



GEL CONTAINING ETHOSOMAL VESICLES FOR TRANSDERMAL DELIVERY OF ACECLOFENAC

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ABSTRACT

The current investigation aims to evaluate the potential of ethosomes to increase the transdermal transport of Aceclofenac. Effects of different concentrations of lecithin (2, 3, 4, 5 and 6% w/w) and ethanol (20, 30 and 40 % w/w) on different properties of ethosomes (EF-1 to EF-7) were studied. The size of the vesicles was found to have increased with increasing lecithin concentration (2-6%). Also it was observed that the size of the vesicles decreased significantly with increasing ethanol concentration (20-40%). EF-2 ethosomes with 3% lecithin and 20% ethanol were found to have shown highest Aceclofenac release ($92.72 \pm 1.04\%$). In final phase of formulation development, EF-2 Ethosomes containing Aceclofenac were converted to gel using three different carbopol concentrations (1, 1.5 and 2% w/w). The gel containing Aceclofenac encapsulated in EF-2 ethosomes in 1.5% gel was found to be the optimized formulation (G-2). G-2 was found to have shown excellent *in-vitro* drug release and *in-vivo* activity comparing gel containing free Aceclofenac drug and marketed gel (Hifenac[®]).

Keywords: Ethosomes, Deformability, Rheumatic disease, Carbopol gel, Aceclofenac, Lecithin

INTRODUCTION

Aceclofenac is a non steroidal anti-inflammatory drug (NSAID) used in treatment of pain and inflammatory disorders, such as rheumatoid arthritis. However like other NSAIDs, oral administration of this drug is also associated with severe gastrointestinal side effects like ulceration and gastro intestinal bleeding. Further as it is required for chronic use in the conditions like rheumatoid arthritis, these drawbacks become disabling factors of such therapy. The solution of this problem lies in the fact that, topically applied NSAIDs are safer than and as efficacious as oral NSAIDs¹. Furthermore, the transdermal route of administration has a high patient compliance, which derives from it being non-invasive and the long interval between applications. Transdermal administration also provides a means to obtain constant systemic drug levels². However, penetration through transdermal route always remained an area of concern.

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 structure³.

In present study we have investigated the suitability of ethosomal system in enhancement of Aceclofenac transport across the skin. This study also focuses on making the formulation more pharmaceutically acceptable by converting it into carbopol gel.

MATERIALS AND METHODS

Materials

Aceclofenac was a kind gift from Tirupati Medicare Limited, Himachal Pradesh, India. Phosphotidyl choline (soyabean) and L-carrageenan were purchased from Sigma-Aldrich Co., USA.

Carbopol[®] 934 was obtained from SD Fines Chemical Ltd., Mumbai. Ethanol absolute (99.9%) was purchased from Merck, Mumbai. Propylene glycol was purchased from Qualigens Fine Chemicals, Mumbai. Water used for all experimental purposes was type-I (Millipore[®]). Animals (*wistar* rats) and animal skins were obtained from animal house, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi. All animal experiments were carried out as per approved protocols by the Institutional Animal Ethical Committee (IAEC).

Analysis of drug excipients interaction

Drug excipients interaction was studied by using Fourier Transform Infrared (FTIR) analysis and Differential Scanning Calorimetric Analysis (DSC) of the freeze dried final gel formulation. FTIR analysis was carried out on JESCO-420, Japan in the region of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and the data was analyzed by Spectra manager[®] software. DSC analysis was performed on DSC-60 (Shimadzu, Japan) at temperature 40-400 °C at the rate of 10 °C/min. Nitrogen gas was introduced at a pressure of 2 bars and a flow rate of 20 ml/min and the data was analyzed by using TA-60 collector[®] software.

Preparation of Aceclofenac loaded ethosomal vesicles

Ethosomal formulation was prepared according to the method reported by the Touitou et al.³. Phospholipid and Aceclofenac was dissolved in ethanol in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. This mixture was heated to 30 °C in a water bath. The water was heated to 30 °C in a separate vessel and was added to the mixture drop wise in the centre of the vessel, which was stirred for 5 min at 700 rpm in a covered vessel. Ethosomes were formed spontaneously by the process. Composition of different ethosomal formulations are given in Table 1.

