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Formulation and Evaluation of Ethosomes for Transdermal Delivery of Etodolac

Bhale Shweta[1], Bhandari Govind[1], Pal Pradeep [1],Mahajan S.C.[1]
1.Mahakal Institute of Pharmaceutical studies, Ujjain (M.P)

ABSTRACT

The objective of work was to formulate, evaluate the potential of ethosomes for delivering etodolace, a potent, water insoluble non-steroidal anti-inflammatory drug via skin to enhance skin permeation after topical application. The effects of pH and ethanol contents on etodolac solubility were evaluated to find out the suitable dispersion medium for the ethosome preparations. Drug loaded ethosomes had been prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, zeta potential, particle size and size distribution. The ethosome were prepared by using the thin film hydration method. The optimized formulation composed of 0. 4% w/v Soya lecithin and 0.1 % pluronic F127 as lipid component and 30% v/v ethanol in phosphate buffer pH 7.4 as dispersion medium. The vesicular size and zeta potential of ethosome was found to be 458.7nm,-36.6 mV respectively The entrapment efficiency was found to be 68.4±0.4%. In final phase of formulation optimized ethosome containing drug, was converted to gel using three different carbopol 930 concentrations(0.5, 0.7 and 1% w/w).The gel of 0.7% carbopol was found to be the optimized gel which shown excellent *in-vitro* drug release

Keywords: Ethosome; Ethanol; Transdermal; Phospholipid; In vitro skin permeation, drug loading; flux.

*Corresponding Author Email: bhaleshweta11@gmail.com
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INTRODUCTION

Etodolac is a racemic acetic acid derivative, non selective cyclooxygenase (COX) inhibitor with potential analgesic and anti inflammatory activities. It is effective in the treatment of osteoarthritis, gout, rheumatoid arthritis and traumatic injury. Administration of non-selective COX inhibitor by oral route causes many gastrointestinal (GI) side effects like nausea, vomiting, dyspepsia, gastric irritation, peptic ulceration and bleeding.[1] Owing to these common gastrointestinal side-effects, the NSAIDs frequently cause gastrointestinal injury and increase the risk of ulcer complications. There were some data suggesting that etodolac, like other NSAIDs, produces some gastrointestinal side effects.[2] GI side effects produced by NSAIDs are either due to direct contact or indirect effect of the drug on the GI mucosa. [3]

Therefore, there is a need to develop topical dosage forms of etodolac to minimize the gastrointestinal side-effects of oral administration, and to provide relatively consistent drug levels at the application site for prolonged periods.[4] Transdermal and topical deliveries also provide an increased bioavailability by avoiding first pass metabolism by the liver and a consistent delivery for an extended period. Topical delivery vehicles (creams, gels) and transdermal delivery agents (dermal patches) can improve patient compliance due to decrease in the dosage frequency. [5]

Novel drug delivery systems (NDDS) have revolutionized the methods of medication and provided therapeutic benefits. The interest of both the pharmaceutical and cosmetic industry for skin delivery has prompted the development and investigation of a wide variety of vesicular systems. Ethosomes are one such vesicle system, which are elastic in nature due to the presence of ethanol in the bilayer structure.[6] In present study we have investigated the suitability of ethosomal system in enhancement of etodolac transport across the skin. This study also focuses on making the formulation more pharmaceutically acceptable by converting it into carbopol gel.

Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesiclesas well as by the presence of ethanol in ethosomes which allows for better solubility of many drugs. Ethosomes were reported to improve in *vivo* and in *vitro* skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions.[7,8]

MATERIALS AND METHODS

Chemicals Required

Etodolac(Ranbaxy Pvt. Ltd),ethanol (merck, India), Carbopol 930(CDH chemicals), Soya lecithin(Himedia Laboratories, Pvt. Ltd., Mumbai, India),pluronic F127(CDH chemicals) Methyl paraben(S.D Fine Chemicals Ltd, Mumbai), propyl paraben (loba chemi), triethanolamine (S.D Fine Chemicals Ltd, Mumbai).

