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Research Article **Novel drug delivery system**

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FORMULATION AND EVALUATION OF LIPOSOMAL GEL FOR TREATMENT OF PSORIASIS.

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ABSTRACT

The aim of this study was to formulate and evaluate liposomal gel of Diflurasone diacetate for topical application. Liposomes were prepared by using Soya PC and Egg PC by the lipid film hydration method. It was characterized by DSC and Surface morphology of Liposomes by SEM. assessed for particle size, entrapment efficiency, and their effect on *in vitro* drug release. The Batch B4 containing Soya PC with Cholesterol in 9:1 was found to be optimized .The Stability study of optimized formulation was done as per ICH guidelines. The Optimized liposomes were incorporated into Carbopol gel (2%) and were evaluated for *ex vivo* drug permeation studies. It was observed that Diflurasone diacetate loaded liposomes bearing hydrogel was more efficient in the treatment of Psoriasis.

KEYWORDS: **Diflurasone diacetate; Liposomes; Topical gels; Topical delivery**

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INTRODUCTION

During the past several years, Liposomes began to act as a topical carrier for pharmaceutical molecules. Liposomes have emerged as an alternative to conventional carriers such as cream, tincture and emulsion[1,2]. Liposomes combine their advantages such as controlled release, *in vivo* good toleration and protection of active compounds[3]. Liposomes can favor drug penetration into the skins[4, 5], maintain a sustained release to avoid systemic absorption[6], act as a depot system[7] and reduce irritation[8, 9]. Diflurasone diacetate is a topical Corticosteroids acts by the induction of phospholipase A_2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A_2. The drug is primarily used as a topical treatment for Psoriasis[10, 11]. The treatment for psoriasis includes topical therapy, systemic therapy and phototherapy. The systemic therapy leads to systemic toxicity as psoriasis requires long term therapy and phototherapy is expensive as well as leads to poor patient compliance. Topical corticosteroid formulations are available in market in conventional form having disadvantage of low transdermal penetration which leads to low therapeutic effectiveness. The rationale behind this work was to develop liposomal Topical Drug Delivery System with enhance permeation and to sustain the drug release at the localized area. The aim of this study was to develop topical gels containing liposomal dispersions loaded with Diflurasone diacetate.

MATERIALS AND METHODS

Diflurasone diacetate was gifted by Micro Lab Bangalore, India. Phospholipid (Soya PC) was obtained from Lipoid GmbH, Germany. Cholesterol, Carbopol 934P were purchased from SD Fine Chemicals, Mumbai, India. All the other chemicals were of the analytical grade. Water was used in double-distilled quality.

Preparation of Liposomal Dispersion [12]

The liposomal dispersions were prepared using Lipid film Hydration method. Table1 reports the composition of the prepared liposomal dispersions. Drug, Soya PC/Egg PC, Cholesterol was dissolved in Chloroform: Methanol (3:1 v/v) & solvent was evaporated in Rota evaporator (Eqitron Roteva) under reduced pressure at 60 ^{o} c & 60 rpm for 15 min until a dry film was formed. Film was hydrated with 10ml Phosphate buffer pH6.8 & rotated for 10 min. to get uniform dispersion. It was sonicated at 4 ^{o} c using probe sonicator (at 40 w, vibra cell, model no.CV 334, SONICS) in 3 cycles of 5 min providing 5 min rest between each cycle.

Table I
Composition of various Liposomal formulations. Particle size, Polydispersity Index (PI), Zeta potential and % Drug entrapment, %Drug Release parameters of different Liposomal formulations

Batch code	Drug : Soya PC ratio	Drug : Egg PC ratio	PC:Cholesterol ratio	Particle size (nm)	PDI	Zeta potential (mV)	Drug entrapment (%)	Drug Release (%)
B1	**1:1**	-	**9:1**	200.90±2.10	0.386	-40.3 ± 0.73	75.23 ±0.92	72.16±0.81
B2	**1:2**	-	**9:1**	210.90±1.23	0.299	-44.4 ± 2.57	76.65 ±0.81	65.24±0.92
B3	**1:3**	-	**9:1**	218.00±1.65	0.684	-50.2 ± 3.13	82.60 ±0.90	68.09±0.53
B4	**1:4**	-	**9:1**	238.06±1.40	0.362	-40.52 ± 0.87	84.28 ±0.53	80.06±0.53
B5	**1:5**	-	**9:1**	312.50±1.24	0.652	-49.2 ± 0.75	78.24 ±0.36	74.08±0.54
B6	-	**1:1**	**9:1**	212.9±1.67	0.804	-50.7 ± 0.87	74.58 ±0.57	71.2±0.68
B7	-	**1:2**	**9:1**	274.7±2.10	0.625	-32.53 ± 1.67	75.54 ±0.88	67.58±0.95
B8	-	**1:3**	**9:1**	285.70±1.50	0.589	-33.33 ± 1.84	80.25 ±0.57	67.66±0.43
B9	-	**1:4**	**9:1**	356.20±1.63	0.371	-35.83 ± 1.58	81.30 ±0.64	64.39±0.85
B10	-	**1:5**	**9:1**	488.40±1.60	0.386	30.21±1.25	77.94 ±0.48	59.44±0.54

