Development and In Vitro Evaluation of Flurbiprofen Ethosomal Drug Delivery System

Nansri Saha, Shireesha, A Saritha
Department of Pharmaceutics, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District

ABSTRACT :
Flurbiprofen, a non-steroidal anti-inflammatory drug (NSAID), suffers from poor water solubility and limited skin permeation, restricting its therapeutic efficacy in topical or transdermal applications. The present study aimed to develop and evaluate flurbiprofen-loaded ethosomal formulations to enhance skin delivery and anti-inflammatory activity. Ethosomes were prepared using the cold method, employing phospholipids, ethanol, and distilled water. The formulations were characterized for particle size, zeta potential, entrapment efficiency, and morphology. In vitro studies included drug release and skin permeation analysis using Franz diffusion cells. Stability studies were conducted under different temperature and humidity conditions to evaluate vesicle integrity and drug content over time. Results indicated that the prepared ethosomes had a nanometric particle size (153–263 nm), negative zeta potential (-20 to -29 mV), and high entrapment efficiency (above 70%), confirming their suitability for transdermal delivery. In vitro drug release and skin permeation studies demonstrated enhanced flurbiprofen flux compared to conventional gel formulations, indicating improved bioavailability. Stability studies confirmed that the optimized formulation was physically and chemically stable over the study period. Overall, the developed flurbiprofen ethosomal system shows promise as an effective transdermal delivery platform, providing controlled drug release, improved skin permeation, and potential reduction of systemic side effects associated with oral administration.
Keywords: Flurbiprofen, Ethosomes, Cold method, FTIR Studies, Phosphotidyl choline, In vitro drug release studies

I.INTRODUCTION

Ethosomes are soft, malleable lipid vesicles composed of phospholipids, ethanol, and water, which demonstrate significantly improved transdermal permeation compared to conventional liposomes.[1] The synergistic mechanism of ethanol-induced membrane fluidization and vesicle deformability enables ethosomes to pass through deep skin layers, making them a promising platform for topical and transdermal delivery of both hydrophilic and lipophilic drugs.[2] Their enhanced entrapment efficiency, stability, biocompatibility, and capability to deliver drugs at controlled rates make ethosomes suitable for the treatment of localized inflammatory disorders, pain management, and dermatological conditions.[3] Transdermal drug delivery systems (TDDS) have gained considerable attention as an alternative to conventional oral and parenteral routes due to advantages such as avoidance of first-pass metabolism, sustained drug release, improved patient compliance, and reduced systemic side effects. [4] However, the major challenge associated with transdermal delivery is the limited penetration of drugs across the stratum corneum, which acts as a primary physiological barrier. To overcome this limitation, novel vesicular carriers such as liposomes, niosomes, transferosomes, and ethosomes have been developed to enhance dermal and transdermal transport of therapeutic agents.[5] Flurbiprofen, a nonsteroidal anti-inflammatory drug (NSAID), is widely used in the management of osteoarthritis, rheumatoid arthritis, dental pain, and soft tissue inflammations. Therefore, formulation of flurbiprofen into an ethosomal carrier system presents a strategic approach to enhance drug penetration, localize drug action at the inflammation site, and reduce systemic adverse effects.[6] In recent years, several studies have demonstrated the potential of ethosomal formulations in improving solubility, dermal retention, and therapeutic efficacy of anti-inflammatory agents.[7] The present research focuses on the formulation, optimization, and in vitro evaluation of flurbiprofen-loaded ethosomes to investigate their particle size, entrapment efficiency, vesicular stability, drug release profile, and permeation characteristics.[8] This study aims to establish ethosomes as a promising platform for improved transdermal delivery of flurbiprofen with enhanced bioavailability and therapeutic benefit.

