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Formulation and Characterization of Flurbiprofen Loaded Transethosomal Gel by Employing Design of Experiment, For Novel Topical Drug Delivery System

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ABSTRACT: Flurbiprofen, a nonsteroidal anti-inflammatory drug (NSAID), is commonly used to treat pain and inflammation. However, its poor skin permeability limits its effectiveness. To overcome this, we developed a transethosomal gel of flurbiprofen, which utilizes transethosomes as a carrier system to enhance skin penetration.total 9 batches of transethosomal suspension was prepared by employing factorial design using DOE software.the 9 batches showcased the particle size between 126.8nm to 386.2 nm and %EE between 59.36% to 87%. optimized batch of TE was incorporated into the gel base and further evaluated for pH,spreadability,drugcontent,appearance and in-vitro diffusion study.Our results show that the transethosomal gel significantly improved the in-vitro diffusion rate of flurbiprofen, compared to plain gel formulations. This novel formulation offers a promising topical treatment option for pain management, with potential applications in various clinical settings.

KEYWORDS: Penetration, DOE, Transethosomes, NSAID, pain management.

I. INTRODUCTION-

During the course of treatment, novel vesicular drug delivery systems seek to route the API to the intended site and release API determined by the body's needs. The term "/Bingham bodies" regards biological origin, which was 1st documented by \^Bingham} in 1965. In order provide VDDS incorporating several routes of administration have arisen. Targeted medication delivery is a technique that increases therapeutic efficacy and minimizes adverse effects by transport API to targeted site while minimizing API loss.Drug targeting is process of delivering medication to a receptor, organ, or other particular site of the area where API dosage is needed. Paul Ehrlich created site specified drug method in 1909, allowed the material to be sent straight to the sick cells. Since then, a variety of carriers have been used to carry the medication to the intended location. These carriers include erythrocytes, liposomes, niosomes, synthetic polymers, immunoglobulin's, and serum proteins. Vesicular drug delivery systems are among the most well-known among many carriers. Additionally, bioavailability, solvancy, stabilities, all enhanced by these systems.(1,2)

Since SC create the strongest hurdle to passage for most medications, skin serves as an excellent barrier to molecular transport. There have been several attempts has been used to enhance deposition of medications. Drug transfer via the skin is difficult, even if there aren't many ways as appealing as TDDS. To enhance the permeability's of topical applications into the skin ,The researchers has developed the Vesicular systems that accumulate in the layers of the skin include transethosome, an ultra-deformable vesicle (UDV). Lipid vesicles called transethosomes are synthesized from a combination of transfersomes and ethosomes.(3)Song et al. initially reported on it in 2012, describing its high ethanol concentration (up to 30%).Transethosomes combine the benefits of ethosomes with transfersomes. According to studies on vesicles elasticity and skin penetration/permeation, transethosomes have a regular spherical form and higher values. This is because combination of ethyl alcohol and surfactant activator causes lipid bilayer to reorganizeTransethosomes are different from other vesicular systems in that they have small particle sizes and are quickly able tochange the shape of vesicles.They have the ability to cross skin barriers. Because of this, the medication enclosed in transethosomes can quickly reach its intended location. Ethanol, phospholipids, and an edge activator make up transethosomes. Transethosome skin penetration is improved by ethanol and edge activator Different types of medications, including anticancer, corticosteroids, proteins and peptides, NSAID, and anti-fungal can be delivered by transethosomes.Anti-inflammatory drugs such as

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flurbiprofen are used to treat edema, inflammation, and joint paints.(4)

Even though flurbiprofen is a BCS class 2 medication with non-selective cyclooxygenase 1,2 inhibition and anti-inflammatory properties, its low water solubility makes it difficult to create topical and oral formulations. The principal barrier in topical dose methods has trouble reaching the deeper layer of skin, and long-term usage of NSAIDs also leads to gastric ulcers. This can be circumvented, however, by loading flurbiprofen into transethosomes, which can facilitate drug deposition into the skin's deeper layers.(5)
Incorporating the prepared transethosomal suspension into the gel provides better topical application and increases the adherence of formulation onto the skin, better patient compliance as gels are easy to apply.

II. MATERIALS AND METHODS:

MATERIALS:

Flurbiprofen was purchased form Tokyo chemicals inida ltd haidrabad, Soya-phospotediaycholine, Carbopol 934, Glycerine, Triethanolamine were purchased from LobachemiePvt. Ltd. Mumbai; Ethanol, were purchased from Gemini Associates, Goa.span-60 was obtained from pallav chemicals and solvents pvt.ltd.,Mumbai. Propyl Paraben, Methyl Paraben were obtained from Research Lab Fine Chem. Mumbai. All other chemicals and reagents were of analytical grade.