Characterization of Liposomal Dispersion

Particle size and zeta potential determination [25]

Particle size and size distribution measurements of the liposomal dispersions were performed using photon correlation spectroscopy (PCS). The average particle size (z-average size) and polydispersity index (PDI) were measured by photon correlation spectroscopy Malvern Zetasizer at 25 ^{o} C under a fixed angle of 90^{o} in disposable polystyrene cuvettes. Zeta potential was measured by using Zetasizer. Samples were placed in clear zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment.

%Drug entrapment efficiency [30]

The amount of encapsulated Diflurasone diacetate was calculated by subtracting the free amount of the drug from Diflurasone diacetate liposomal dispersion by ultracentrifugation at 20,000 rpm at 10 ^{o} C for 30 min. After centrifugation, 1 ml of supernatant was diluted with the addition of 9 ml phosphate saline buffer (pH 7.4) and then the absorbance was measured using UV–Vis spectrophotometer at 238 nm..Entrapment efficiency (EE %) was calculated from the following equation

$$EE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug laded expected}} \times 100$$

Scanning electron microscopy (SEM) [31]

The morphology (shape and surface characteristics) of Liposomes was studied by scanning electron microscopy (SEM) (model JSM-5610LV Scanning Microscope; Jeol, Tokyo, Japan). The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA. The condenser lens position was maintained between 4.4 to 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm.

DSC Analysis [31]

DSC studies of pure Diflurasone diacetate, cholesterol, soya pc & mixture was carried out. Accurately weighed samples were carefully placed in DSC boats and *heating curves* were recorded in temperature range of *25–250 ◦C* at a heating rate of *10 ◦C/min* under inert atmosphere (*N_2 30 ml/min*).The study was carried out using Differential Scanning Calorimeter (DSC, Mettler

Toledo Star[e],SW 7.01 Japan).

In Vitro Drug Release [32]

The in vitro drug release profile of Diflurasone diacetate loaded Liposome-bearing hydrogel and marketed formulation were studied using a dialysis bag. Formulations were taken into a dialysis bag (molecular weight cut-off, 8000) and placed in a beaker containing 20 ml of PBS (pH 6.8) Then, the beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 1°C throughout the study. Samples (2 ml) were withdrawn at definite time intervals and replaced with equal amounts of fresh buffer. The samples were analyzed for drug concentration by UV-VIS spectrophotometer at 239 nm.

Stability Studies[32]

In order to determine the physical stability of vesicles, the Optimized vesicles (Batch B 4) was stored at 4 ^{o} c and 40 ^{o} c for up to 3 months under light protection. In predetermined time intervals, the particle size and Entrapment Efficiency of the vesicles was measured

Preparation and Characterization of Liposomal-Based Hydrogel [32]

For the preparation of hydrogel, the gel-forming polymer Carbopol 934P(2%) was dispersed in distilled water, stirred for 10 min at 1500 rpm and neutralized by triethanolamine until pH 6.0. Hydrogel were further allowed to equilibrate for 24 hours at room temperature and then used to disperse a freshly prepared Liposomal suspension. Liposomal dispersion and hydrogel were mixed in a high speed stirrer (Remi, Mumbai, India) at 1000 rpm for the next 5 min. The gel was allowed to stand overnight to remove entrapped air.

