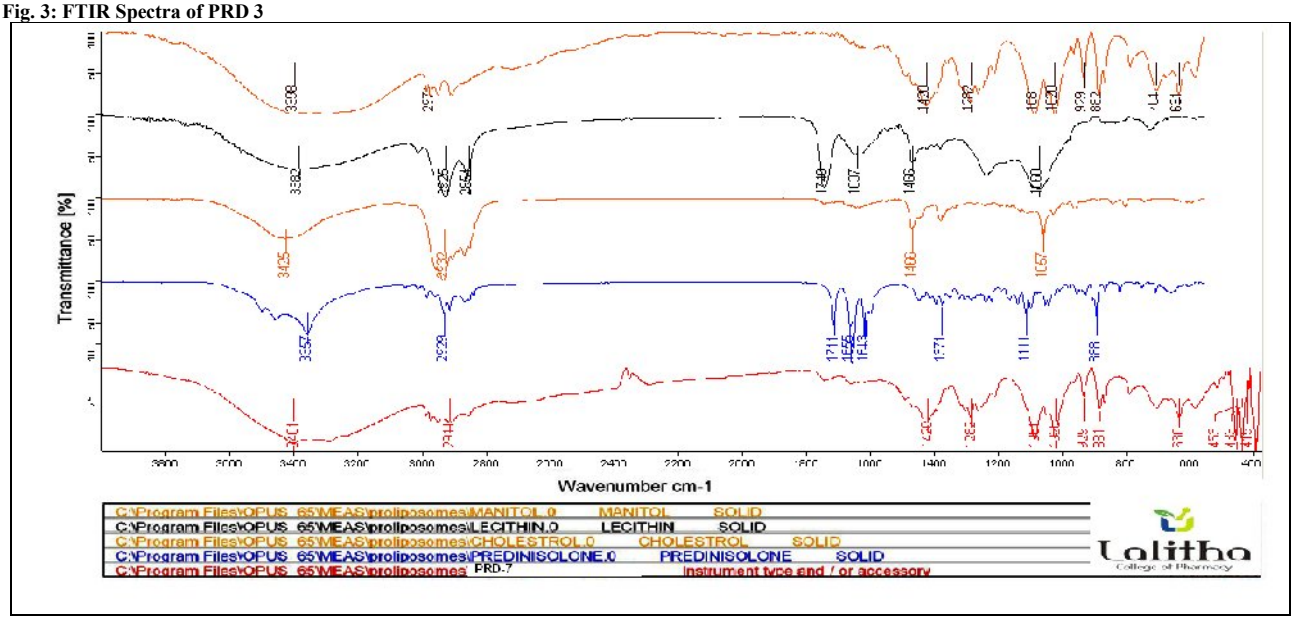


Fig. 4: FTIR Spectra of PRD 7

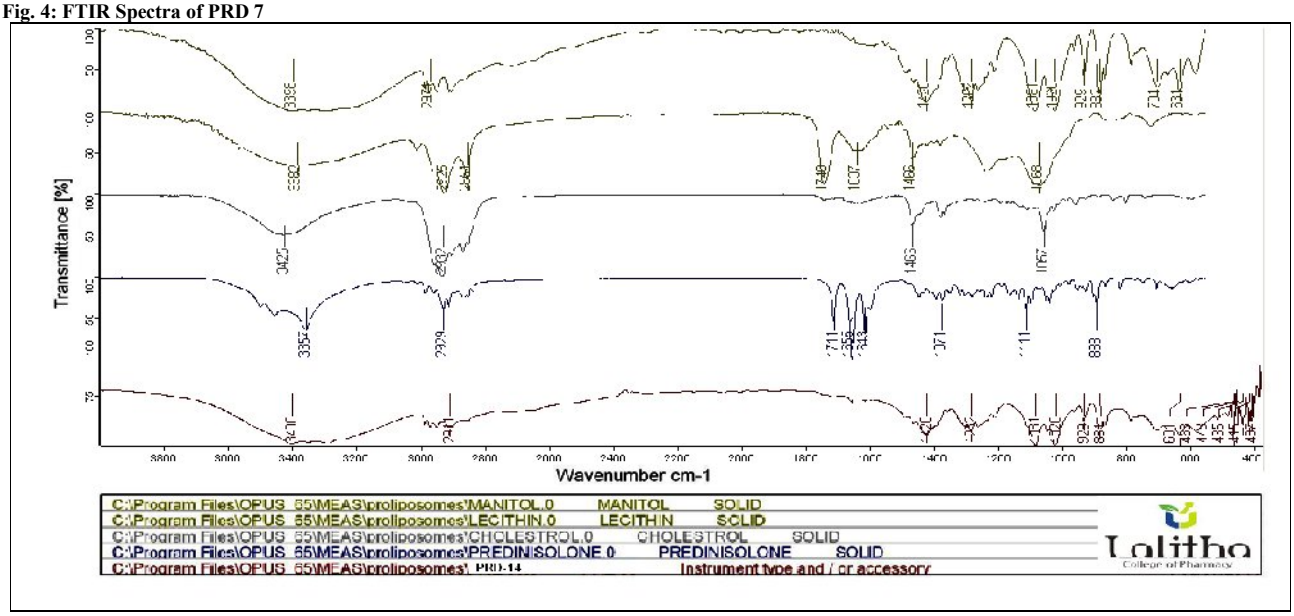