Table 1: Compositions of different ethosomal formulations

Ethosome formulation	Composition (% w/w)				
	Lecithin	Ethanol	Propylene glycol	Drug	Water
EF-1	2	20	10	1.5	q.s.
EF-2	3	20	10	1.5	q.s.
EF-3	4	20	10	1.5	q.s.
EF-4	5	20	10	1.5	q.s.
EF-5	6	20	10	1.5	q.s.
EF-6	2	30	10	1.5	q.s.
EF-7	2	40	10	1.5	q.s.

Vesicle shape

Ethosomes were visualized using a Transmission Electron Microscope (TEM) (Philips CM 12 electron microscope), with an accelerating voltage of 100 kV. Samples were negatively stained with a 1% aqueous solution of phosphotungstic acid. Vesicle suspension was dried on a microscopic carbon-coated grid for staining. The

excess solution was removed by blotting. After drying, the specimen was viewed under the microscope at a 100 k fold enlargement.

Entrapment efficiency

The Aceclofenac entrapment capacity of ethosomal vesicles was determined by ultracentrifugation method⁴. The entrapment efficiency was determined after separating the untrapped drug by

centrifugation at 4° C at 15,000 rpm for 2 h. the vesicles were lysed using Triton-X 100 (0.1% v/v) and analysed for drug content. Entrapment efficiency was expressed as percentage of total drug entrapped.

The entrapment capacity was calculated using the formula:

$$(T - C)/C \times 100$$

Where T is the theoretical amount of Aceclofenac that was added and C is amount of drug detected only in the supernatant.

Vesicle size and size distribution

The vesicles size and size distribution were determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetasizer, Nano- ZS, Malvern, U.K.) with DTS® (nano) software. For vesicles size measurement, vesicular suspension was diluted with distilled water and the measurements were conducted in triplicate⁵.

Zeta potential determination

Zeta potential of the vesicles was determined using Zeta Sizer (Nano- ZS, Malvern, U.K.). The measurements were made in triplicate.

Degree of deformability

In this study, the flux of ethosomes through the pores of known size (through a sandwich of different polycarbonate filters with pore diameter between 200 and 50 nm, depending on the starting

suspension of ethosomes) was driven by an external pressure of 2.5 bars. The amount of ethosome suspension, which was extruded during 5 min, was measured, and the vesicle size and the size distribution were monitored by DLS measurement before and after filtration. The experiment was performed in triplicate. The degree of deformability is calculated by using the following formula, as reported by⁶.

$$D = J \left(\frac{r_v}{r_p} \right)^2$$

Where, D is deformability of vesicle membrane, J is amount of suspension passed in 5 min, r_v is size vesicles (after passed), and r_p = pore size of barrier.

Preparation of ethosomal and free drug gels

The best achieved ethosomal vesicle suspension, formula EF-2, was incorporated into carbopol gel (1%, 1.5%, and 2% w/w). The specified amount of Carbopol® 934 powder was slowly added to ultrapure water and kept at 100 °C for 20 min. Triethanolamine was added to it drop-wise. Appropriate amount of formula EF-2 containing Aceclofenac (1.5%w/w was then incorporated into gel-base. Water q.s. was added with other formulation ingredients with continuous stirring until homogeneous formulations were achieved (G-1, G-2 and G-3). Gel containing free Aceclofenac was prepared by similar method using 1.5% carbopol (Table 2).

Table 2: Composition of gels

Gel formulation	Composition (% w/w)			
	Aceclofenac (in vesicles)	Carbopol	Triethanolamine	Phosphate buffer (pH 7.4)
G- 1	1.5	1	0.5	q.s.
G- 2	1.5	1.5	0.5	q.s.
G- 3	1.5	2	0.5	q.s.
G-4*	1.5	1.5	0.5	q.s.

*G-4 contains free drug

Content uniformity of gel

Carbopol gel (1g) was kept in a beaker containing 1000 ml of phosphate buffer (pH 7.4) for 48 h with constant stirring using magnetic stirrer. Solution was filtered and observed with UV spectrophotometer at λ_{max} 273 nm. The measurements were made in triplicate.

In-vitro drug release studies

The *in-vitro* release of Aceclofenac was carried out in the modified Franz diffusion cell. Vesicle suspensions were placed over the dialysis membrane. The donor compartment was clamped over it with the help of springs. Samples of 1.5 ml were withdrawn at predetermined (5, 10, 15, 30, 60, 120, 240, 360, 720, and 1440 min) time intervals from the receptor compartment. Fresh receptor fluid was added to the receptor compartment each time to maintain a constant volume. Both dialysis membrane and rat skin were used for *in-vitro* release of gels. The samples were then analyzed using UV double beam spectrophotometer at λ_{max} 273 nm. Measurements were conducted in triplicate.