METHODS:

The transethosomal formulation of flurbiprofen were prepared by cold method containing varying range of soya phophatidly choline (260-300mg) ,span-60 (30 mg) and ethanol(20-40 ml). the concentration of drug used in the formulation was 1000 mg. span-60, soya-pc and drug was dissolved in ethanol to prepare the organic phase, resultant organic phase was stirred at 700 rpm for 45min at 30^{0}C temp, with continue drop wise addition of aqueous phase(distilled water),Then the formed transethosomal suspension was sonicated for 10 minutes to reduce the vesicular size.composition of transethosomes are mentioned in table 1.

Table 1: Composition of transethosomes

Ingredient	TE1	TE2	TE3	TE4	TE5	TE6	TE7	TE8	TE9
Flurbiprofen (mg)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Soya pc(mg)	300	300	300	280	280	280	260	260	260
Span 60(mg)	30	30	30	30	30	30	30	30	30
Ethanol(ml)	40	30	20	40	30	20	40	30	20
Purified water(ml)upto 100	(Q.S)	(Q.S)	(Q.S)	(Q.S)	(Q.S)	(Q.S)	(Q.S)	(Q.S)	(Q.S)

OPTIMIZATION OF TRANSETHOSOMAL BATCHES:

A 3^2full factorial design was used in the preparation of transethosomes, with help of design software. A 3^2 design consist of 2 independent factor with 3 level, concentration of soya phosphatidylcholine (X1) and the conc. Of ethanol (X2) was selected as, the 2 independent factors. The dependent variables investigated was the particle size (Y1), and entrapment efficacy (Y2). The variable levels and proposed formulations are mentioned in Table 2

Table 2: Design layout of 3^2 factorial design

variables	code	Low level	Medium level	High level	units
Independent variables- Soya -pc conc.(mg) Ethanol conc.(ml)	 X1 X2	 260 20	 280 30	 300 40	 mg ml

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Dependent variables(response)-		Constraints			
Particle size	Y1	Minimum			nm
Entrapment efficacy	Y2	Maximum			%

Evaluation of Flurbiprofen loaded transethosomes-

Particle size determination and poly-dispersivity index-

The average particle size was determined using HORIBA PS 100 particle sizer which works on light scattering principles, the 1ml sample from each prepared transethosomal batches were diluted 10 times with distilled water before being analysed. The results were shown as z mean. PDI which characterizes and measures the uniform Size distribution of particles, was also evaluated.

% Entrapment efficacy-

Entrapment efficacy of prepared transethosomes were determined via measuring the free unentrapped drug content concentration using UV spectrophotometry. 5 ml sample from prepared transethosomal batches were centrifuged using cooling centrifugation at 4^0 C and 15000 rpm for 1 hr to form supernatant, then the 1 ml of unentrapped drug suspension supernatant was collected, diluted with methanol and analysed at 247 nm taking methanol as blank. Following equation was used to calculate percentage entrapment efficacy,

$$\% \text{ Entrapment Efficiency } = \frac{\text{Initial drug} - \text{final drug}}{\text{Initial drug}} 100$$

Statistical Analysis of Data by Design Expert Software-

3^2 full factorial designs was selected and the use of the two factors was evaluated at three levels each. concentrations of soya phosphatidyl Choline(X1) and ethanol concentration(X2) were selected as independent factors, and the dependent variables were particle size (Y1) and Entrapment Efficiency(Y2) of flurbiprofen loaded transetheosomes. Data collected and Design Expert was used for the statistical analysis (ANOVA). 3D response surface graph is usual to investigate the relationship between the independent variables concentrations of soya phosphotidylcholine (X1) and ethanol concentration (X2),dependent variables Y1, and Y2 Quadratic terms were used to evaluate the response.

Zeta potential-

The zeta potential measurement of optimized transethosomal suspension was carried out using Zetasizer (HORIBA ZA 100).light scattering is measured by Doppler velocitemtry and phase analysis at an electrode voltage of 3.3 V.

Anti-inflammatory activity-

Determination of anti-inflammatory activity of prepared transethosomal suspension was carried out using the protein denaturation method, reaction mixture total 5ml consisting of 0.2 ml egg albumin,2.8ml phosphate buffer pH 6.4 and 2ml of optimized batch of prepared transethosomal suspension with concentration of 1,10 and 100ug/ml. this prepared solution was named as test solution and for control blank transethosomal suspension used,then mixtures were incubated at 37^0 C in Biochemical oxygen demand(BOD) incubator for 15 min to promote the growth of protein afterwards heated for 5 min at 70^0C Then their absorbance was measured at 660 nm using UV spectroscopy taking vehicle as blank.

%inhibition of protein then calculated by using following formula,

Inhibition= AC-AT/AC*100

Where,AC=absorbance of control solution,AT=absorbance of test sample

TEM –

Shape and morphology of optimized transethosomal batch were visualized using TEM (model 1230:JEOL,Tokyo,japan). The optimized batch was diluted 10 times and placed on carbon-coated copper grid for one minute, the excessive dispersion was cleaned. 1% solution of phosphotungistic acid was used as staining agent, acceleration voltage of 200kV was provided, images was captured by digital monograph and soft imaging software were used to analyse the images.