Ex vivo skin permeation Studies [21]

Ex vivo permeation of Diflurasone diacetate from Liposome based gel and marketed formulation (Diflorate cream) were performed using the modified Franz Diffusion Cell. The animals were sacrificed by an overdose of chloroform inhalation. The hairs on the dorsal side of animal were removed with the help of 0.1 mm animal hair clipper, in the direction of tail to head. The shaven part of the skin was separated from the animal and the hypodermis including blood vessels were surgically removed using a surgical blade. The dermis part of the skin was wiped off with a wet cotton swab soaked in iso propanol 3–4 times to remove any adhering fat material. The skin membrane surface area exposed to receptor phase was 5.7226 cm^2 the prepared formulations each equivalent to 1.0 mg of drug was applied onto the prepared mice skin facing the donor chamber. An aliquot of 5 ml of samples was withdrawn at suitable time interval and replaced with same amount of medium to maintain the receptor phase volume as 45 ml. The samples were analysed by UV spectrophotometer at 238 nm.

Fitting into Kinetic Model

The diffusion data obtained from diffusion profile was fitted by to various kinetic models (Zero order, First order, Higuchi, Koresmeyer peppas) and the best fit model was obtained by regression analysis. The release mechanism is determined by finding out n value form peppas model.

RESULTS & DISCUSSION

- ***Particle size and Zeta potential determination***

The average particle size analyzed by Malvern Particle size Analyzer was found to be between 200.90±2.10 to 488.40±1.60 nm which is in the desired range as shown in table No.1. The Poly dispersity index (PDI), is a measure of distribution of molecular mass in a given sample. The PDI calculated is the weight average molecular weight divided by number average molecular weight. The PDI has value less than 0.5, this indicates good dispersion. It shows that as the concentration of lipid increases the particle size also increases .Zeta potential is an indication of the stability of the vesicle and indicates charge present on the vesicular system. Here zeta Potential was found to be -- 40.52 ± 0.87 mV for B4 formulation which indicates a negative surface charge on

Liposomes this shows stability of Preparation because of the anticipated surface repulsion between similar charged particles hence inhibiting aggregation of Liposomal vesicular particle.

- ***%Drug entrapment efficiency***

Batch B4 shows highest % Entrapment efficiency of 84.28±0.53. From the data obtained for entrapment efficiency we can say that as the concentration of lipid increases the entrapment of the drug also increases for lipophillic drug as the nature of the lipid is also lipophillic. Cholesterol also plays an important role in entrapment efficiency. Here as the concentration of cholesterol increases the entrapment increases upto certain extent but further increase in cholesterol causes decrease in entrapment

Scanning electron microscopy – SEM

SEM Photographs indicated that the prepared Liposomes were nearly spherical in shape as shown in fig.No.1

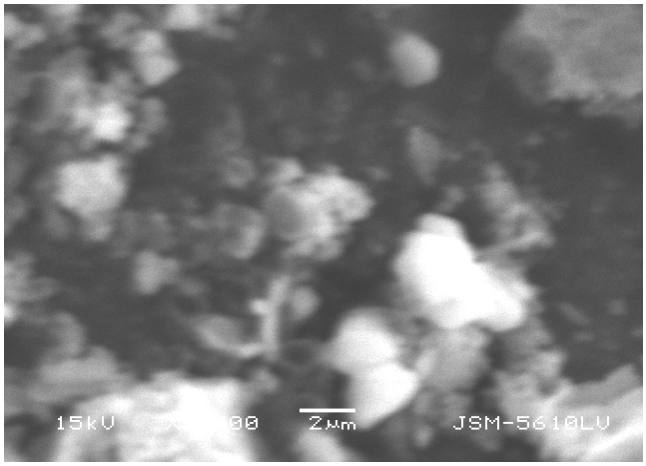


Figure No 1
SEM of Liposomes

- ***DSC Analysis***

DSC studies of pure Diflurasone diacetate, cholesterol, soya pc & mixture was carried out and results were given in fig. No.2 and fig.No.3. From the thermograms, it was concluded that drug and excipient are compatible with each other.