Fig. 5: FTIR Spectra of PRD 14

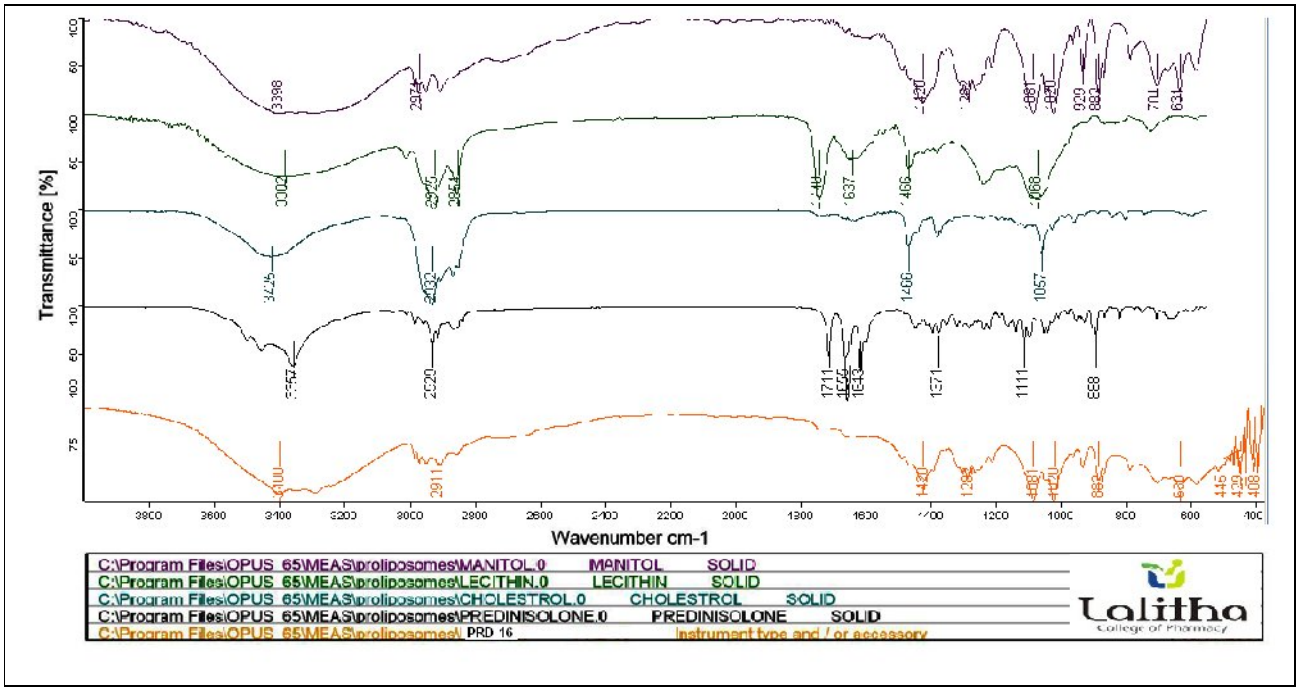
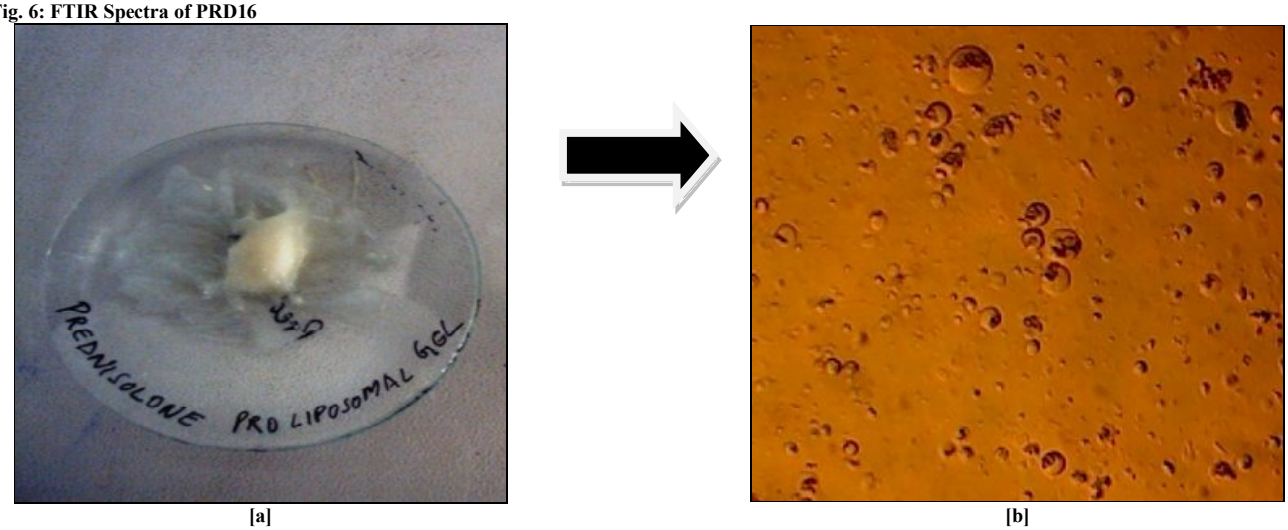