II. EXPERIMENTAL WORK

MATERIALS

Flurbiprofen was procured from Hetero Labs, HYD. Phosphotidyl choline, Ethanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY

Compatibility study (IR spectroscopy)

FTIR analysis was performed in order to study the compatibility of ingredients used in the preparation of nanoparticles, using a Shimadzu FTIR spectrophotometer (Prestige21, Shimadzu Corporation, Kyoto, Japan). Atomoxetine and Excipients their mixture with ratio (1:1) was evaluated using FTIR spectrophotometer using potassium bromide disc technique where 1mg of the sample is mixed with 100 mg of dry powdered KBr, the mixture is pressed into a transparent disc and was inserted in the apparatus for IR scan.[9,18]

Formulation development

Table-1: Composition of Flurbiprofen Ethosomes (F1 to F8)

S.No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Flurbiprofen	75	75	75	75	75	75	75	75
2	Phosphotidyl choline	100	200	300	400	500	600	700	800
3	Ethanol	5	5	5	5	5	5	5	5
4	Water	10	10	10	10	10	10	10	10

Procedure

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation. Phospholipid and drug and other pharmaceutical ingredient listed in table were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to 30^0C ± 10C and a fine stream of distilled water was added slowly, with constant mixing at 100 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 30^0 C ± 10C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4^0 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator.[10]

CHARACTERIZATION

Particle size:

All the prepared batches of ethosomal gel were viewed under microscope to study their size. Size of Ethosomes from each batch was measured at different location on slide by taking a small drop of ethosomes on it and average size of ethosomes were determined.[11]

SEM analysis

The morphology of Ethosomes was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the Ethosomes was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.[12]

Zeta Potential:

The zeta potential means the charges which are present on the surface of Ethosomal. The many time the charge is present on the surface of Ethosomal. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all transferosome present in formulation, due to some charge all Ethosomal particle repeal to each other and coagulation of particle are avoided. The zeta potential of Ethosomal was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25ºc with the angle of detection 90º. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of Ethosomal particle.[13]

Drug entrapment efficiency

Prepare ethosomal dispersion (known total drug concentration). Record volume and total drug added Transfer aliquots into centrifuge tubes. Include blank/control tubes. Centrifuge at 15,000 rpm (~20,000 × g) for 30–45 min at 4°C. (Adjust if pellet not forming; nanosized ethosomes may need ultracentrifugation. Carefully collect the supernatant (avoid disturbing pellet). This supernatant contains **free (unentrapped) drug.** If desired, wash pellet once with small volume of buffer, recentrifuge and combine washes with supernatant (to quantify any loosely bound drug). Determine drug concentration in supernatant using a validated analytical method (UV at λmax). Multiply by supernatant volume to get mass of free drug.[14]

$$\text{EE}\ (\%) = \frac{\text{Total drug added} - \text{Free (unencapsulated) drug}}{\text{Total drug added}} \times 100$$

***in-vitro* Drug release studies:**

The *in-vitro* study of drug permeation through the Dialysis membrane was performed using a modified Franz type glass diffusion cell. The modified cell having higher capacity is (10 ml) is used to maintain sink condition. The samples were analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.[15]

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Release kinetics[16]

The release kinetics can be understood basically by applying the obtained data to the release kinetics models.

Zero order kinetics

$$C = K0t$$

K0 - rate constant for Zero-order (concentration/time) t - Time (h).

First orderkinetics

$$\text{Log } C = \text{Log}C0 - Kt / 2.303$$

Where C0 - Initial concentration of drug K = constant first order and t = Time (h)

Higuchi Model

$$Qt = Kt1/2$$

Where Qt - Amount of the drug release drug in time t K- Kinetic constant and t- is time in hrs **Korsmeyer PappasModel**

$$Mt / M = Kt\ n$$

Where, Mt - amount of the released drug at time t, M- Overall drug amount released after 8 hrs. K- Diffusion constant n- Diffusion exponent mechanism of release of drug.

Stability studies[17]

Optimized medicated niosomes were subjected to short term stability testing. The niosomes were sealed in aluminium foils and kept in a humidity chamber maintained at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months as per ICH guidelines. Changes in the appearance and drug content of the stored patches were investigated after storage at the end of every month.