Figure No 2
DSC- Diflurasone diacetate

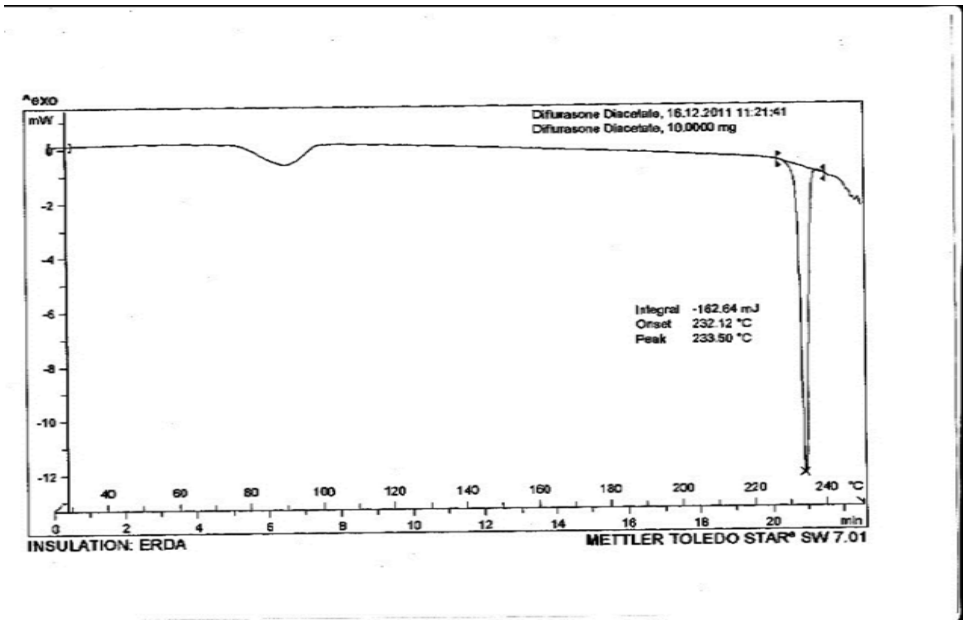
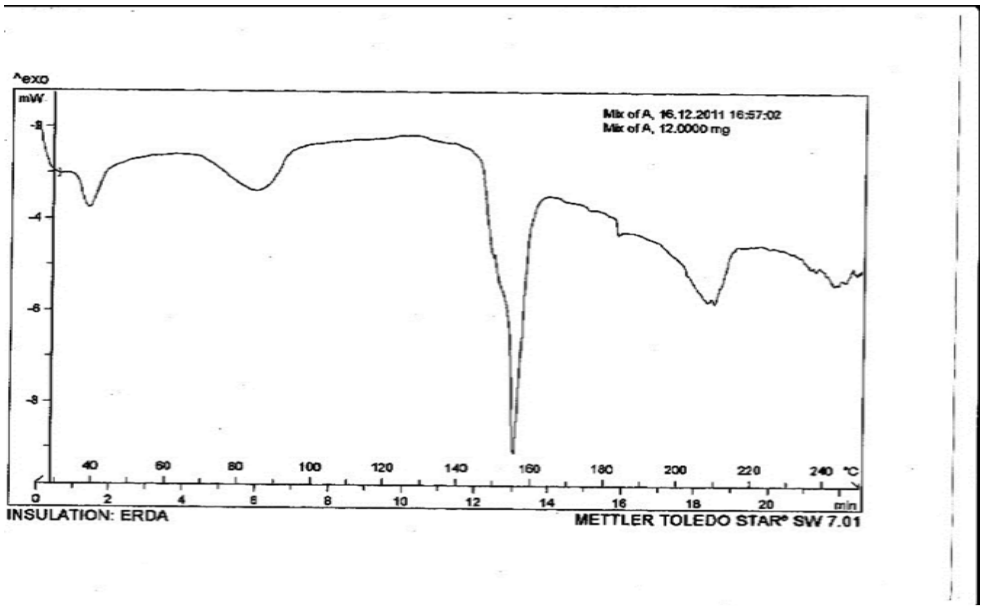


Figure No 3
DSC- Liposomes



- ***In Vitro Drug Release***

The graph of drug diffusion shows that drug release of batch B4 containing Soya PC was found to be maximum release of 80.06±0.53 after 24 hrs.

DISCUSSION

• Depending on the% Entrapment Efficiency, particle size, Zeta Potential and in vitro drug release studies Batch B4 Containing Soya PC from liposomes vesicles showed desirable results and it was selected for further studies

Stability Study

Stability study was carried out for 3 months as per ICH guideline for Batch B4. The vesicle size, entrapment efficiency of liposomal gel was monitored for a period of 3 months. After time interval there was no any appreciable change in vesicle size, entrapment efficiency and gel viscosity. It indicates the optimized formulation was stable.

Sr.no.	Parameters	Result obtained before 3months at	
		4 ˚ C	40 ˚ C
1	Particle size	238.06	237.63
2	Entrapment efficiency	84.28	83.25
	Parameters	Result obtained after 3 months at	
		4 ˚ C	40 ˚ C
1	Particle size	239.85	238.54
2	Entrapment efficiency	84.69	82.96

Table No 2
Data for Stability studies.