Solubility studies

The effects of pH and ethanol content on etodolac solubility were evaluated to find out the suitable dispersion medium for the preparations of ethosome containing etodolac. The study was conducted by adding an excess amount of etodolac to glass vials containing 10 ml of the hydro-ethanolic solution with 0-50% v/v ethanol and the desired media which include the buffer solutions pH 4.5, 5.5, 6.8, 7.4 that were prepared according to I.P. The samples were sonicated for 30 minutes and observed at room temperature for 48 h and 72 h to ensure that the solubility of etodolac reached equilibrium. The excess drug was filtered and the solubility was determined using spectroscopy at 278 nm wavelength. All determinations were performed in triplicate. [9]

Analysis of Drug-Excipients Compatibility Study

This study was done to observe any physical change in the drug when kept in contact with various formulation excipients. The drug was mixed with excipients in the 1:1 a ratios and kept in glass vials properly capped and sealed with teflon tape. Two vials of each sample were kept at room temperature and any change in physical appearance and color of the contents was observed.

Measurements of differential scanning calorimetric of drug, polymer and excipients were obtained on a TA Instruments 2910 thermal analysis system. Samples of approximately 2-4 mg were accurately weighed into an aluminum DSC pan, and covered with an aluminum lid that was crimped in place.

Preparation of etodolac loaded ethosomal vesicles

Ethosomes of etodolac were made by thin film hydration method based on 3^2factorial design. In which three levels and two variables (factors) so, there are nine formulation prepared. In formulation of ethosome soya lecithin and Pluronic F 127 were two independent variables, both at three different levels (−1, 0, +1). In the design, the lower concentration of soya lecithin, i.e., 0.2% w/v has been assigned at −1 level whereas 0.4% w/v concentration was assigned the level 0. The concentration at highest level +1 was 0.6% w/v. In case pluronic F 127 concentrations at three levels from lowest to highest (−1, 0, +1) were 0.1% w/v, 0.25% w/v, and 0.5% w/v, respectively. Based on this factorial design, nine ethosomal formulations were developed.

Ethosomes were prepared in a completely dried round bottomed flask (RBF) SPC in varying concentration was dissolved in a mixture of chloroform and methanol (2:1 v/v). A thin lipid film was then formed on the wall of the RBF using Rotavapour, by maintaining the temperature above the lipid transition temperature (55 ± 2 C) and the organic solvent was completely evaporated by vacuum, followed by hydration (6 h) with 30%, w/v of ethanol in PBS 7.4 of hydroethanolic mixture containing etodolac (1.0% w/v).The preparations were further subjected to sonication for 3cycles of 5 min at each 5 min interval.[9] (Table1)

Table 1: Compositions of different ethosomal formulations

S.No.	Formulation Code	Ingredients in levels			
		Drug (% w/v)	SPC (% w/v)	Ethanol (% w/v)	Surfectant (% w/v) Pluronic f127
1.	ET_1	1	0.2	30	0.1
2.	ET_2	1	0.2	30	0.25
3.	ET_3	1	0.2	30	0.5
4.	ET_4	1	0.4	30	0.1
5.	ET_5	1	0.4	30	0.25
6.	ET_6	1	0.4	30	0.5
7.	ET_7	1	0.6	30	0.1
8.	ET_8	1	0.6	30	0.25
9.	ET_9	1	0.6	30	0.5

Characterization of ethosomal systems

Visualization of vesicles by TEM and by SEM:

Vesicular shape of the ethosome preparations were assessed by using Transmission Electron Microscope (TEM). Samples were dried on carbon-coated grid and negatively stained with aqueous solution of phospho tungstic acid. After drying the specimen was viewed under the microscope The size and shape of the vesicles were observed in the Scanning Electron Microscopy (SEM). One drop of ethosomal suspension was mounted on a clear glass stub. It was then air dried and gold coated using sodium aurothiomalate to visualize under scanning electron microscope at 10,000 magnifications.[10]

Vesicle Size and Zeta Potential Determination:

Vesicle size, zeta potential, and polydispersity index were measured by Zetasizer by diluting one drop of ethosomal suspension with hydroethanolic solution at 25°C in clear disposable zeta cells. All the measurements were done in triplicate for each sample.