III. RESULTS AND DISCUSSION

FT-IR studies

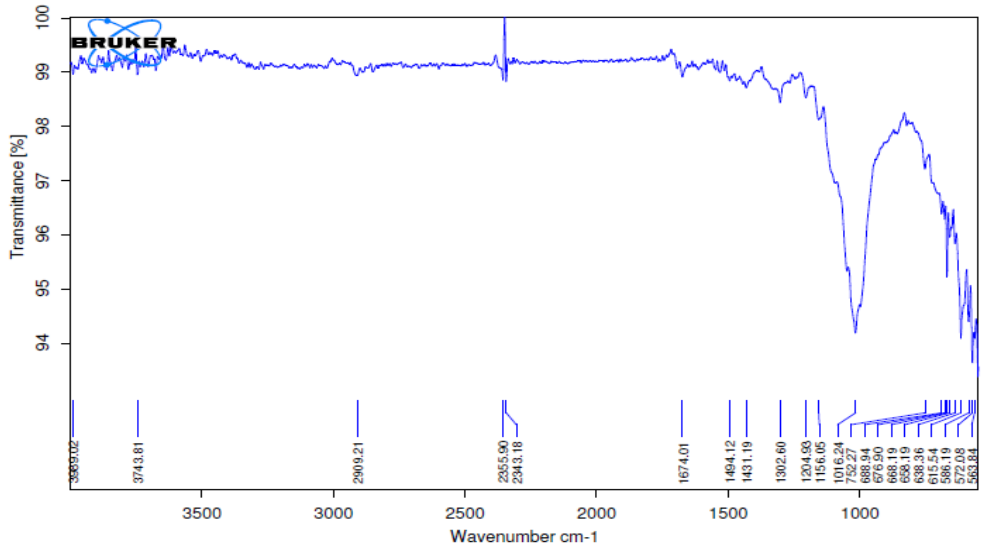

Fig-1:FT-IR graph for Flurbiprofen

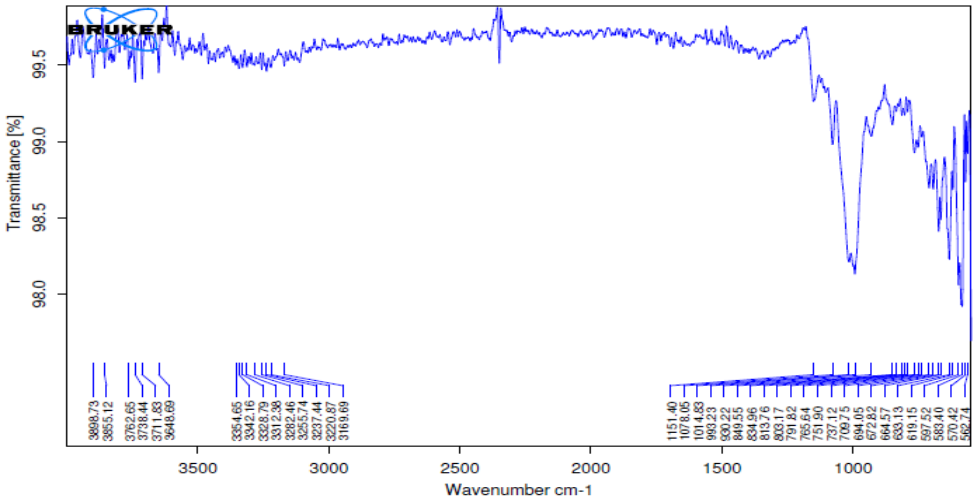

Fig-2: FTIR Spectra of physical mixture of drug and excipients

EVALUATION PARAMETERS:

DETERMINATION OF VESICLE MORPHOLOGY AND SIZE

The morphological characteristics of formulated ethosomes were carried by using Scanning electron microscopy (SEM). A small drop of Ethosomal gel was placed between two rivets fixedon a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^{0}c and voltage of 5kV.

TABLE-9: EVALUATION STUDIES OF PARTICLE SIZE AND ZETA POTENTIAL ETHOSOMES

F. No	Particle size (nm)	ZETA POTENTIAL (MV)
F1	249	-20
F2	263	-22
F3	257	-25
F4	250	-23
F5	198	-29
F6	153	-27
F7	220	-25
F8	218	-24

