Development and In Vitro Evaluation of Indomethacin Transferosomal Gel

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ABSTRACT:

The present study was focused on developing and evaluating Indomethacin- containing transfero some formulation for in vitro studies. Transfero some formulations were prepared by using the thin film hydration method and were evaluated for in vitro characteristics, and stabilitystudies. Transferosome formulation displayed the highest entrapment efficiency with the desired particle size. SEM analyses showed that Transferosomes formulation was spherical in shape. Transferosomes containing lipids higher percentage of drug release after 12h as compared to other formulations.F8 formulation was found to be stable at the end of the study on storage conditions. The present study suggested that Transferosomes gel formulations provide sustained and prolonged delivery of drugs with enhancedbioavailability.

Keywords: Transferosomes, Indomethacin, bioavailability, thin film hydration method, In vitro drug release studies.

I. INTRODUCTION

Transdermal delivery of drugs through the stratum corneum is a challenge. One of the most important factors to consider for a successful transdermal formulation is the penetration of the drug through the skin, which is mostly dependent on the physicochemical properties of the drug. Drugs with optimal lipophilicity are best suited for transdermal delivery. Hence, a hydrophilic drug must penetrate the skin to elicit a systemic effect. As a result, in the last decade, lipid-based vesicles or carriers have been routinely studied for topical drug delivery.[1]

Transferosomes is commonly known as "Ultra Deformable Vesicles" and it contain a lipid vesicle made up of Phospholipids and an edge activator.[2] Transferosomes passes stratum corneum layer by squeezing themselves many times smaller than its size owing to its elasticity nature which is achieved by mixing suitable surface-active components and lipids.[3] A gel consists of a polymer which swells in the presence of fluid and perhaps within its structure. The rigidity of the gel is determined by the amount of fluid it entraps. The clarity ranges from clear to whitish translucent. [4] The extensive studies on release properties orgels have revealed that the active ingredients in gel-based formulations are better percutaneous absorbed than from creams and ointment bases. Thus, facts have clearly indicated that a formulation and development of a gel based topical dosage form for dermal conditions will be proved to be worthwhile.[5]Indomethacin is a non-steroidal anti–inflammatory drug used in thesymptomatic management of painful and inflammatory conditionssuch as rheumatoid arthritis. The most frequent adverse effect is gastro– intestinal (GI) and central nervous system disturbances. The traditional dosage forms of indomethacin such as tablets and capsules have totaken three times per day.[6]

II. EXPERIMENTAL WORK

MATERIALS

Indomethacin was procured from Hetero Labs, HYD. Soya lecithin, Cholesteroland Span 80 was obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY

Fourier transform infrared spectroscopy:

Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 cm^{-1} and the resolution was 4 cm^{-1}.[7]

Formulation development

Table-1: Formulation table

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Indomethacin	25	25	25	25	25	25	25	25
Soya lecithin	50	100	150	200	250	300	350	400

Cholesterol	50	50	50	50	50	50	50	50
Span 80	2	2	2	5	2	2	2	2
Chloroform	3	3	3	3	3	3	3	3
Methanol	1	1	1	1	1	1	1	1
HPMC	250	500	750	1000	-	-	-	-
Carbopol 940	-	-	-	-	250	500	750	1000

Preparation method Trasferosomes

A thin-film hydration technique was employed to form transferosomes. Specific amounts of lipids were dissolved in a mixture of organic solvents consisting of methanol and chloroform (1:3) in a dry round-bottom flask. The evaporation of organic solvents was performed under vacuum using a rota evaporator at 100 rpm, at 48-50 °C. The thin lipid film thus obtained was then hydrated using water containing surfactant and drug by rotation for 1 hour at 50 °C. Each formulation was placed in an ultrasonicator bath at 150 W for 20 seconds. A dialysis bag was used to remove the free drug, and the final formulation was stored at 4 °C for further use. Blank transfersomes were prepared using the same method for each formulation.[8]

Preparation of topical Transfersome gel:

As a vehicle for incorporation of transfersomes for topical delivery, carbopol gels were prepared. Transfersomes aqueous dispersion was utilized for the formulation of topical gel. Gel polymer such as carbopol 940 was utilized to prepare transfersome gel. 2g of carbopol- 940 powder was dispersed into vigorously stirred (stirred by magnetic stirrer Remi 5MLH) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 24 hrs. The dispersion was neutralized with tri ethanolamine to adjust the pH 7.4 by using pH meter (Lab India Sab 5000).[9]

Characterization

Vesicle size

Transfersomes vesicles can be visualized by SEM and optical microscope. The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by using optical microscope and SEM.[10]

SEM Analysis

The shape, surface characteristics, and size of the Transferosomal gel were observed by scanning electron microscopy. Once again, 0.2 g of the Transferosomal gel in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The Transferosomal gel were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.[11]

Determination of pH:

The value of pH of topical Transferosomal gel wasmeasured by using digital pH meter(Lab India Sab 5000 pH meter) at the room temperature.[12]

Determination of entrapment efficiency percentage:

The amount of Indomethacin entrapped in transfersomal gel was estimated by centrifugation method. 1gm of Transfersomalgel was taken and diluted with 10ml phosphate buffer (pH 7.4). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted before going for absorbance measurement using UV spectrophotometer (UV-3200 Lab India)[13]. This gives us the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

% Entrapment = Total drug – Diffused drug/ Total drug × 100

***In-vitro* drug release studies**

Modified Franz diffusion cell with a receiver compartment volume of 18ml and effective diffusion area of $2cm^2$ was used for this study. *In-vitro* drug study was performed by using egg membrane in phosphate buffer solution (pH 7.4). To perform *in-vitro* drug release study, egg membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was $2cm^2$ and capacity of receptor compartment was 30ml. The receptor compartment was filled with 30ml of phosphate buffer (pH 7.4) maintained at 37± 0.5^0C and stirred by a magnetic bar at 100rpm. Transfersomal gel formulation equivalent to 80mg drug was placed on the cellophane membrane and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 7.4) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ max 320 nm. [14]

Kinetics of drug release:[15]

To study kinetices data obtained from invitro releasase were plotted in various kinetic models.

- **Zero-order equation :**

$$\% R = Kt$$

This model represents an ideal release profile in order to achieve the pharmacological prolonged action.

First order equation:

$$\text{Log \% unreleased} = Kt / 2.303$$

This model is applicable to study hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms.

Higuchi equation :

$$\% R = Kt^{0.5}$$

- **Korsmeyer-Peppas equation :**

$$\%R = Kt^{n}$$

This model is widely used, when the release phenomenon could be involved.

Stability Studies of transfersomal gel [16]

After measuring the initial percentage entrapment of the drug in the optimized formulation, the three batches of the same formulation were stored in sealed glass ampoules (one each) at refrigeration temperature (4±2°C), room temperature (25±2°C) and body temperature (37±2°C) for a period of at least 3 months. The percentage entrapment of the drug and % drug content was determined in the formulations after 15, 30, 45 and 90 days to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100%.

III. RESULTS AND DISCUSSION

FT-IR Spectrum of Indomethacin

FT-IR Spectra ofIndomethacinand F8 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between drug and excipients.FTIR spectra of drug in KBr pellets at moderate scanning speed be 4000-400 cm-1 was made.