Entrapment efficiency:

Entrapment efficiency of ethosomal vesicles was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 20,000rpm for 90 minutes in the temperature maintained at 4°C .The sediment and supernatant liquids were separated amount of

drug in the sediment was determined by lysing the vesicles using 30 % v/v triton 100X. From this, the entrapment efficiency was determined by the following equation,[12]

Entrapment efficiency = DE/DT x 100

Where,

DE - Amount of drug in the ethosomal sediment

DT - Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment)

Preparation of ethosomal gel

The ethosomal formulation,having high entrapment efficiency and smaller vesicular size was centrifuged in the temperature 4 C at 20,000rpm for 90 minutes to separate the ethosomal vesicles. The ethosomal sediment which contains only the entrapped drug was collected and dispersed in the carbopol 930 gel base with gentle stirring to obtain the total drug equivalent to 1%w/w of etodolac. To the final homogeneous mixture triethanolamine was added to adjust the pH between 6-7.A clear gel was formed [12](Table 2)

Table 2 Composition of ethosomal gel

S.NO.	Ingredients(% weight)	Formulation code EG1	EG2	EG3
1	Carbopol-934	0.5%	0.7%	1%
2	Propylene Glycol	15%	15%	15%
3	Disodium EDTA	0.1%	0.1%	0.1%
4	Ethosome	1%	1%	1%
5	Propyl Paraben	0.001%	0.001%	0.001%
6	Methyl Paraben	0.05%	0.05%	0.05%
7	Triethanolamine	q.s	q.s	q.s
8	Distilled Water	q.s	q.s	q.s

Evaluation of Gel

Viscosity and Appearance, Determination:

Viscosity of different formulation was measured using Brookfield Viscometer (DV-E (RV), USA). Viscosity was determined by using spindle number-06; viscometer was set at 10 RPM. The viscosity of different formulation was measured. Appearance, was determined visually.

Determination of pH:

The pH of the gels was determined using a digital pH meter by dipping the glass electrode completely into the gel system so as to cover the electrode.

Drug Content:

A specified quantity, i.e., 1gm of gel was taken and dissolved in 100 ml of phosphate buffer 6.8.

The volumetric flasks were shaken well to mix it properly. The solution was filtered and the absorbance was measured after suitable dilution at 278 nm and the drug content was determined from calibration curve

Permeation study:

Cumulative amounts of drug (µg) penetrating the unit diffusion surface (cm^2) was plotted against time (h). The in vitro skin permeation rate or flux (*J*) was calculated from the slope of the regression line fitted to the linear portion of the profile. Extrapolation of this line will intercept with the x-axis at a time equal to the lag time. The permeability coefficient, k*p*, was estimated from the flux and donor drug concentration. The cumulative drug permeation (*Q*t) was calculated from the following equation:

$$\mathbf{Qt=VtCt+\sum_{t-0}^{t-1} VsCi}$$

Where

Ct = the drug concentration of the receiver solution at each sampling time

Ci= the drug concentration of the *i* th sample

*V*r = the volumes of the receiver solution

Vs=the volumes of the sample

Apparent permeability coefficients (K*p*) were calculated according to the equation:

$$\mathbf{Kp = Jss /Co}$$

Drug Diffusion Studies:

The drug diffusion studies of prepared gel were carried out in Franz diffusion cell using an egg membrane. Phosphate buffer 6.8 was used as bathing solution in the receptor compartment. The membrane was mounted between the donor and receiver compartments of the diffusion cell. The donor cell was filled with 1 g of gel. The receiver medium is continuously agitated with a magnetic stirrer at a temperature of $37^oC \pm 2^oC$ maintained thermostatically. Samples 1ml in each case was withdrawn at regular intervals and fresh receptor fluid was added to maintain a constant volume of receptor fluid. The samples withdrawn from the receptor compartment were diluted and then analyzed spectrophotometrically at 278 nm and the drug content was determined from the calibration curve.