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Preparation of transethosomal gel-

The optimized transethosomal suspension was incorporated in the gel for better topical application.

Carbopol-934 was used as gelling agent, 1%,1.5% and 2% w/v concentration of carbopol was prepared. Varying concentrations of carbopol-934 was added into the 90ml water 10 ml propylene glycol then solution was agitated continuously at 800 rmp for 1hr and then allowed to soak overnight. Methyl paraben was added as preservative and triethanolamine to adjust the pH,1% carbopol 934 concentration showed better appearance so in this prepared gel base optimized batch of transethosomal suspension was incorporated.

Table no 3- Composition of optimized Gel

Sr. No.	Ingredients	Quantity Taken	Role
1	Carbopol 934	1gm	Gelling Agent
3	Triethanolamine	0.01 ml	pH modifier
4	Distilled Water	90 ml	Vehicle
5	Propylene glycol	10ml	Plasticizer
6	Methyl paraben	0.02w/w	Preservative
7	Propyl paraben	0.03w/w	Preservative

Characterization of transethosomal gel-

Organoleptic Characteristics:

Organoleptic characteristics were assessed from color and odor.

Homogeneity:

Homogeneity was analysed by visual inspection for the appearance and existence of any clog.

pH Determination:

Determination of gel pH was carried using digital pH meter, 500 mg of gel was dissolved into the 20 ml distilled water and stirred for 30 min with help of magnetic stirrer at roomtemperature then after 30 min pH meter was dispersed into the solution and pH was recorded which was shown on the screen of pH meter, the readings were recorded in triplet order.

Viscosity:

Viscosity of transethosomal gel of flurbiprofen was determined by Brookfield Viscometer DV III model using spindle no S-64 at 100 rpm and temperature of 25 °C. The results were recorded after the viscometer shows a stable number.

Drug content:

1gm of prepared gel was added into the 50ml volumetric flask and volume was made up by methanol, then sonicated to dissolve the gel and to get clear solution. Solution was then filtered and diluted with methanol.at 247 nm wavelength the solution was scanned to get drug concentration, methanol as reference. Following equation was then used to get drug content.

Drug content=Practical yield/theoretical yield*100

Spredability-

The spredability of prepared transethosomal gel was determined by using the glass slide method, an 1 gm. weighed gel of transethosomes was placed at the middle of glass slide and another glass slide was situated above it which was tied with weight of 30gm,then time was recorded for glass slide to separate. The following formula was used to calculate the spreadability,

S=M x L/T

Where,M=mass,L=length and T=time

In-vitro diffusion study-

Invitro-diffusion srtudy of transethosomal gel and normal prepared gel of flurbiprofen was carried out using the Franz diffusion cell, here normal gel was used to compare the diffusion rate with respect to that of transethosomal gel. In donor compartment 1gm of gel was kept. Dialysis membrane which was previously soaked in buffer pH 6.8 for 24 hr used to separate the donor and receptor compartment.

The receptor compartment was continuously agitated with 50 rmp speed and temperature was maintained at 37±0.5°C. samples were withdrawn from the receptor compartment at interval of (0.5,1,2,4,6,8,12,and 24hr) each time 3ml sample was taken and replaced with 3ml buffer pH to maintain the sink condition then withdrawn samples were diluted and analysed at 247 nm using the UV-spectroscopy.

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Kinetic analysis-

The kinetics of drug from the gel preparations were evaluated using linear regression coefficient. The in-vitro release of obtained transethosomal gel was put according with various kinetic models: zero order,first order, and higuchi. An Korsmeyer-Peppas model which estimate the drug release mechanism was plotted log time vs %log C,R.The 'n' exponent value gives the mechanism of drug release.

Stability-

The 3 month stability study of prepared transethosomal gel was carried at Room temperature(25^{0}C) ± 2^{0}C and evaluated for its appearance, drug content and pH at interval of 30 days.

III. RESULT AND DISCUSSION-

Transethosomes-

Particle size and PDI-

Table 4 shows particle size and PDI of prepared transethosomes. The particle size of formulated transethosomal suspensions of TE1 to TE9 batches observed between 126.8 to 386.2nm. batch showing the particle size below to 150nm which are having low concentration of soya-pc.The mean particle size was influenced by the 2 factors, with increase in concentration of soya-pc the particle size increased while particle size was decrease with increase in concentration of ethanol, the optimized batch of transethosomes shown the particle size 173.6 nm and PDI 0.376 which indicates that good polydispersitivity among the transethosomes.

Table 4:Particle size and PDI

Sr.no	Batch code	Particle siz(nm)	PDI
1	TE1	225.2	0.726
2	TE2	371.9	0.544
3	TE3	386.2	0.431
4	TE4	146.9	0.378
5	TE5	173.6	0.376
6	TE6	197.3	0.468
7	TE7	126.8	1.78
8	TE8	141.8	0.241
9	TE9	134.9	0.363

%Entrapment efficiency-

The result shows that as there is increase in conc. of soya-pc, Increase in %entrapment efficiency, the increase in ethanol concentration at optimum levels also favors the %entrapment efficiency of transethosomes, but as ethanol conc. increases upto 40ml,it causes the leakage of vesicles thus further increase in ethanol concentration showed decrease in %E.E. The transethosomal batches showed %E.E ranging from 59.36% to 87%.TE7 batch with minimum and TE2 with maximum %E.E. optimized batch of TE showed %E.E of 74.17(TE5).