Particle size analysis

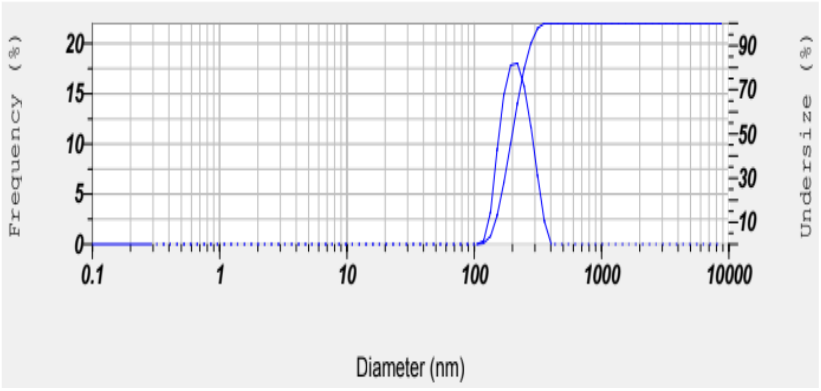

Fig-3: Particle size analysis of Optimized formulation of Ethosomes

Zeta potential

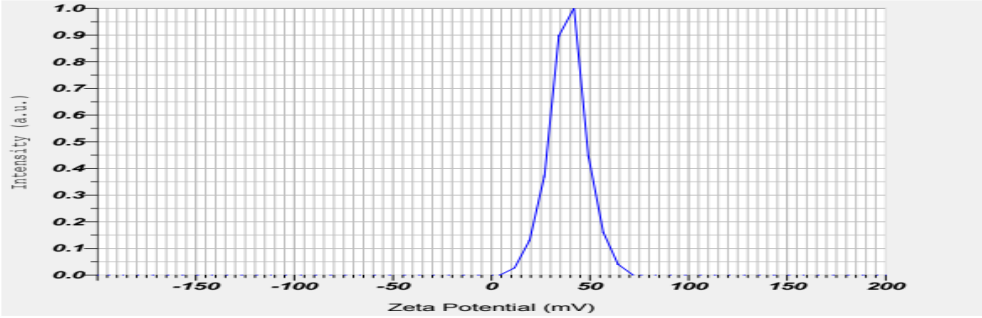

FIG-4: ZETA POTENTIAL OF ETHOSOMES

The particle size of the ethosomal formulations ranges from 153 nm (F6) to 263 nm (F2). F6 (153 nm) is the smallest vesicle, suggesting high ethanol concentration or optimized lipid content, which reduces vesicle aggregation. F2 (263 nm) is the largest, which may indicate slightly lower ethanol or higher phospholipid concentration, leading to larger vesicles. All formulations are in the nanometer range (<300 nm), which is suitable for transdermal delivery, as smaller vesicles penetrate the stratum corneum more efficiently. Formulations F5 and F6, with smaller particle sizes, may have higher surface area and potentially better skin permeation. Slight variations in particle size can be attributed to changes in lipid-to-ethanol ratio, sonication time, or homogenization conditions during preparation.

Zeta potential measures the surface charge and stability of the vesicles. Values range from -20 mV (F1) to -29 mV (F5). All formulations have a negative charge, which is typical for phospholipid-based ethosomes due to ionized phosphatidylcholine and ethanol. A zeta potential of ±25–30 mV or higher generally indicates moderate to good stability due to electrostatic repulsion preventing aggregation. F5 (-29 mV) and F6 (-27 mV) are expected to have better physical stability, whereas F1 (-20 mV) might be slightly more prone to aggregation over time

SEM Analysis

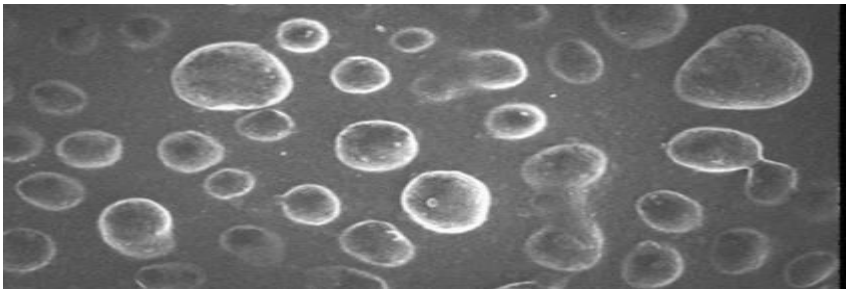

Fig-5: SEM Analysis of Ethosomes

***In vitro* release study:**

Table-12: *In vitro* drug release profiles of Flurbiprofen Ethosomes (F1-F8)