RESULTS AND DISCUSSION

The thin film hydration method is used in the preparation of etodolac ethosomes in this study. Etodolac is very slightly soluble in water (1.6 mg/L), because it is a lipophilic drug. However, the solubility properties have influenced on formulation and preparation of ethosome containing

etodolac. We need to find an appropriate dispersion medium that is able to avoid drug precipitation in the formulation. In this case, the effects of pH on etodolac solubility were evaluated. Since etodolac is a weak organic acid with greater solubility in alkaline than in acidic medium (pKa = 4.5) and its solubility increases as pH increases. At pH 4.5, 5.5, 6.8, 7.4 and the solubility of etodolac increase to about 2.24, 5.5,14.1 & 22.1 mg/mL, respectively. Therefore, the buffer solutions pH 7.4 was selected for use in preparing ethosome formulation. (Figure 1)The solubility profile of etodolac in various ethanol concentrations showed that the solubility of etodolac increased as ethanol concentration increased, Owing to ethanol as a solubilizing agent and a component of the ethosome formulation, the skin permeability is enhanced. Thereby, in this study, 10-50% v/v ethanol was added in dispersion media of ethosome formulations and significant differences in the solubility of etodolac in various pH and ethanol concentration between 48 and 72 h ($p > 0.05$) which demonstrated that the solubility of etodolac reached equilibrium It was suggested that ethanol had a fluidzing effect on phospholipid bilayers as compared with common liposomes. Furthermore, ethanol probably caused an alteration of the net charge of the system and conferred it some extent of steric stabilization that might finally lead to a reduction in the mean particle size.. As concentration of ethanol goes beyond 30% w/v, the entrapped drug tends to leak out. Additionally, the repeated topical application consisting of high percent of ethanol may affect the skin adversely by causing skin irritation and contact dermatitis Hence 30% hydroetanolic solution of ethanol with 7.4 PBS is used.(Figure 2)

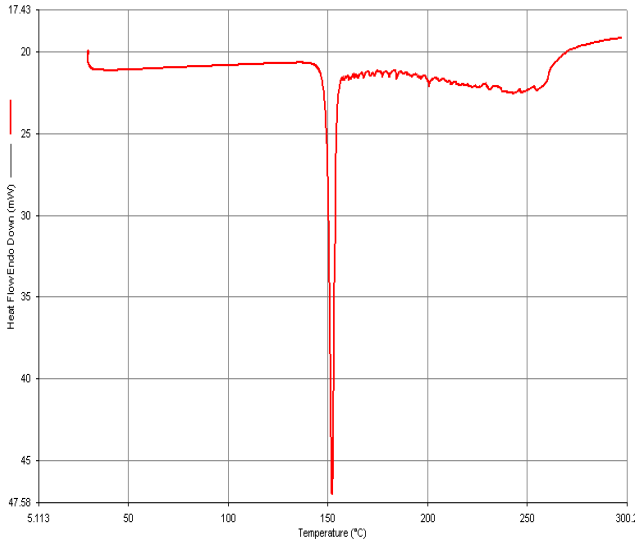


Figure 1: The DSC thermogram of etodolac. Etodolac showed a characteristic exothermic peak at150^{o}C, which corresponds to its melting point.

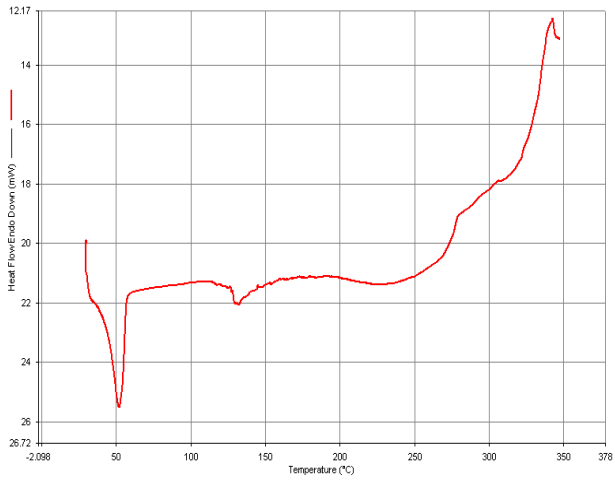


Figure 2: The DSC thermogram of etodolac, soya lecithin and pluronic F127

It has been shown that ethosomes exhibit high encapsulation efficiency for a series of lipophilic molecules and deliver effectively drugs through the skin ,the diffusion of drug through skin take place as below first, the stratum corneum (SC) lipid multi layers were highly conformationally ordered and densely packed at physiological temperature. Ethanol might interact with lipid molecules in the polar head group region inducing a reduction in the Tm of the SC lipids. Thus the organization of the SC lipid bilayer was disturbed, and their fluidity was enhanced. Second, the intercalation of ethanol into the polar head group environment could lead to an increase in the membrane permeability. The third contribution to the high skin penetration from the ethosomal system could have connection with the interaction between ethanol together with phospholipid vesicles and SC which was also proposed by Kirjavainen et al. . It has also been suggested that mixing of phospholipids with SC lipid so the intercellular layers enhanced the permeability of the skin. In summary, the effect of ethanol on SC lipids and on vesicle fluidity as well as a dynamic interaction between ethosomes