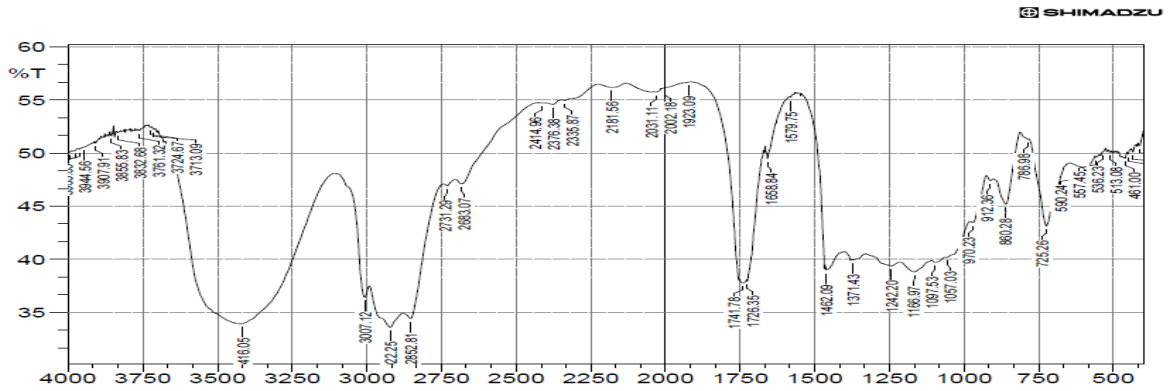

Fig-1: FTIR Studies of Indomethacin

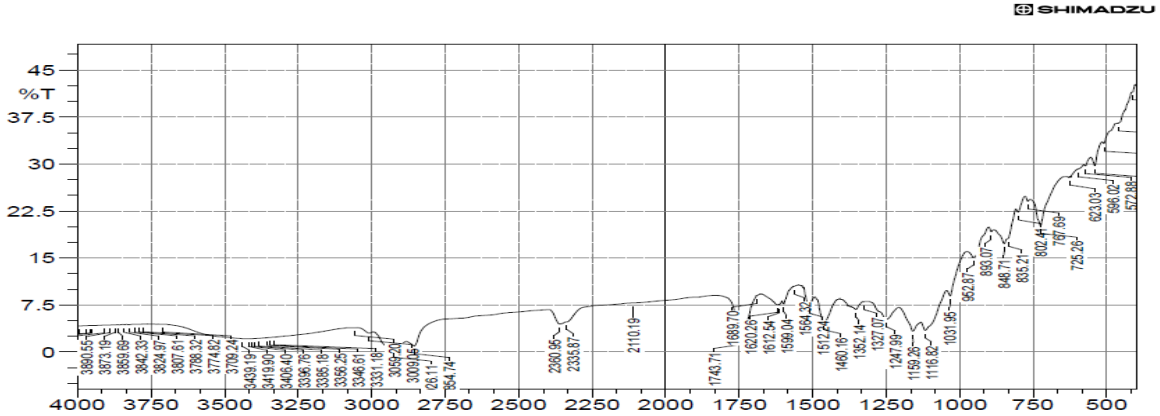

Fig-2: FTIR Studies of optimized formulation

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Puredrug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks were obtained as above and as they were in official limits

(±100 cm^{-1}) the drug is compatible with excipients.

Particle size of Transferosomes

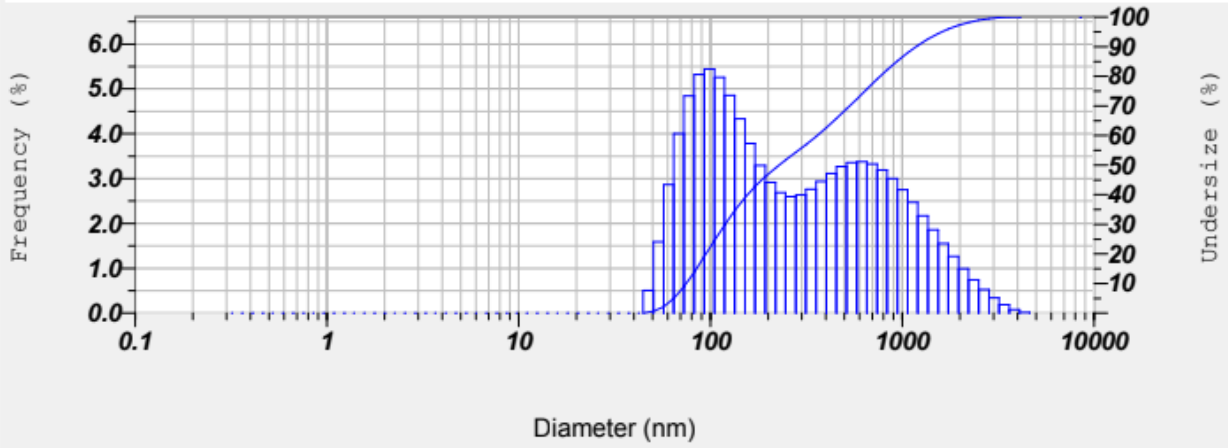

Fig-3: Particle size of Optimized formulation

The mean particle size of optimized Polymeric nanoparticles was found to be 229 nm

pH value of topical transferosome gel:

The value of pH of topical transferosome gels was measured by using digital pH meter (LabindiaSab 5000 pH meter) at the room temperature. The pH of all topical Transferosomal gels were found to be in the range of 6.2 to 7.1

Table-2:pH value of topical Transferosomal gel

F. No	pH
F1	6.6
F2	6.9
F3	6.3
F4	7.1
F5	6.5
F6	7.0
F7	6.2
F8	6.8

SEM Analysis

Scanning electron microscopy (SEM) SEM revealed that the Indomethacin transfersomes were smooth and spherical without any aggregation.

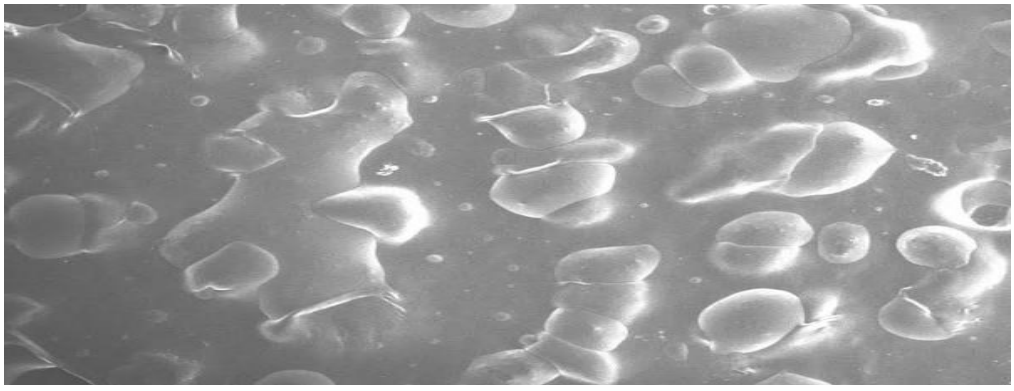

Fig-4: SEM Analysis of Transferosomal gel

Entrapment efficiency

Table-3: Percentage entrapment efficiency

F. No	% EE
F1	78.56
F2	80.15
F3	79.68
F4	81.69
F5	83.77
F6	89.65
F7	85.65
F8	90.21

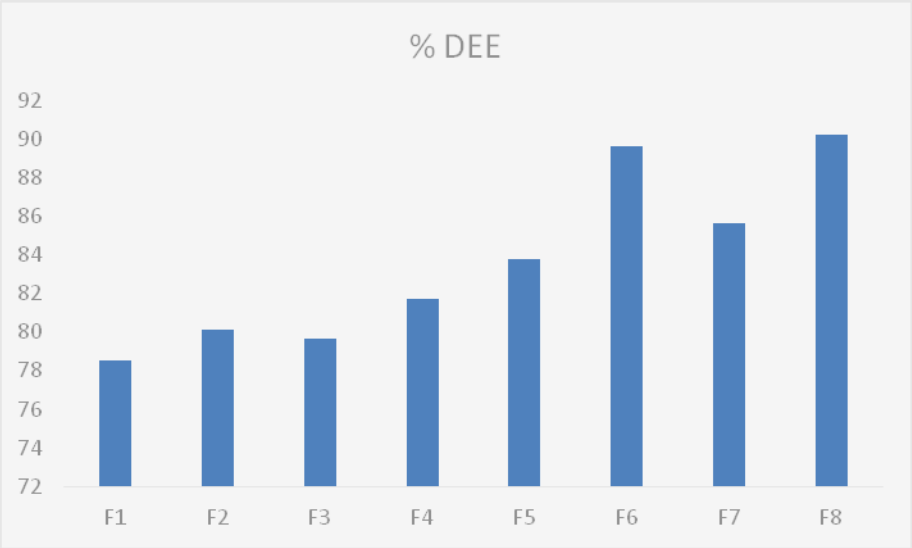

Fig-5: Entrapment efficiency of all formulations

In vitro drug release studies

The diffusion medium used was phosphate buffer solution pH 7.4 . In the Franz diffusion cell, the Dialysis membrane was mounted between donor and receptor compartment of diffusion cell. Egg membrane separated both the compartments. Area of membrane separating two compartments was 1.7662 cm^2. Transfersomalgel(20 ml)volume was accurately pipetted into donor compartment which was then covered with aluminum foil to avoid any evaporation. Diffusion medium (20 ml) was maintained at 37 ±1 ^{o} C, so that the membrane just touches the receptor medium surface. A magnetic bar continuously stirred in the diffusion medium at 100 rpm. Each of 1 ml volume was withdrawn at required time intervals and replaced by 1 ml volume of receptor medium (phosphate buffer pH 7.4) to maintain the sink condition. These samples were analyzed by UV spectrophotometer at maximum wavelength 320 nm.

Table-4: Diffusion studies of Transfersomal gel

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	17.13	16.82	17.10	18.10	17.10	18.75	16.12	16.85
2	26.39	24.58	25.92	26.92	24.98	25.62	26.75	26.51
3	33.45	34.79	37.85	38.20	38.22	37.41	38.18	38.13
4	46.98	52.61	55.93	57.15	53.46	54.60	55.25	57.45
6	55.69	63.75	65.86	66.89	66.89	67.45	67.89	67.19
8	77.80	76.82	78.10	79.15	76.14	75.10	77.53	76.89
10	82.87	83.75	85.96	86.82	81.56	82.69	83.67	85.72
12	93.30	94.58	95.82	91.25	95.66	96.34	97.52	98.90

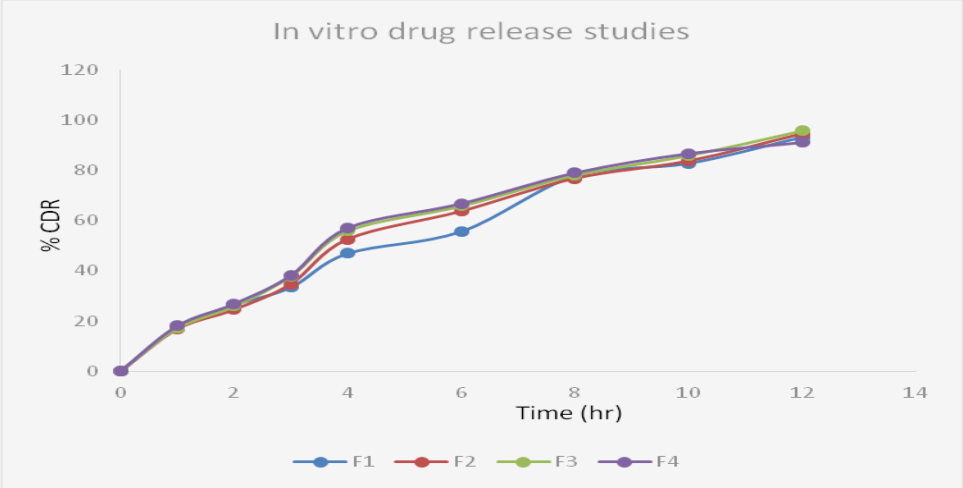

Fig-6: Diffusion studies of Transfersomes (F1-F4)