Drug release kinetic modelling

The kinetics of Aceclofenac release from the ethosomal vesicles and from the gels formulated was determined by finding the best fit kinetic model among: zero order⁷, first order⁸, and Higuchi^{9, 10}. In order to better characterize the drug- release behavior Korsmeyer-Peppas^{11,12} semi- empirical model was further applied.

In vivo study

Wistar rats of either sex weighing between 150-200 g were divided in four groups. The animals were starved overnight with water being provided *ad libitum*. The formulations were administered by oral or topical route (as per the group). After 30 min rats were challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan on a sub-plantar surface of both the paws. The paws were marked with ink at the level of lateral malleolus and immersed in mercury column of a plethysmometer and each paw volume measured. The paw volume was measured prior to carrageenan injection and then at 1- 6 h of administration¹³⁻¹⁵. The percentage

inhibition of carrageenan-induced paw edema was calculated for each formulation by using the following equation:

$$\% \text{ inhibition of edema} = (V_{(\text{control})} - V_{(\text{treated})}) / V_{(\text{control})} \times 100$$

Stability study

Stability of the optimized gel was determined by physical appearance content uniformity and release study at 5 °C ± 3 °C and 25 °C ± 2°C at different time intervals (0- 3 months).

Statistical analysis

Analysis of variance (Kruskal-Wallis One way ANOVA) along with multiple comparison test (Student-Newman-Keuls Method) and t-test (for two samples) were employed in the statistical analysis of the determined parameters in this study. Statistical significance was defined at P < 0.05. SigmaStat® 3.5 software was used for all the statistical testing.

RESULTS AND DISCUSSION

Analysis of drug excipients interaction

An infrared spectrum of the gel containing Aceclofenac loaded ethosomes was found to have shown peaks at 1281.47 cm^{-1} due to C-N stretching, N-H stretching at 3275.50 cm^{-1} , C-H stretching at 3026.73 cm^{-1} , C=C aromatic stretching at 1417.42 cm^{-1} and C-O stretching at 1256.40 cm^{-1} which are characteristics of Aceclofenac^{16,17} (Fig. 1).

In DSC curve of the gel no significant shift in endothermic peak was found compared with those of pure drug, which was found to be at 153.18 °C (Fig. 2a). In DSC curve of gel containing Aceclofenac loaded ethosomes, peak was found at 149.23 °C (Fig. 2b). This result further confirmed the absence of any interaction between Aceclofenac, lecithin and carbopol.

Characteristics of vesicles

The outer membrane of the vesicle was not properly visible by transmission electron microscopy (Fig. 3). One possible reason for this might be the effect of ethanol, which leads to thinning of the outer membrane.

Amounts of ethanol and lecithin, used for ethosome preparation, were found to have influenced the entrapment efficiency. The

entrapment efficiency of ethosomes was found to have decreased with the increase in ethanol concentration significantly ($p < 0.05$). One possible reason for this could be the increase in fluidity and presence of thinner membrane with higher ethanol concentration. Maximum entrapment was observed with EF-1 formulation ($73.85 \pm 0.40\%$). However, it was not found to be significantly higher than EF-2 formulation ($71.55 \pm 1.16\%$). Data on particle size (Fig. 4) showed that the size of the vesicles increased with increasing

lecithin concentration (2-6%) significantly ($p < 0.05$). Also it was observed that the size of the vesicles decreased significantly ($p < 0.05$) with increasing ethanol concentration (20-40%). These data are in perfect agreement with previous finding³. The largest vesicles were observed in the formulation EF-5 (324.77 ± 1.85 nm), while the smallest vesicles were observed in the formulation EF-7 (131.7 ± 1.36 nm).