The composition of different ethosomal formulations is summarized in (Table1). TEM photomicrograph of ethosomes revealed the presence of spherical vesicular structures. The three dimensional nature of formulated ethosomal vesicles were confirmed by scanning electron microscopy shown in (Figure 3,4). The entrapment efficiency for etodolac ethosomal formulations was given in (Table 3). On the basis of entrapment efficiency, vesicles size, size distribution and Zeta potential was measured given in(Table 4)..The DSC thermogram of drug with soya lechithine and pluronic F127 was given in (Figure 2).

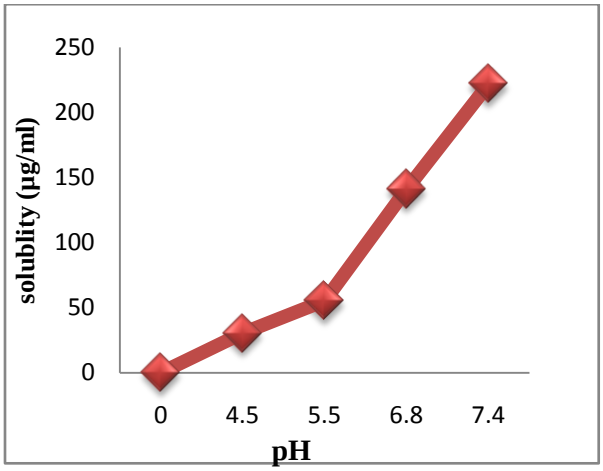


Figure 3 pH solubility profile of Etodolac

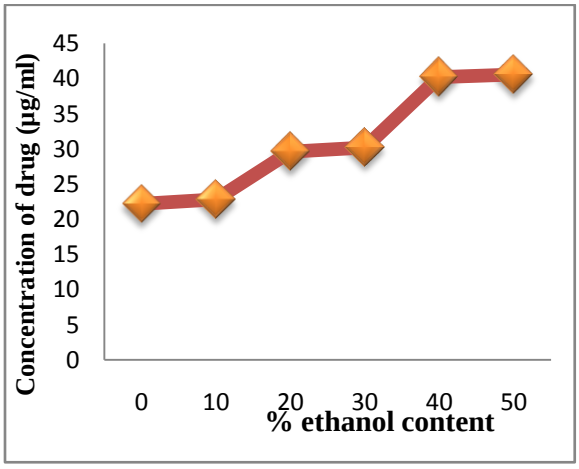


Figure 4 Solubility profile of etodolac in phosphate buffer 7.4 with various conc. of ethanol

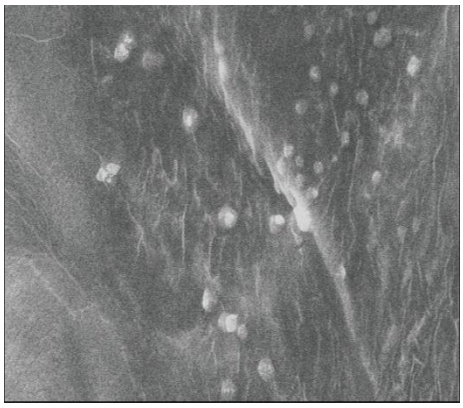
Table 3 Entrapment efficiency of various ethosome formulations

S.no	Formulation code	% entrapment efficiency
1.	ET_1	28.18±0.8
2.	ET_2	36.3 ±0.3
3.	ET_3	41.7±0.5
4.	ET_4	68.7±0.4
5.	ET_5	42.8±0.2
6.	ET_6	32.6±0.842
7.	ET_7	42.2±1.7
8.	ET_8	37.8±0.9
9.	ET_9	65.4±0.8