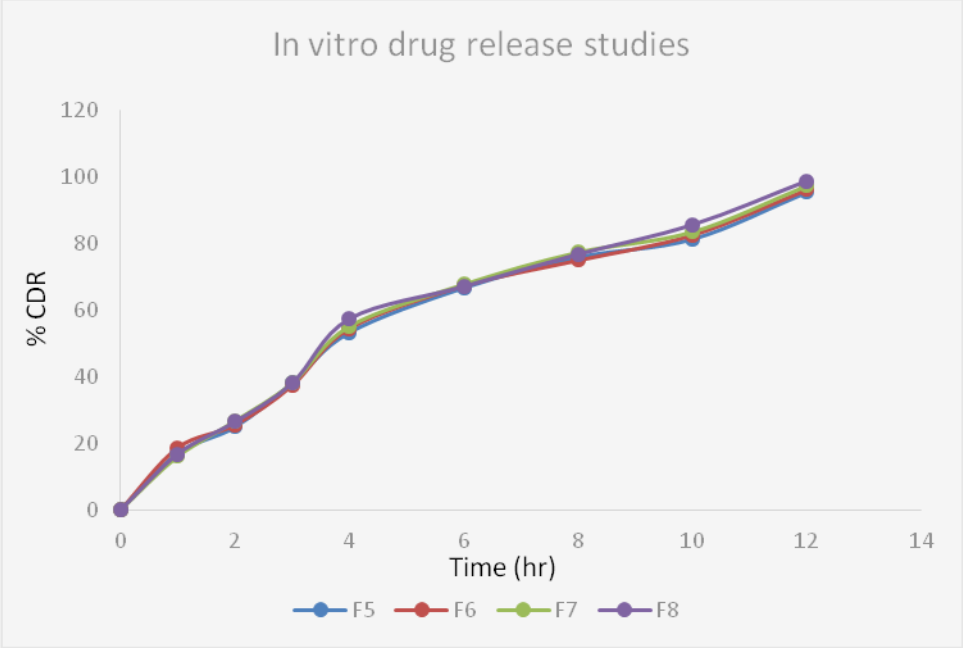

Fig-7: Diffusion studies of Transfersomes (F5-F8)