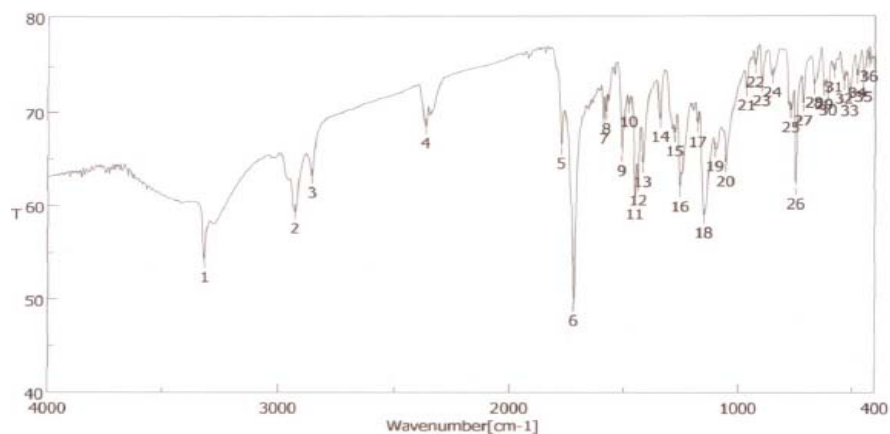


Fig. 1: FTIR spectra of the Aceclofenac-lecithin-carbopol physical mixture

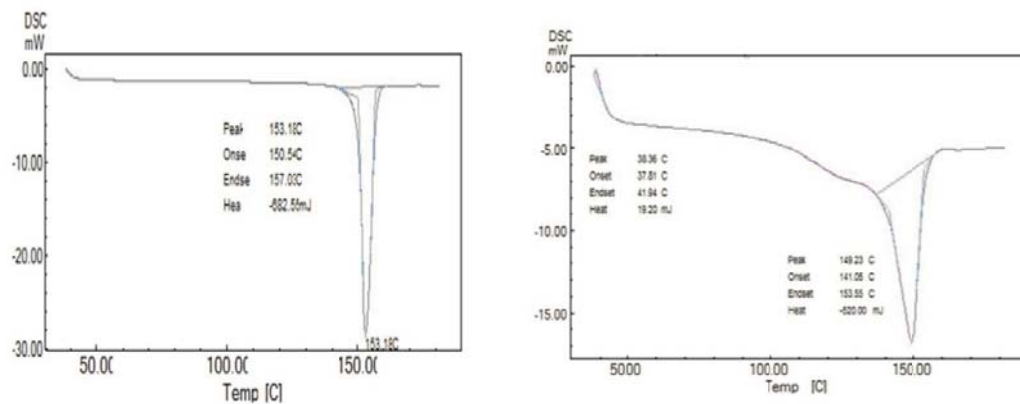


Fig. 2: DSC scans of a) Aceclofenac and b) Aceclofenac-lecithin-carbopol physical mixture.

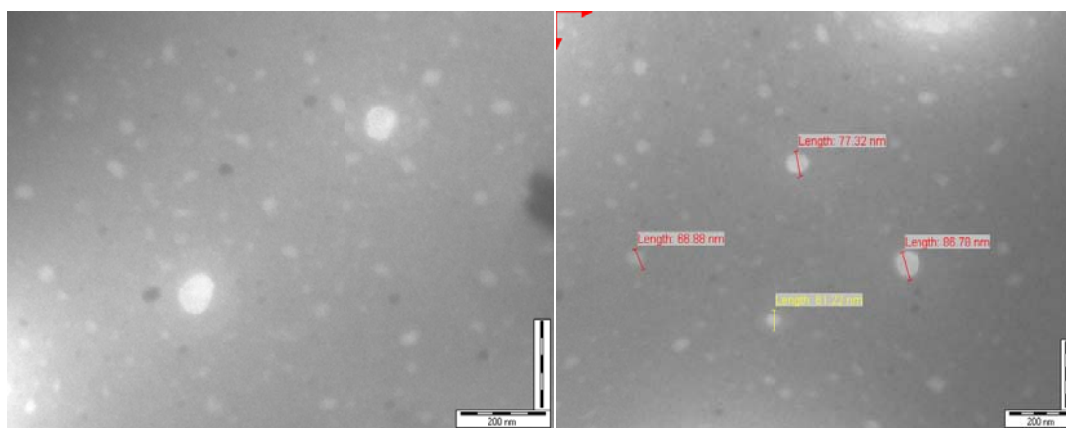


Fig. 3: Transmission electron microscope photograph of Aceclofenac ethosomal vesicles (magnification $\times 100,000$).