Table 4 Vesicle size, zeta potential, and polydispersity index of optimized ethosome formulation

S.no	Formulation code	Entrapment efficiency	Zeta potential	Particle size(nm)	Poly dispersiblity index
1	ET4	68.7%	-36.6	458.7	0.998
2	ET5	42.8%	-20.3	622	0.712
3	ET9	65.4%	-34.6	515.3	0.987

The ET4 formulation was selected as optimized batch. The entrapment efficiency particle size, zeta potential and polydispersiblity index, were found to be 68.7%,458.7 nm,-36.6 and 0.998 respectively. The optimized ethosomal formulation was then subjected to incorporated into gel bases with different concentration of carbopol 930 i.e 0.5%,0.7%,and 1.0%.The gel bases was optimized by pH, viscosity ,drug release, drug content and permeability, and EG2 formulation was selected as optimized batch. The pH, viscosity ,drug release, drug content and permeability 6.77± 0.1, 18243 ± 122.43, 74.48±2.24, 85.65%±1.9 and 74.90±2.24 respectively.



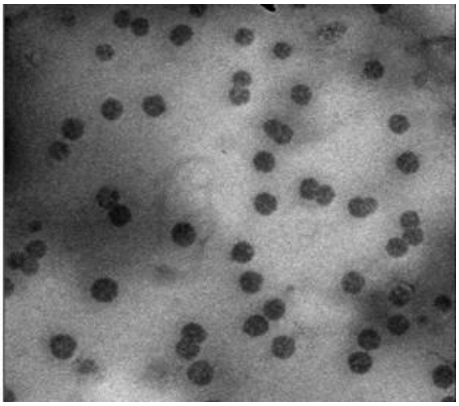


Figure 5 SEM image of etodolac ethosome .Figure 6 TEM image of etodolac ethosome.

At the end the release kinetics of the optimized gel was analysed on the basis of different models suggested for release kinetics such as zero-order model. First order model, higuchi model, Hixson-Crowell model and korsmeyer- peppas model. the values was put into the equations and various graphs were plotted given below that resulted into the linear equations and regression values which was R2=0.986for zero order model, R^2 = 0.930for first order model, R2= 0.923for Higuchi model, R2= 0.972for Hixson-Crowell model; and R2= 0.982 for Korsmeyer-peppas model. On the basis of above figures and data obtained, the optimized formulation was showing the release pattern of Zero order model that shows that the drug release pattern is sustained release. Diffusion exponent was found to be 0.915 which shows super case II transport

Table 5: pH, appearance, viscosity drug content of optimized gel formulation

Gel code	pH	Viscosity (cp)	Appearance	drug content	Flux(mg/h/cm^2)	Apparent Permeability coefficients (K*p*)
EG1	6.7 ±0.1	14220 ±75.38	Translucent	81.68%±1.4	10.80	0.108
EG2	6.77± 0.1	18243±122.43	Translucent	85.65%±1.9	11.73	0.117
EG3	6.71± 0.1	18252 ± 54.35	Transparent	82.04%±0.09	9.98	0.0998

Table 6 Drug release studies of ethosomoal gel formulation

S.No	Time (hrs)	Cumulative % drug release		
		EG1	EG2	EG3
1	1	19.93±0.81	15.75 ±1.43	13.51±0.51
2	2	27.01±1.58	21.93 ±1.76	20.43±1.36

3	3	35.87±1.69	38.03±1.62	35.39±1.85
4	4	45.84±2.15	44.38 ±2.14	43.24±1.39
5	5	58.77±1.84	58.71±1.36	55.16±1.15
6	6	72.13±1.38	74.48±2.24	60.25±1.71

Table 7cumulative drug permeation study of different formulation

S.No	Time	cumulative drug permeationEG1 (mg/cm^2 /hr)	cumulative drug permeation EG2 (mg/cm^2 /hr)	Cumulative drug permeation EG3 (mg/cm^2 /hr)
1	1	19.93 ±0.81	15.75±1.43	13.51±0.51
2	2	27.21 ±1.58	20.87±1.76	20.57±1.36
3	3	36.15 ±1.69	38.09 ±1.62	35.60±1.85
4	4	46.21±2.15	44.00±2.14	43.60±1.43
5	5	59.24 ±1.84	58.27±1.36	55.60±1.15
6	6	74.33 ±1.38	74.90±2.24	60.81±1.71