Drug release kinetics
Zero order kinetics

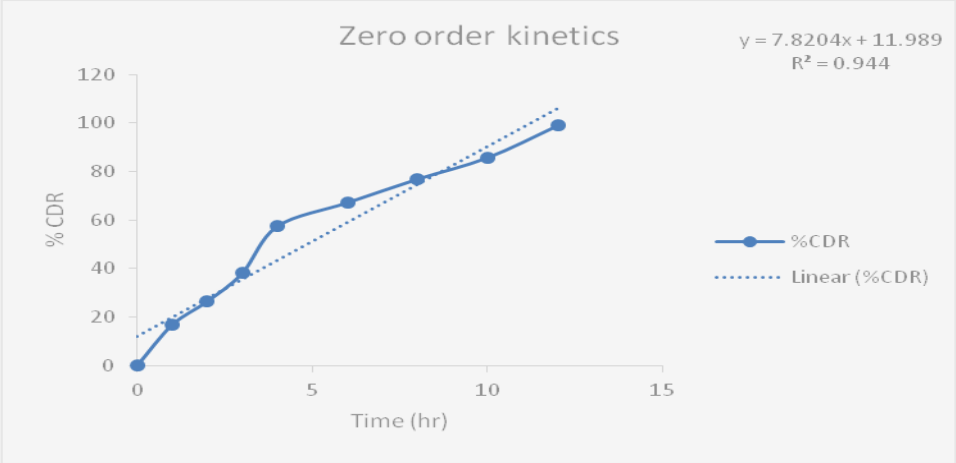

Fig-8: Zero order kinetics optimized formulation

First order kinetics

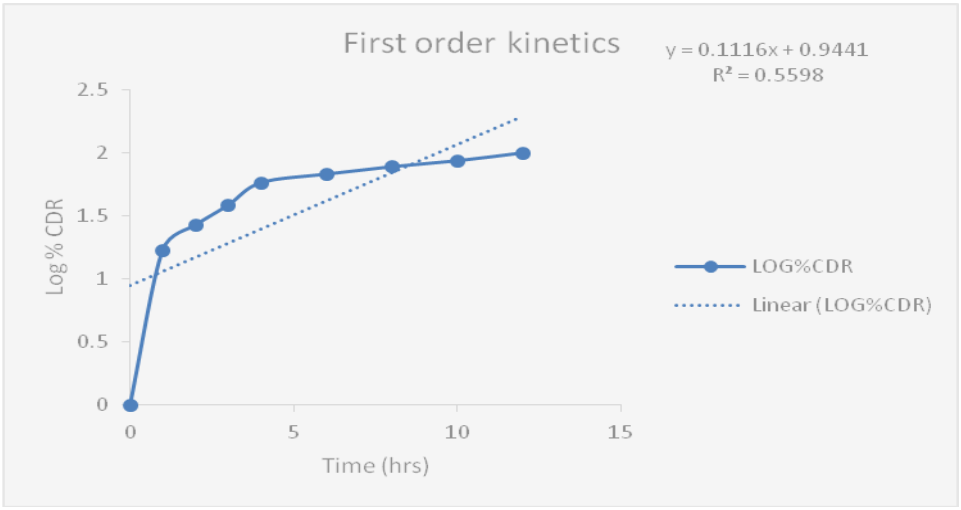

Fig-9: First order kinetics optimized formulation

Higuchi model

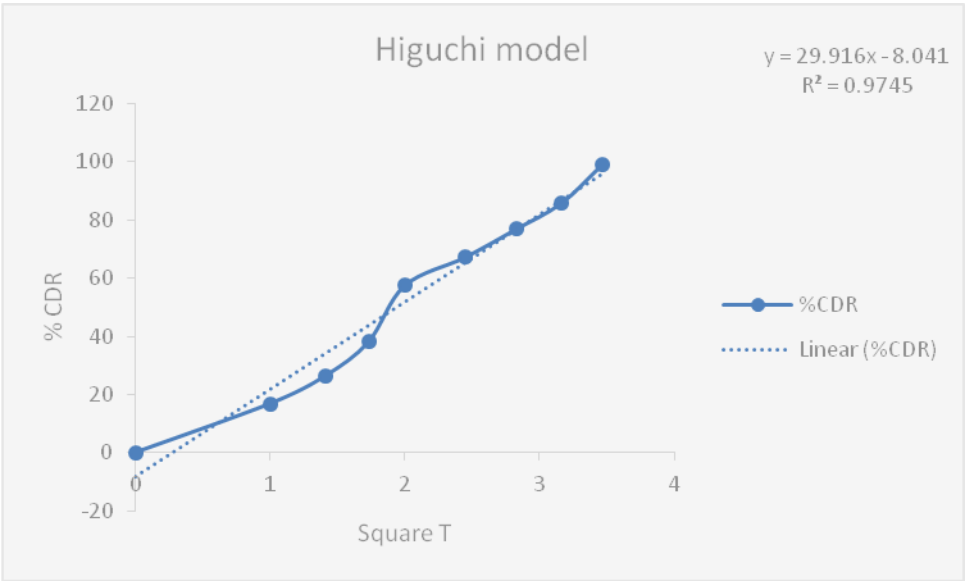

Fig-10: Higuchi model optimized formulation

Korsmeyer peppas

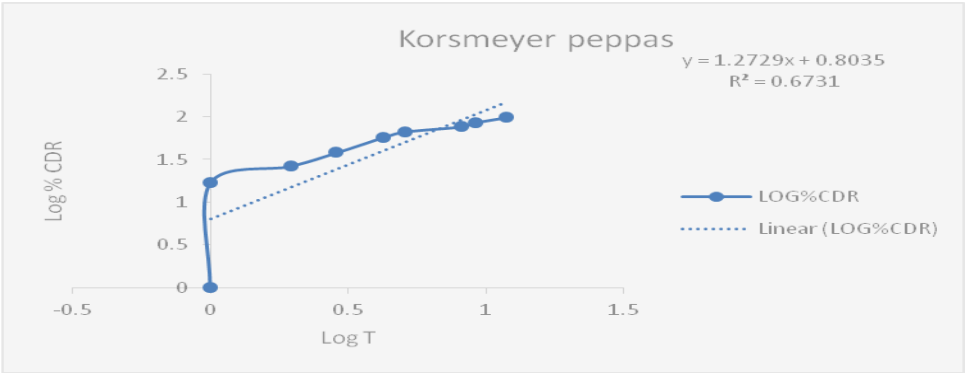

Fig-11: Korsmeyer peppas of optimized formulation

Stability studies:

There was no significant change in physical and chemical properties of the nanoparticle's formulation F8 after 3 months. Parameters quantified at various time intervals were shown.

Table-5: Results of stability studies of optimized formulation F-8

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	25^0C/60%RH % Release	98.90	97.68	96.81	95.26	Not less than 85 %
F-8	30^0C/75% RH % Release	98.90	97.75	96.57	95.24	Not less than 85 %
F-8	40^0C/75% RH % Release	98.90	97.52	96.52	95.13	Not less than 85 %

CONCLUSION

Finally, it can be concluded from the results of present study that Transferosomalgel improve the transdermal delivery, prolong the release, and improve the site specificity of the drug Indomethacin. Transfersomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

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