Fig. 6: FTIR Spectra of PRD16



[a] **[b]**

Fig. 7: Photomicrographs [a]. Proliposomal gel; [b].vesicle formation under microscope

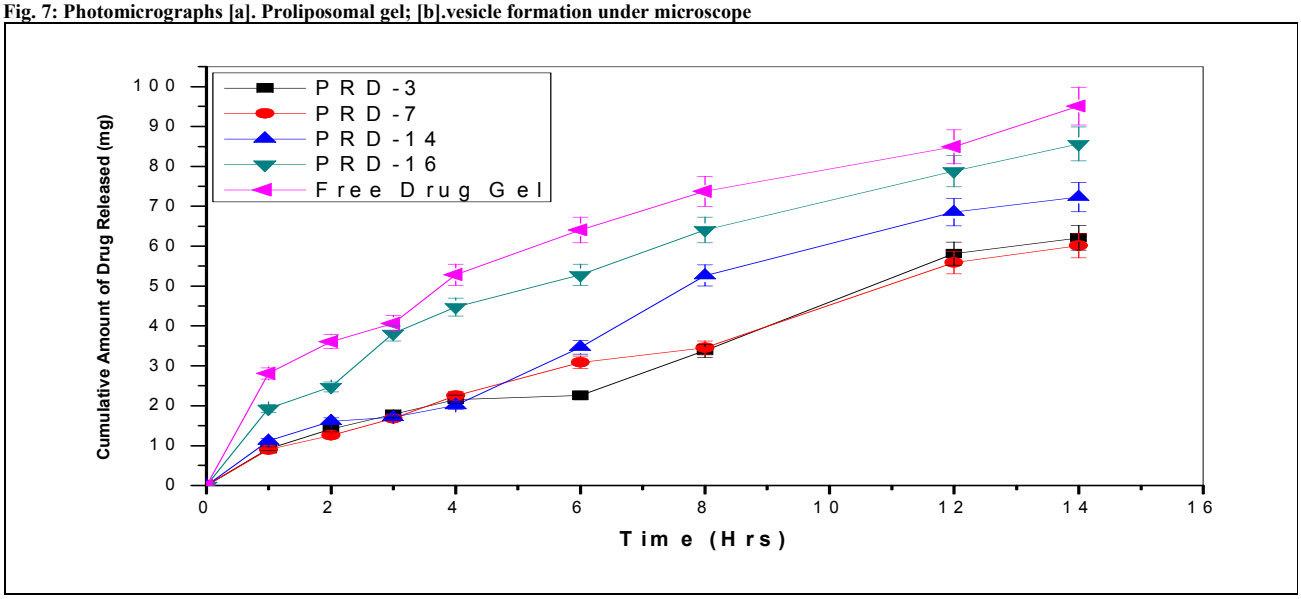


Fig. 8: Percent cumulative amount Prednisolone proliposomal gel release over membrane