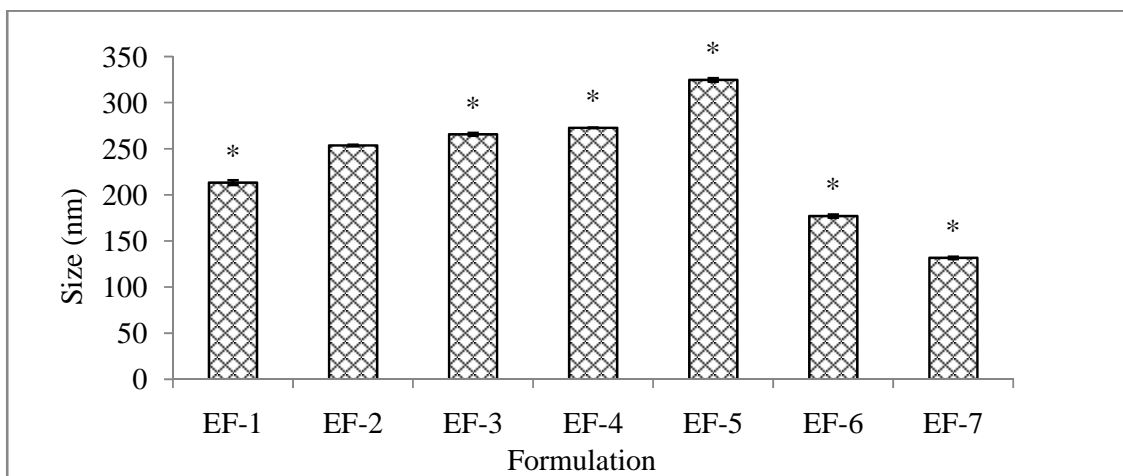


Fig. 4: Variation of size among different ethosomal vesicles.

In case of zeta potential no fixed correlation was observed with variables (ethanol and lecithin concentration). Also it was observed that the differences in zeta potential between different formulations were very small and statistically insignificant. This might be due the reason that lecithin was the major excipient and the charge contributor in all the ethosomes. Therefore, there was no major variability in zeta potentials of different ethosomes. Deformability index of various ethosome formulations were found to vary significantly with lecithin and ethanol concentration. It was

observed that deformability index first increased significantly ($p < 0.05$) with increase in lecithin concentration (From EF-1 to EF-2). However, deformability index was found to decrease on further increase in lecithin concentration (Fig 5). One possible reason for this result could be the rigidization effect of lecithin on higher concentrations. On the other hand deformability was found to increase with ethanol concentration suggesting the major role of the ethanol in vesicle elasticity.

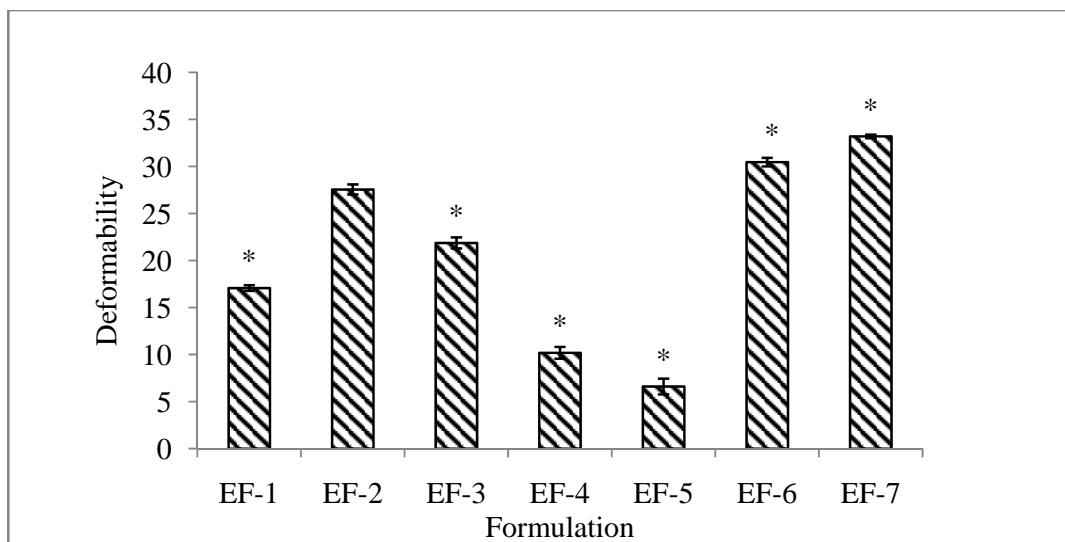


Fig. 5: Variation of deformability among different ethosomal vesicles.