Statistical analysis of data by DOE software-

[Table 5: Summary of experimental design]

Batch code	Soya-pc(X1) mg	Ethanol(X2) ml	Particle size(Y1) nm	%E.E(Y2) %
TE1	300	40	225.7	80.65
TE2	300	30	371.9	87.34
TE3	300	20	386.2	85.08
TE4	280	40	146.9	71.39
TE5	280	30	173.6	74.17
TE6	280	20	197.3	64.71
TE7	260	40	126.8	59.36
TE8	260	30	141.8	62.55
TE9	260	20	134.9	60.69

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Effect of Independent variables on particle size and % Entrapment efficiency-

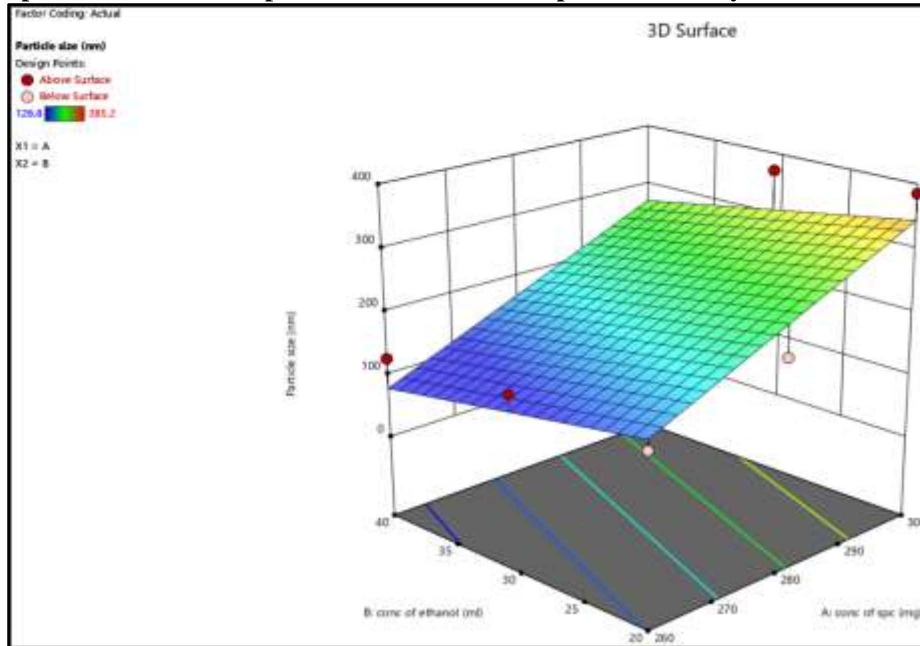

Figure 1:3D plot of particle size

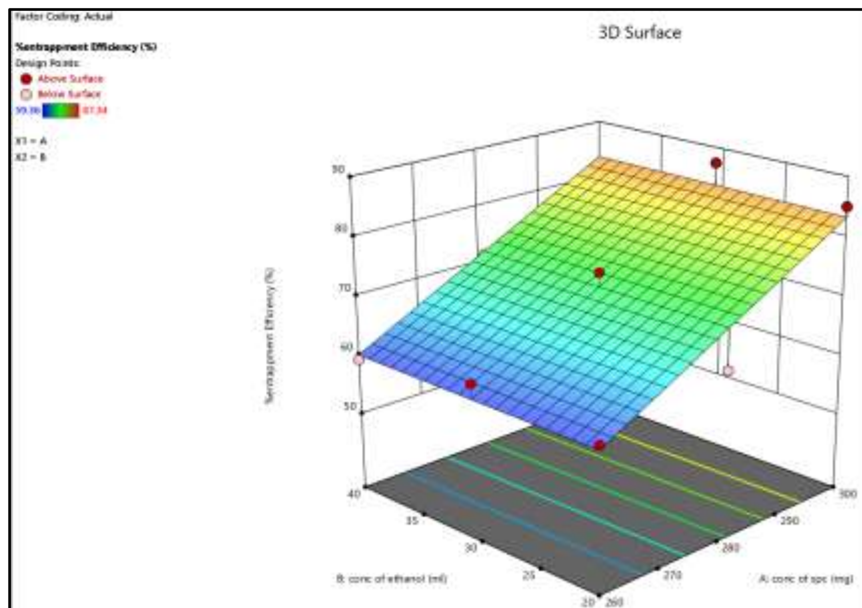

Figure 2:3D plot of%EE

Figure 1 showed that particle size of transethosomes increased with increasing conc.of soya-pc and decreased with increasing conc.of ethanol. Figure 2 showed that %EE of transethosomes increased with increasing conc.of soya-pc and decreased with increasing conc.of ethanol to the 40% but increase in ethanol conc. Upto optimal level showed increase in %EE of transethosomes,the increase in ethanol concentration to the 40% caused the leakage of vesicles which decrease the %EE of TEs.