Table 8 Drug release model

Model	R^2
Zero order model	0.986
First order model	0.930
Higuchi model	0.923
Hixson crowell model	0.92
krosmeyer peppas model	0.982

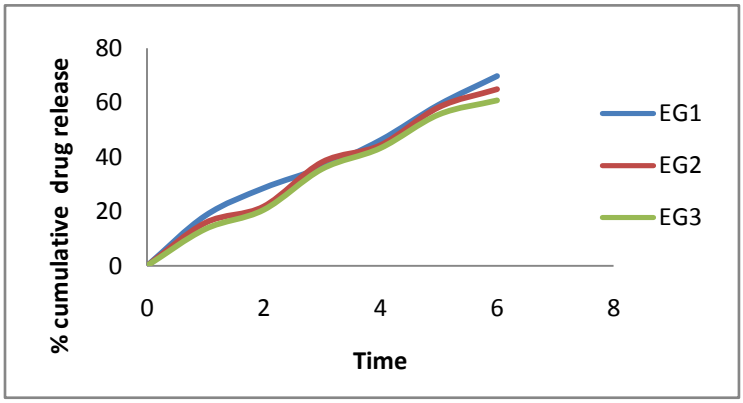


Figure 7: % Cumulative drug release of ethosomal gel formulations

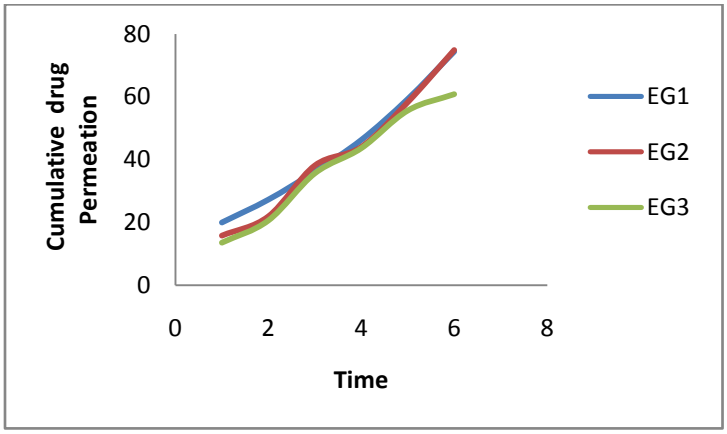


Figure 8 Cumulative drug Permeation study in various ethosomal Formulation

CONCLUSION

This study clearly demonstrated that a significant amount of etodolac transported across the skin when entrapped in ethosomes. The *in-vitro* efficiency of etodolac ethosomal gel was also found to be significant. The results suggest that gel containing ethosomal vesicles of etodolac can be used for transdermal treatment of the diseases like rheumatoid arthritis, where chronic use is needed.

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REFERENCES:

1. Prausnitz MR, Langer R. Transdermal drug delivery, Nature Biotechnology,2008; 26(11):1261-68.
2. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery, Nature Reviews Drug Discovery2004; 3(2):115-124.
3. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 2000;65:403–418
4. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosome for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti inflammatory activity on human volunteers. J Cont Rel; 2005; 106:99-110.
5. Jun-Bo T, Zhuang-Qun Y, Xi-Jing H, Ying X, Yong S, Zhe X, Tao C. Effect of ethosomal minoxidil on dermal delivery and hair cycleof C57BL/6 mice. J Dermatol Sci 2007; 45:135-137.
6. T. Limsuwana , T. Amnuaikit, Development of Ethosomes Containing Mycophenolic Acid ,Procedia Chemistry 2012;4: 328 – 335
7. Touitou E. Compositions for applying active substances to or through the skin. US Patent 5: 538, 934, 19
8. Fry D W, White J.C, Goldman I.D. Rapid secretion of low molecular weight solutes 1from liposomes without dilution. Analytical Biochemistry 1978;90: 809- 815
9. Gregor Cevic, Stefan Mazgareanu, Matthias Rother. Preclinical characterization of NSAIDs in ultradeformable or onventional topical gels. Int. J. Pharm. 2008; 360: 29-39