Total percentage drug release after 24 h was found to be variable among different formulations. A statistically significant increase in total Aceclofenac release ($p < 0.05$) from ethosomes was observed with initial increase in lecithin concentration. However, on further increase of lecithin concentration, the release was found to get decreased. Maximum total release was observed with EF-2 ethosomes ($92.723 \pm 1.047\%$). Comparative characterization of the ethosomal vesicles is given in Table 3.

By kinetic modelling it was found that all ethosomal formulations except EF-1 were found to follow zero order release pattern. EF-1 was found to follow the first order kinetics. According to Korsmeyer-Peppas model all ethosomal formulation under study were releasing the drug through non-Fickian super case-II transport ($n > 0.1$). Therefore, it can be considered that transport of drug from ethosomal suspensions was concentration independent and non-Fickian (Table 4).

On comparing release profile of Aceclofenac from optimized ethosomal vesicles (EF-2) with solution of free Aceclofenac, a significant difference was clearly visible (Fig. 6). The results clearly indicate that ethosomes effects into fast and more complete release of Aceclofenac.

3.3. Evaluation of different gel formulations containing optimized ethosome entrapped Aceclofenac (EF-2)

The drug was found to be uniformly distributed in the G-2 (uniformity of content - $97.817 \pm 0.978\%$). G-1 and G-2 were found to have shown comparable release of Aceclofenac but G-1 was ruled out because less gel consistency. Release from G-3 was significantly less ($p < 0.05$) probably due to detrimental effect of high viscosity due to high concentration of carbopol. Since, formulation G-2 showed maximum release and good consistency a concentration 1.5 % carbopol 934 (G-2 formula) was considered for the final development of the formulation. Therefore, final optimized

transdermal gel formulation of Aceclofenac consists of drug entrapped in EF-2 ethosomes which was ultimately converted to a gel as per the formula followed for G-2.

On drug release kinetic modelling and comparison of the release profile it was found that there was not much difference in the release pattern of drug from all three gels (Table 5). All of them were found to release the drug in accordance to first order kinetics. Also a non-Fickian anomalous transport ($0.5 < n < 1.0$) of drug was involved in all three gel formulations.

A comparative release study of the optimized gel of Aceclofenac (G-2), the gel containing free drug (G-4) and the marketed Aceclofenac

gel (Hifenac®) had shown significant differences (Fig. 7). The total release after 24 h from G-2 was more than two times the release shown by G-4. Also the initial fast release of the drug from G-2 gives it an edge over the G-4 and marketed gel in acute conditions. Therefore, onset as well extent of Aceclofenac release from gel containing novel ethosomes is found to be superior to the free drug gel as well as marketed gel, proving the effectiveness of this system in enhancing the drug transport of Aceclofenac through skin. Release of the drug through rat skin was found to get slightly reduced (Fig. 8). But, the drug release from G-2 was almost thrice the release from G-4.

Table 3: Comparative features of different ethosomal vesicles (n=3)

Ethosome formulation	Entrapment efficiency(%) ± SEM	Size (nm) ± SEM	Zeta potential (mV) ± SEM	Deformability± SEM	Percentage cumulative release(in 24 h)±SEM
EF-1	73.85 ± 0.40	213.33 ± 2.60	-14.6 ± 0.64	17.08 ± 0.30	88.594 ± 0.651
EF-2	71.55 ± 1.16	253.67 ± 0.88	-20.8 ± 1.07	27.57 ± 0.54	92.723 ± 1.047
EF-3	68.37 ± 0.11	265.73 ± 1.59	-15.83 ± 0.68	21.88 ± 0.59	89.854 ± 0.807
EF-4	66.31 ± 1.19	272.73 ± 0.18	-21.1 ± 0.95	10.19 ± 0.63	87.079 ± 0.766
EF-5	61.83 ± 0.77	324.77 ± 1.85	-17.57 ± 1.11	6.61 ± 0.84	82.209 ± 0.437
EF-6	61.07 ± 1.29	177.07 ± 1.76	-15.5 ± 1.26	30.47 ± 0.45	76.382 ± 0.901
EF-7	55.24 ± 1.92	131.7 ± 1.36	-13.5 ± 0.95	33.21 ± 0.19	72.64 ± 0.519

Table 4: Release rate constants (k), correlation coefficients (R²), and release exponent (n) of different ethosomal vesicles