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	14.28	15.68	16.97	17.42	18.13	18.19	17.10	16.32
2	26.89	26.97	23.65	25.49	28.47	29.86	25.56	24.51
3	32.65	33.25	35.79	36.79	37.91	38.15	37.45	35.18
4	43.59	45.97	46.89	48.72	46.38	49.68	45.20	43.25
5	56.18	55.12	56.37	55.68	61.25	63.50	65.17	60.17
6	69.12	68.54	69.87	68.21	73.25	75.19	72.18	74.20
7	78.15	75.98	76.89	77.89	83.19	84.16	81.15	81.20
8	92.25	93.56	95.28	96.83	94.52	98.38	95.80	93.25

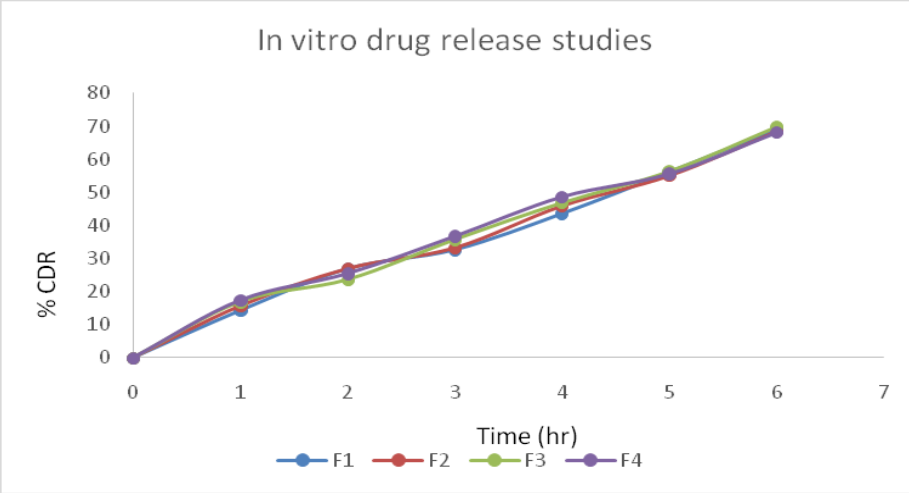

Fig-6: In vitro drug release studies of F1-F4 formulations

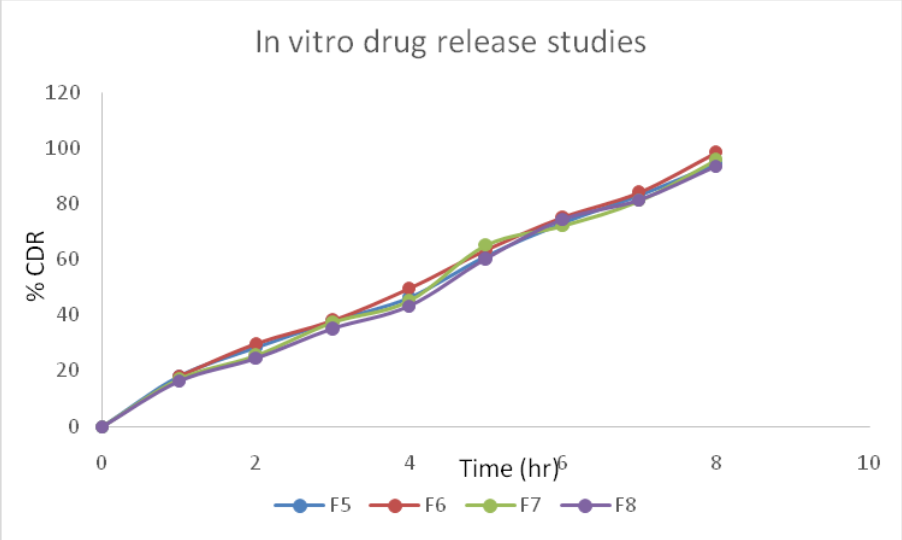

Fig-7: In vitro drug release studies of F5-F8 formulations

Kinetic modelling of drug release

All the 8 formulation of prepared ethosomes of Flurbiprofen were subjected to in vitro release studies these studies were carried out using diffusion apparatus.