Ex vivo skin permeation Studies

The *ex vivo* skin permeation Studies was performed in order to find drug release . This result showed formulation B4 showed around 70.06 % of the drug release after 24 hours.

Time in hr.	% CDR
1	4.56
2	7.86
3	11.56
4	14.78
5	17.56
6	21.54
7	25.99
8	29.89
9	32.02
10	35.45
11	39.87
12	43.57
16	64.25
20	66.34
24	70.06

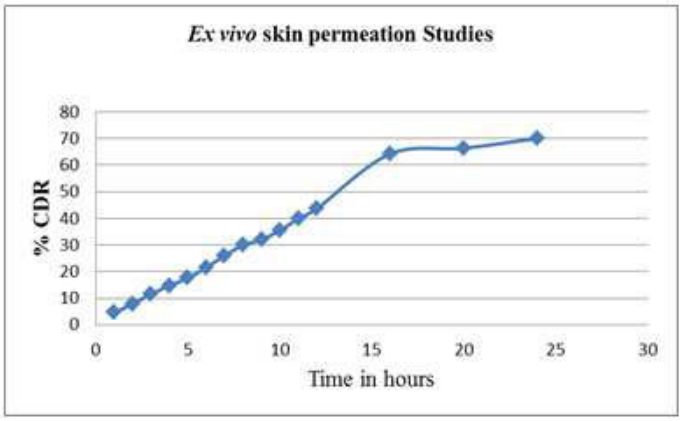


Fig.No.4- Graph of ex vivo skin permeation studies

Table No. 3Data of ex vivo skin permeation studies.

• ***Fitting into Kinetic Model***

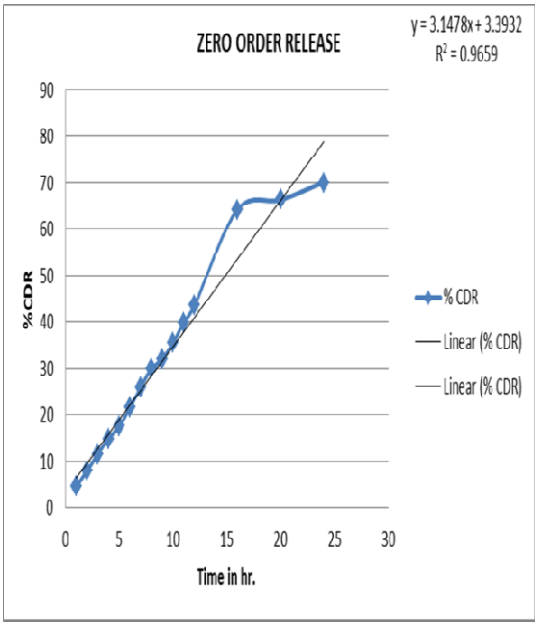
The R^2 value is considered as the tool for repressing the best fitting in a kinetic model. According to data obtained, Liposomes gel shows Zero order release and the release mechanism expected to be anomalous diffusion.

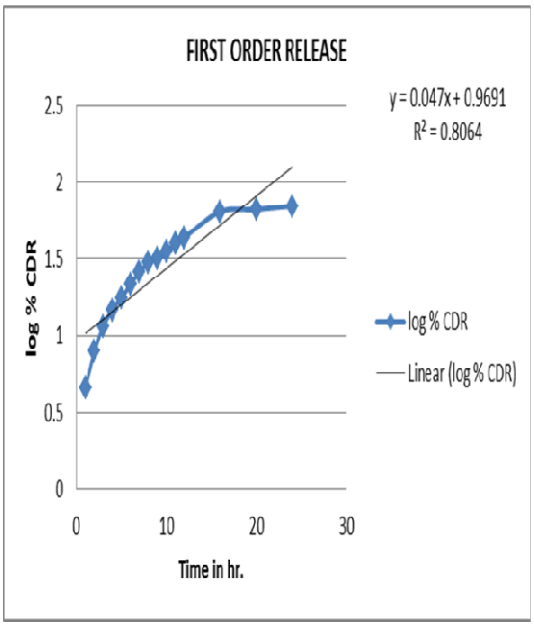
Zero order release		First order release		Higuchi		Korsmeyer Peppas		Release mechanism
K_0	R^2	K_1	R^2	K_H	R^2	N	R^2	
3.147	0.9659	0.047	0.8064	18.928	0.9639	0.9165	0.9936	Anomalous Diffusion