ANOVA for particle size and % Entrapment efficiency-

Anova For particle size-

The significance of each response was assessed by comparing the mean square against the estimate of experimental error. The model-F value

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of 12.00 indicates that the model is significant, with only a 0.80% probability that such a high F-value could be due to random noise. A P-value below 0.0500 shows that the model terms are significant. The predicted R^2 value of 0.8832 is reasonable, while the adjustedR^2 value of 0.8951 is similarly satisfactory. Adequate precision, which evaluates the signal-to-noise ratio, is desired to be above 4. In this case, a ratio of 8.9252 indicates a strong signal.

Particle size=-10.31+4.82750*A-3.633*B……..(Surface linear model)
Where A= conc.of soya-pc and B= conc.of ethanol

Signs + and – shows the synergistic and antagonistic effct,respectively on the response Y1. Response Y1 shows synergistic relationship with soya-pc (X1) and antagonist relationship with ethanol(X2).

Anova for %EE

The significance of each response was assessed by comparing the mean square against the estimate of experimental error. The model-F value of 28.15 indicates that the model is significant, with only a 0.09% probability that such a high F-value could be due to random noise. A P-value below 0.0500 shows that the model terms are significant. The predicted R^2 value of 0.8001 is reasonable, while the adjusted R^2 value of 0.8716 is also satisfactory, with a difference of less than 0.2. Adequate precision, which evaluates the signal-to-noise ratio, is desired to be above 4. In this case, a ratio of 10.7478 indicates a strong signal..

%E.E=-93.09444+0.58717*A+0.015333*B……..(Surface linear model)
Where A= conc.of soya-pc and B= conc.of ethanol
Signs + and – shows the synergistic and antagonistic effct,respectively on the response Y2. Response Y2 shows synergistic relationship with soya-pc (X1) and ethanol(X2).

Optimization of TEs-
Desirability ramp -

Desirability ramp in figure 3 and 4 showed that the optimum conditions to formulate TEs as soya-pc conc.at 282 mg and ethanol conc. to 30 ml to achieve particle size of 224.5 and %EE of 73.34 with desirability value of 0.900 close to 1. TE5 batch has close value of soya-pc and ethanol with these Desirability ramp value.