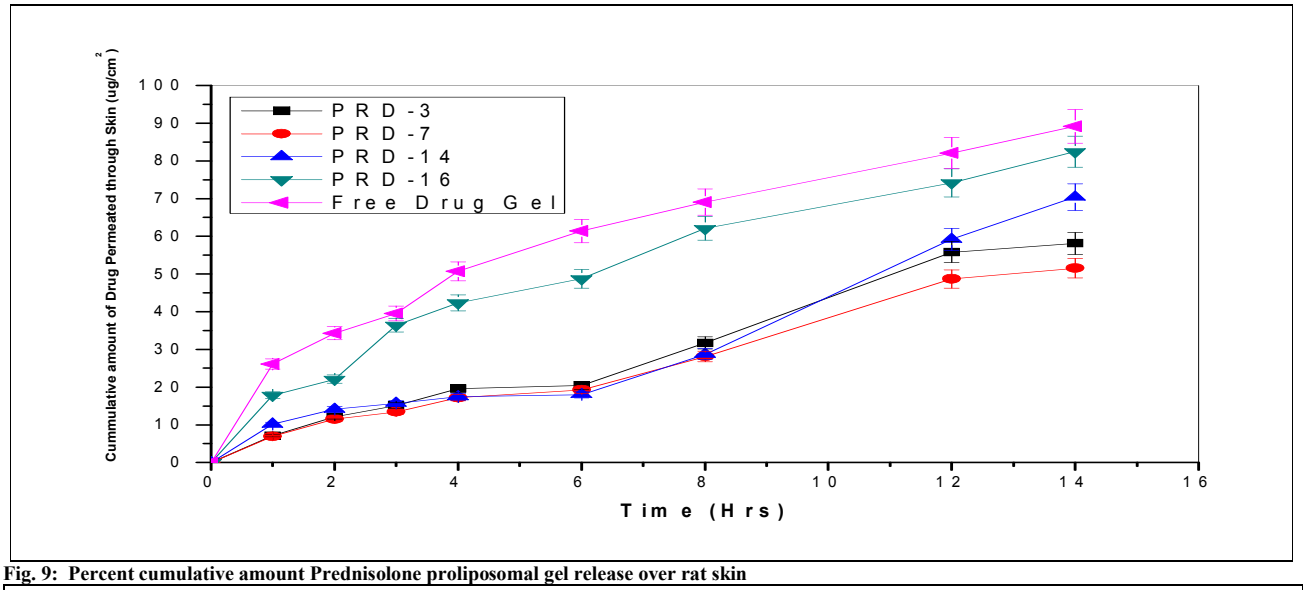


Fig. 9: Percent cumulative amount Prednisolone proliposomal gel release over rat skin