Zero order kinetics

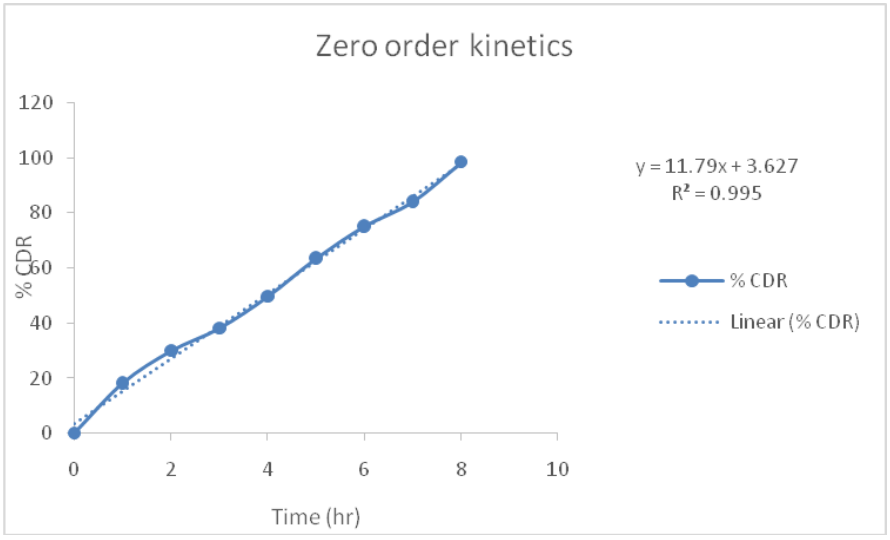

Fig-8: Zero order kinetics of optimized formulation

First order kinetics

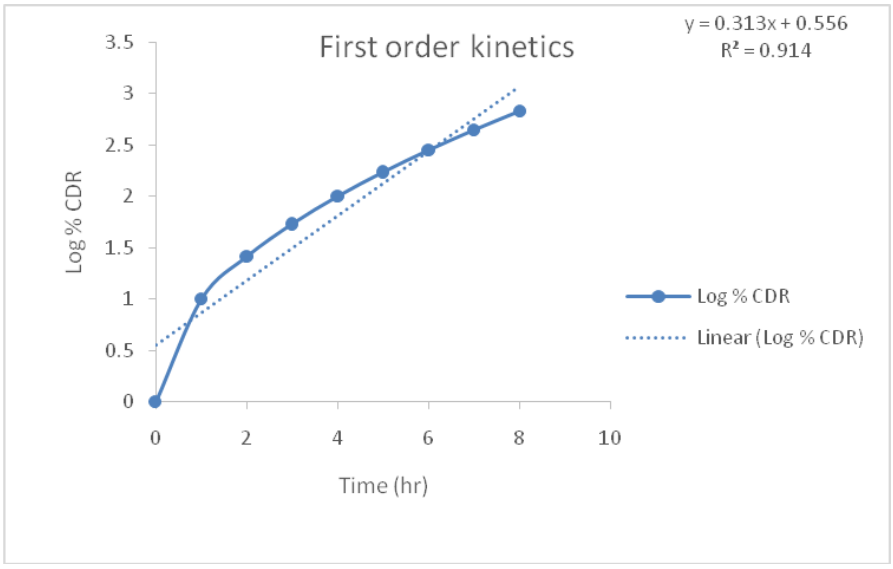

Fig-9: First order kinetics of optimized formulation

Higuchi model

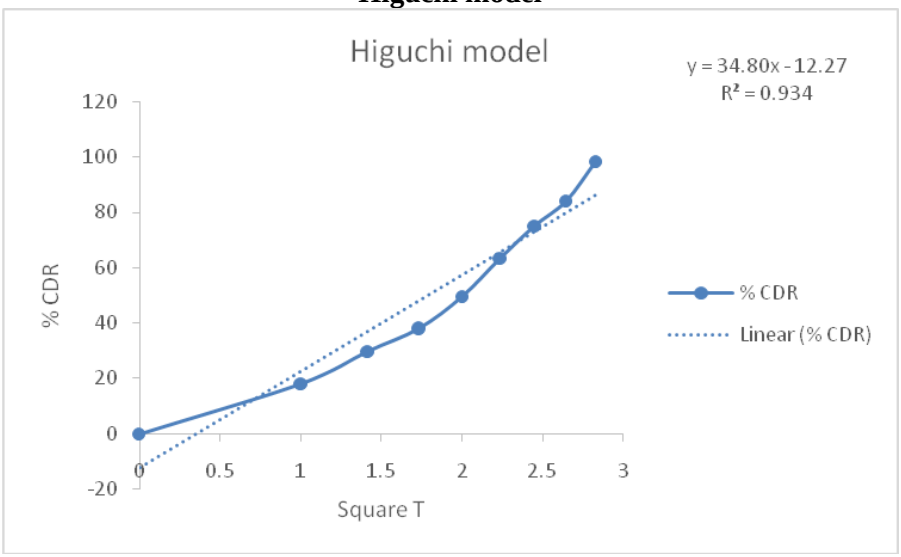

Fig-10: Higuchi model of optimized formulation

Korsmeyer peppas

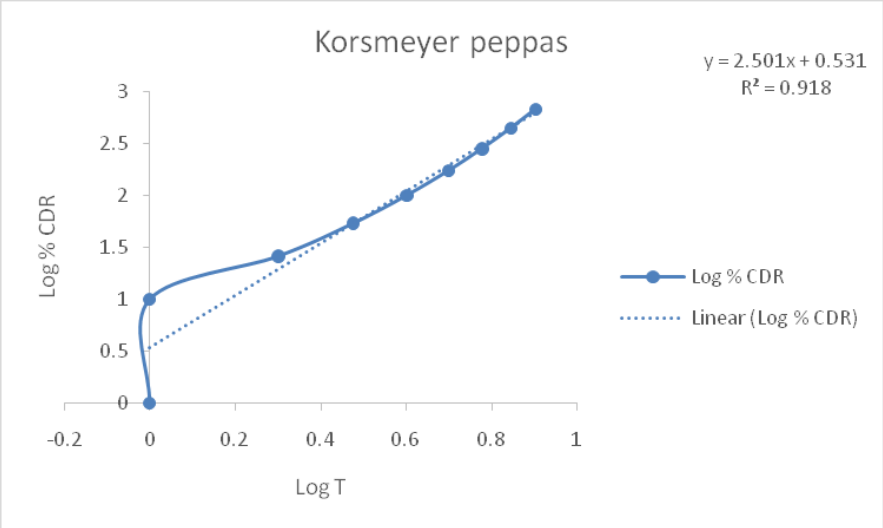

Fig-11: Korsmeyer peppas of optimized formulation

The kinetic values obtained for formulation F6 were shown. The values of in vitro release were attempted to fit into various mathematical models.

Regression values are higher with First order release kinetics. Therefore, all the Flurbiprofen ethosomes follows Korsmeyer peppas release mechanism.

Stability studies

There was no significant change in physical and chemical properties of the Ethosomes optimized formulation after 90 days. Parameters quantified at various time intervals were shown.

Table-15: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	25^{0}C/60%RH % Release	98.38	97.58	96.14	95.48	Not less than 85 %
F-6	30^{0}C/75% RH % Release	98.38	97.62	96.54	95.25	Not less than 85 %
F-6	40^{0}C/75% RH % Release	98.38	97.55	96.33	95.36	Not less than 85 %

CONCLUSION

The study concludes that flurbiprofen-loaded ethosomal formulations are a promising transdermal drug delivery system offering: Enhanced skin permeation due to nanosized vesicles and ethanol-mediated disruption of the stratum corneum. Controlled and sustained drug release, potentially reducing dosing frequency. High physical and chemical stability, ensuring reliable storage and handling. Reduced systemic side effects compared to oral administration, improving patient compliance. Overall, the optimized ethosomal formulation represents a viable and effective strategy for delivering flurbiprofen through the skin, making it suitable for topical anti-inflammatory therapy. Further in vivo studies are recommended to confirm therapeutic efficacy and safety in clinical applications.

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