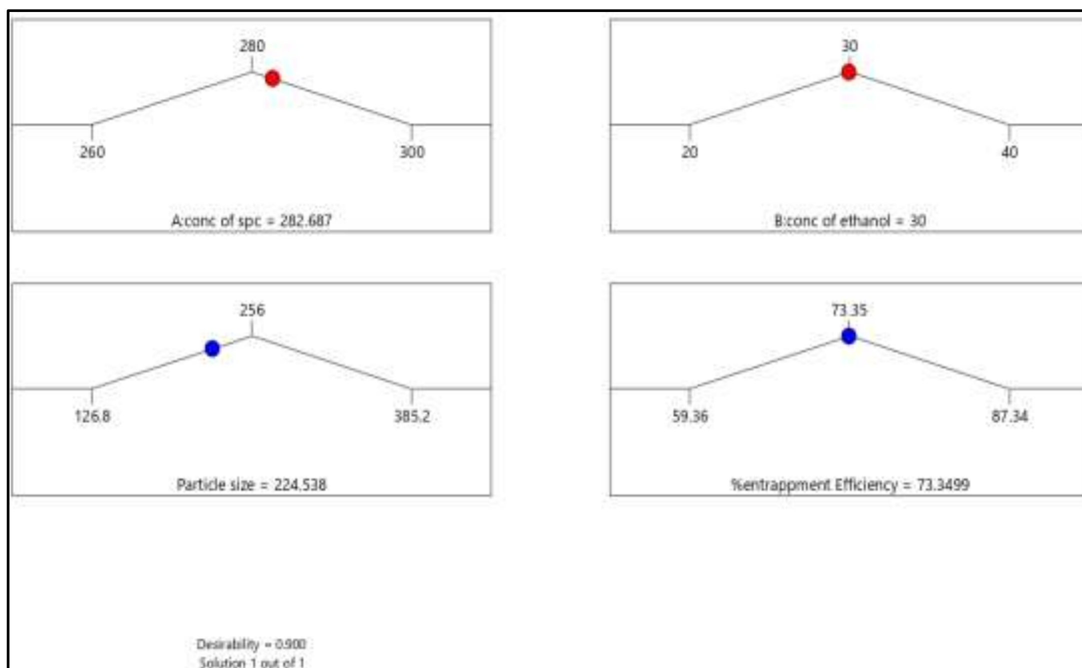

Figure 3: Desirability ramp

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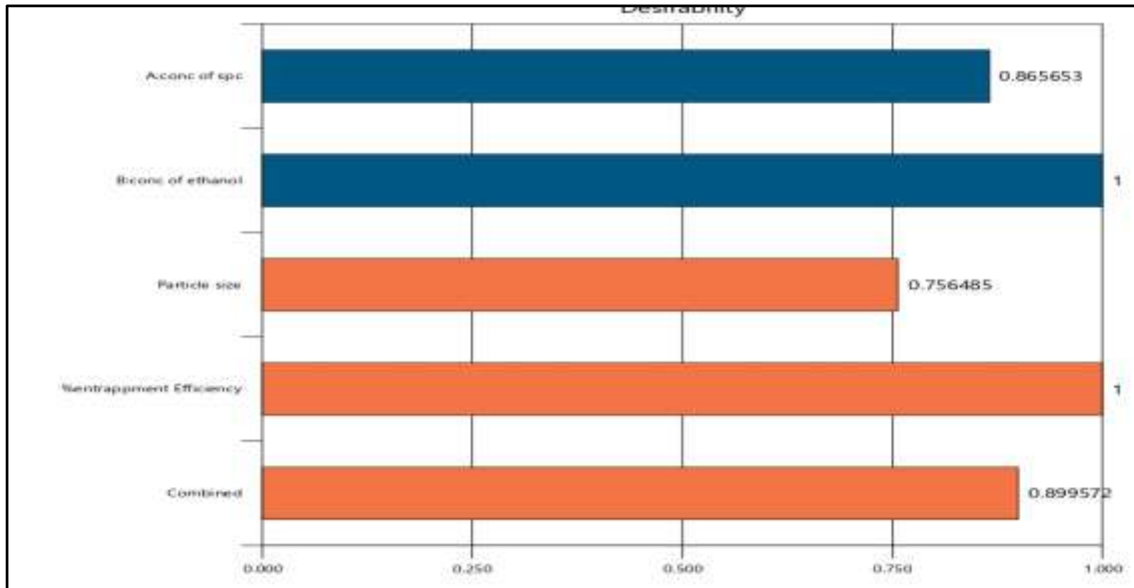

Figure 4:Desirability

Zeta potential-

The zeta potential of optimized batch(TE5) of transethosome was carried out using horiba zeta sizer which was -40.6mv indicating that the transethosome has good stability.

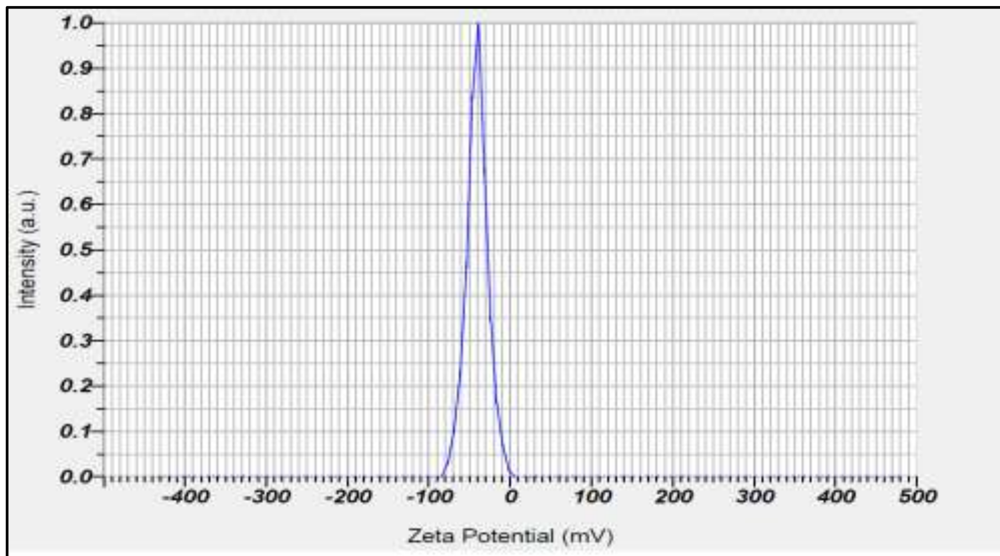

Figure 5: zeta potential of TE5

Anti-inflammatory activity-

Table 6: Absorbance of blank and test]

compound	Conc.	absorbance	%inhibition
blank		0.316	
Test1	1ug/ml	0.290	8.22
Test2	10ug/ml	0.190	39.87
Test3	100ug/ml	0.111	64.87

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The anti-inflammatory activity carried out using protein denaturation study shows that the transethosomal suspension (Batch 5) of conc.100ug/ml showed % protein inhibition of 64.87%,which proves that prepared formulation has good anti-inflammatory activity.

TEM-

Figure 8.40 shows that the prepared transethosomes using cold method. Images of TEM reveals that the transethosomes exhibits spherical shape of ,TE batch 5, exhibit smooth spherical configuration, and a degree of particle stabilization.