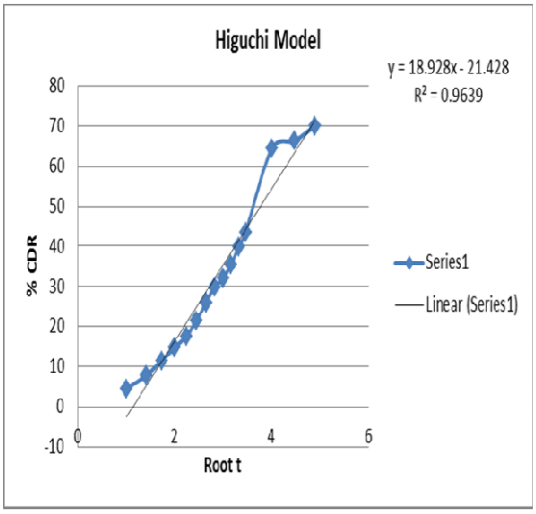
Table No 4
Analysis of In vitro Data

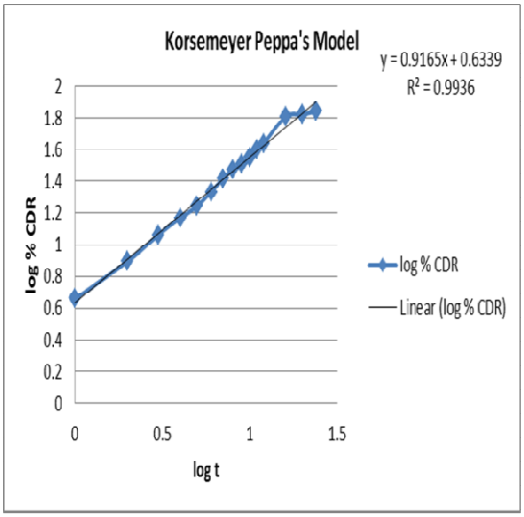
Time in hr.	% CDR	log t	root t	log % CDR	cube root % CDR
1	4.56	0	1	0.658965	1.77
2	7.86	0.30103	1.414214	0.895423	2.22
3	11.56	0.477121	1.732051	1.062958	2.41
4	14.78	0.60206	2	1.169674	2.668
5	17.56	0.69897	2.236068	1.244525	2.884
6	21.54	0.778151	2.44949	1.333246	3
7	25.99	0.845098	2.645751	1.414806	3.107
8	29.89	0.90309	2.828427	1.475526	3.207
9	32.02	0.954243	3	1.505421	3.332
10	35.45	1	3.162278	1.549616	3.476
11	39.87	1.041393	3.316625	1.600646	3.634
12	43.57	1.079181	3.464102	1.639188	3.848
16	64.25	1.20412	4	1.807873	4.02
20	66.34	1.30103	4.472136	1.821775	4.325
24	70.06	1.380211	4.898979	1.84547	4.384

Table No 5
Fitting Analysis









Summary

The Liposomes was prepared by the thin film hydration method. The optimized formulations Batch B4 showed the Particle size of 238.06±1.40 & PDI of 0.362 with acceptable entrapment efficiency of 84.28 and drug release of 80.06 after 24 hrs. SEM Photographs indicated that the prepared Liposomes were nearly spherical in shape. DSC analysis concluded that drug and excipient are compatible with each other. Stability study reveals that Optimizes Liposomal formulation (Batch B4) was found to be stable. The Optimized formulation Batch B4 gel was prepared by using Carbopols974P & was evaluated for *Ex vivo* skin permeation studies and it shows 70.06 % CDR after 24 hours. Various Kinetic model was use to study the release pattern and it shows Zero order release with anomalous diffusion.

CONCLUSION

The Diflurasone diacetate loaded Liposomes could be fabricated with the help of a thin film hydration method and successfully incorporated into hydrogel for topical application. The in vitro and ex vivo data indicate that Diflurasone diacetate loaded Liposomes bearing hydrogel provides an excellent sustained release of Diflurasone diacetate. The obtained results reflect the potential of Liposomes as a carrier for topical administration of Diflurasone diacetate. In conclusion, the developed systems are promising alternative drug carriers for topical pharmaceutics.

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