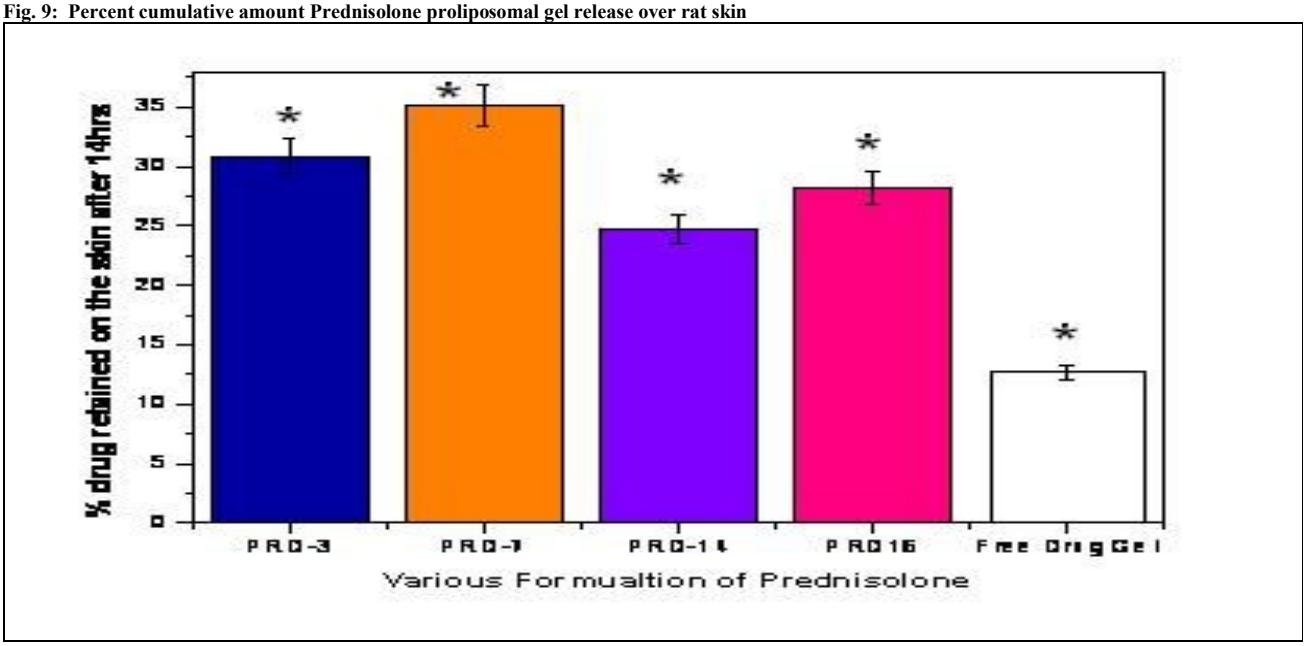


Fig. 10: Percent of prednisolone proliposomal formulation retained over skin
***P value<0.05 is compared with free drug gel**
PRD-7 has more percent proliposomal gel retained on the skin when compared to other PRD-3, PRD-14, PRD-16 and free drug gel

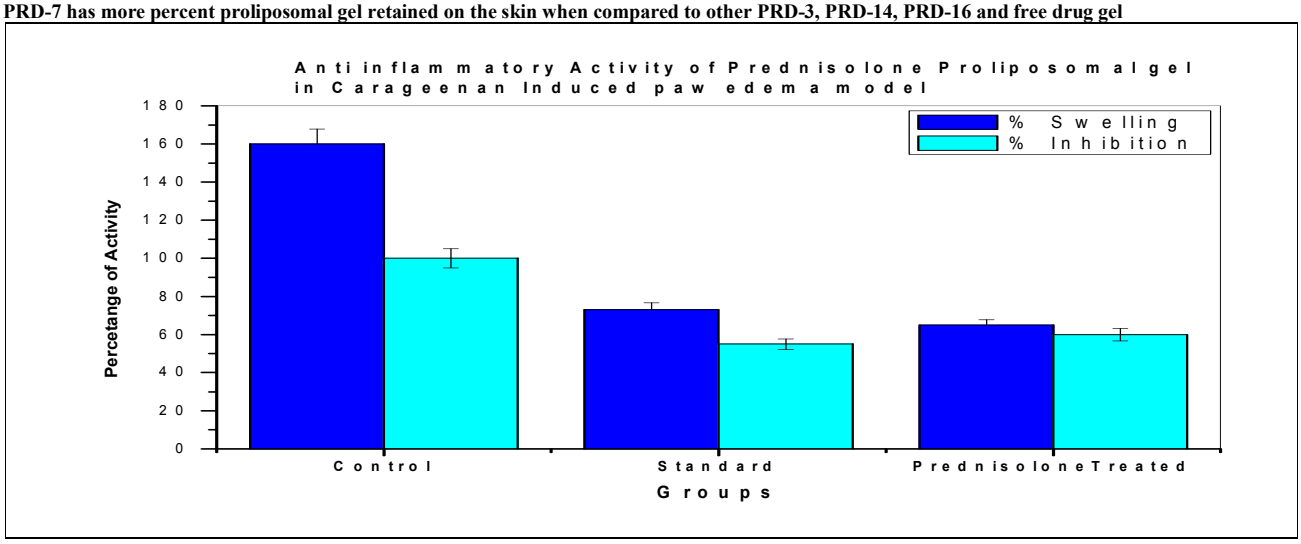


Fig. 11: Anti inflammatory activity of prednisolone proliposomal gel
Prednisolone proliposomal gel shows maximum percent inhibition when compared to diclofenac gel

achieved. Stability studies performed for optimized proliposomal gel formulations indicates that prepared proliposomes have more stability at freezing temperature than that of room temperature. Therefore product should be stored at low temperatures. Proliposomal gel showed greater percentage of inhibition of edema when compared to marketed diclofenac gel. Results indicated the potential of proliposomal gel formulation containing anti-arthritic drug in topical pharmacotherapy for treatment of rheumatoid arthritis. Proliposomes exhibit superior stability when compared to traditional liposomes, thereby increasing its potential application in Transdermal delivery systems.

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