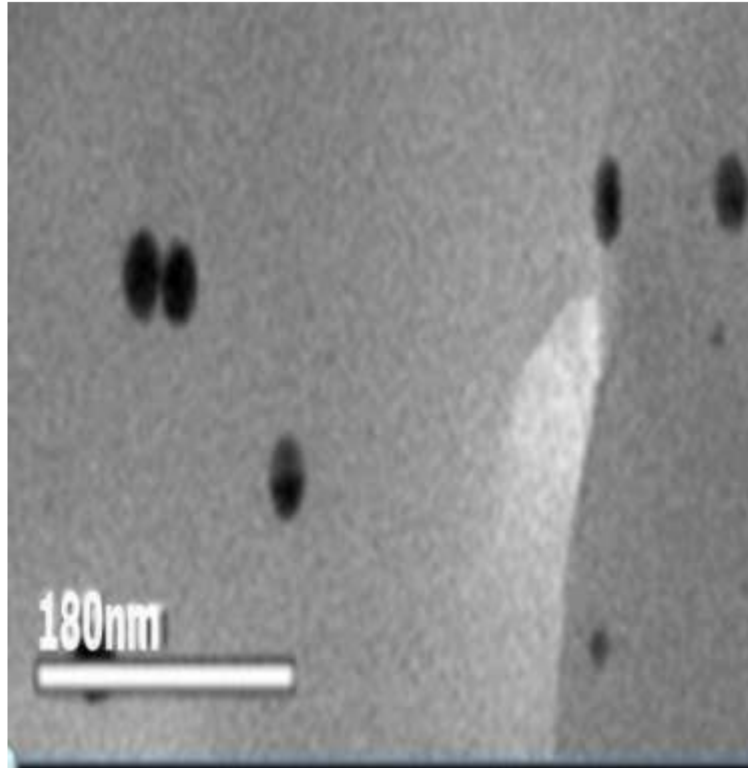

Figure 6:TEM of TE5

Evaluation results of flurbiprofen loaded transethosomal gel-

Table 7: Evaluation result of gel]

Color	White
Odor	Odorless
Homogeneity	Homogenous
pH	6.4±0.04
Viscosity	3462±1.5
Spreadability	6.8±0.08
Drug content	98.90±0.03

In-vitro diffusion

The %C.R of TE and normal gel. After 24 Hr TE gel showed 93.43 %C.R compared to that of normal gel which showed % of 74.15 after 24 Hr.

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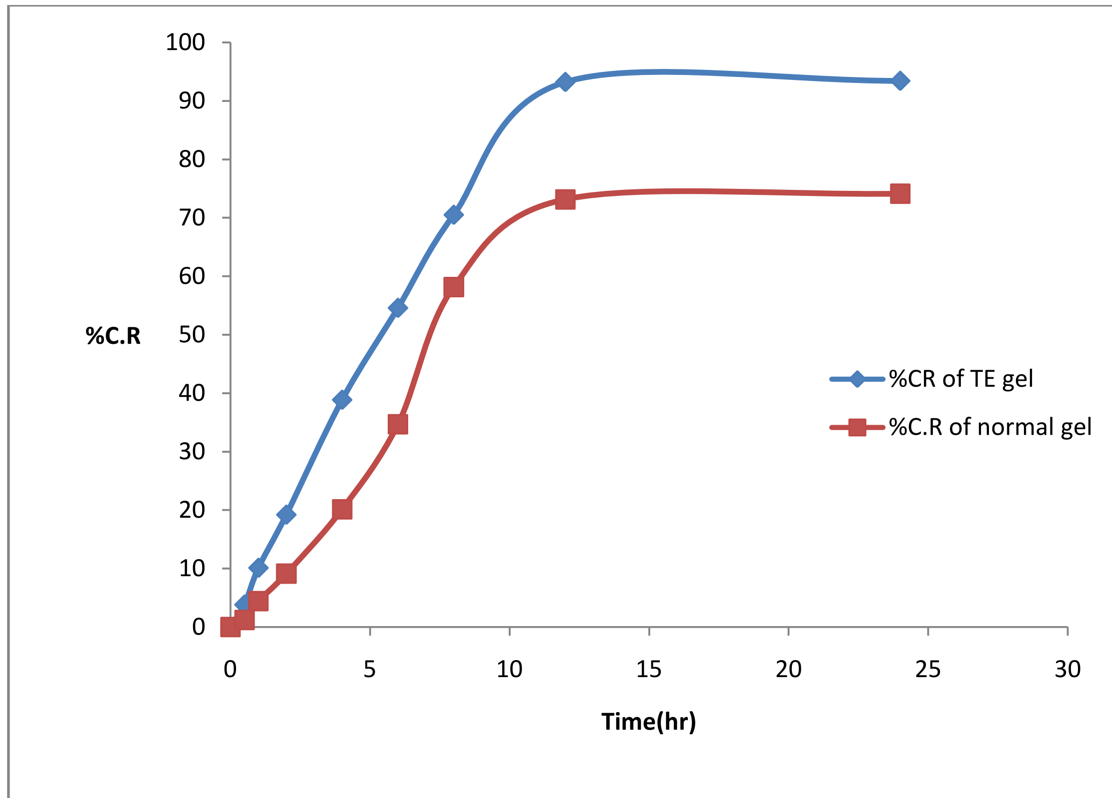
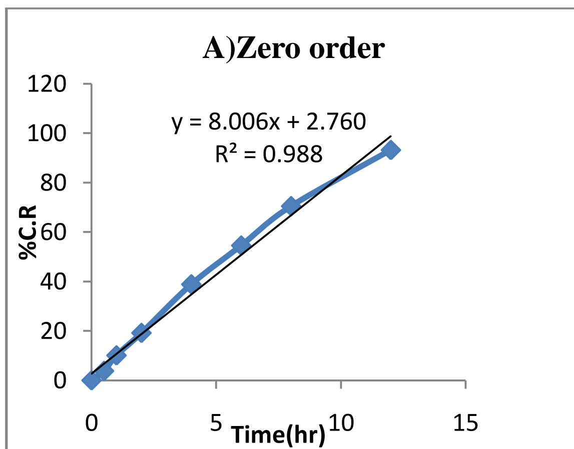
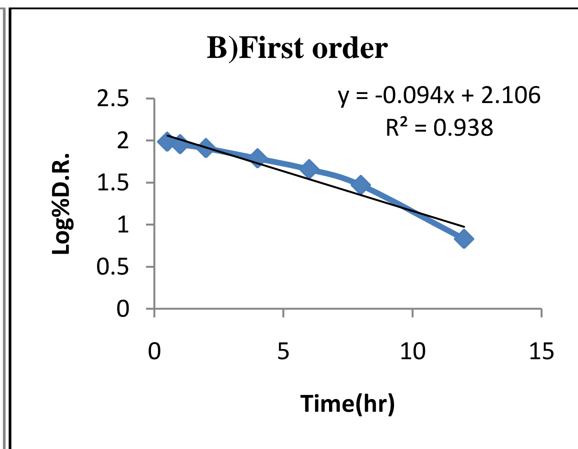


Figure 7: Comparative graph of %C.R

Release Kinetic-

The in-vitro release release kinetic of flurbiprofen loaded TE gel was studied. The regression coefficient R^2 for the different kinetics equation is shown in figure 7. The gel follows zero order and Higuchi model. As shown in figure 7. by comparing value of 'n' which is < than 0.45 the release from gel is fickian diffusion.





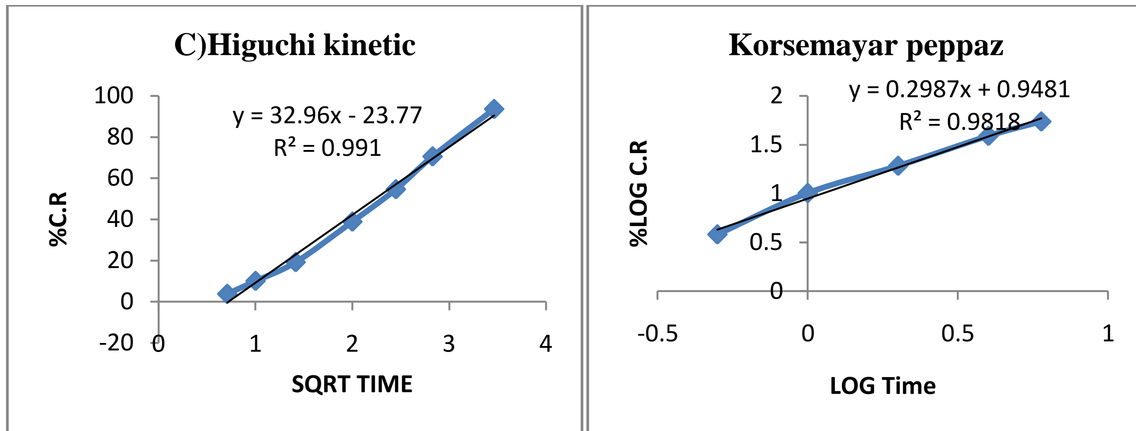


Figure 8: : Models graphs of drug release kinetics profiles A) Zero order kinetics B) Firstorderkinetics C)Higuchikinetics D)KorsmeyerPeppasEquation

Stability study-

Table 8: Stability study result

Months	Room temperature(25±2^{0}C)		
	pH	**Appearance**	**Drug content**
Initial	6.4±0.04	White	98.90±0.03%
1	No change	No change	No change
2	No change	No change	No change
3	6.54±0.01	No change	97.65±0.5%

IV. CONCLUSION-

According to the studies performed TEs of flurbiprofen showcase the increased in-vitro-diffusion rate. The prepared TEs shows small particle size which enhances the permeability of gel across the skin layer. Formulation of TEs gel ease the application of dosage form which enhances the patient compliance. in future in-vivo study can be performed to ensure the formulations toxicity, bioavailability.

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