Ethosome formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	k	R ²	k	R ²	k	R ²	Rel. Expo. (n)	R ²
EF-1	3.867	0.968	-0.037	0.977	18.61	0.951	1.12	0.978
EF-2	4.057	0.984	-0.045	0.963	19.24	0.939	1.192	0.989
EF-3	3.872	0.991	-0.039	0.959	18.18	0.927	1.237	0.985
EF-4	3.718	0.992	-0.035	0.96	17.42	0.924	1.185	0.985
EF-5	3.518	0.991	-0.03	0.974	16.54	0.929	1.145	0.968
EF-6	3.195	0.99	-0.024	0.963	14.84	0.906	1.106	0.964
EF-7	3.073	0.99	-0.022	0.973	14.25	0.903	1.146	0.971

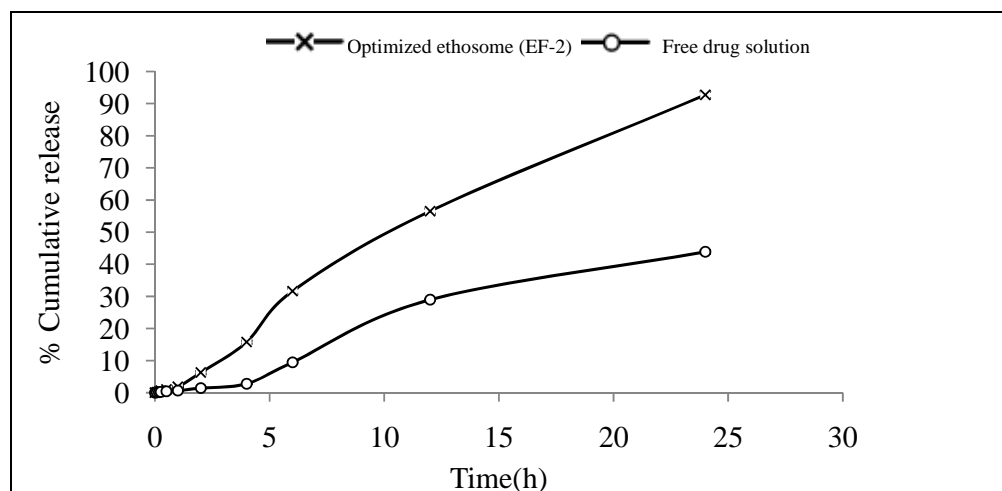


Fig. 6: Comparative release profile of optimized ethosomal vesicles suspension and free drug solution

Table 5: Release rate constants (k), correlation coefficients (R²), and release exponent (n) of gels of different concentrations

Gel formulation	Zero order		First order		Higuchi		Korsmeyer -Peppas	
	k	R ²	k	R ²	k	R ²	Rel. Expo. (n)	R ²
G- 1	2.823	0.989	-0.02	0.995	13.5	0.959	0.665	0.989
G- 2	2.918	0.964	-0.022	0.99	14.3	0.983	0.551	0.989
G- 3	2.225	0.955	-0.014	0.982	10.94	0.978	0.627	0.957

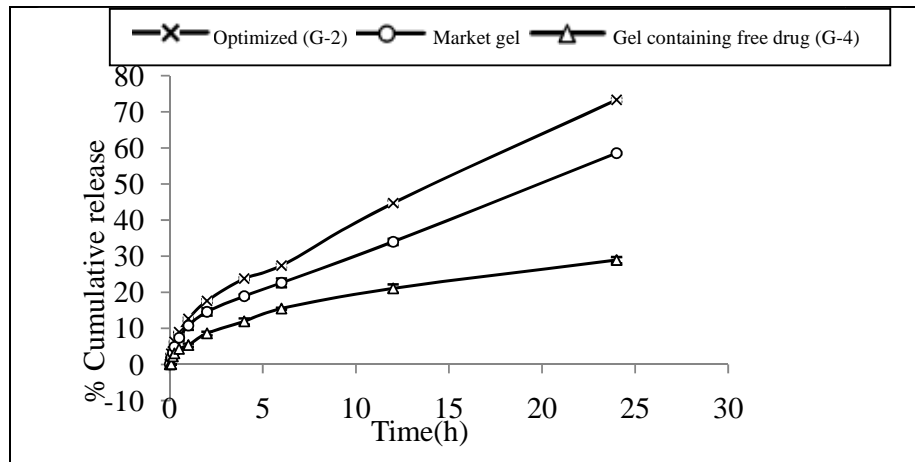


Fig. 7: Comparative release profile (dialysis membrane) of optimized gel formulation, market gel and free drug gel (n=3).

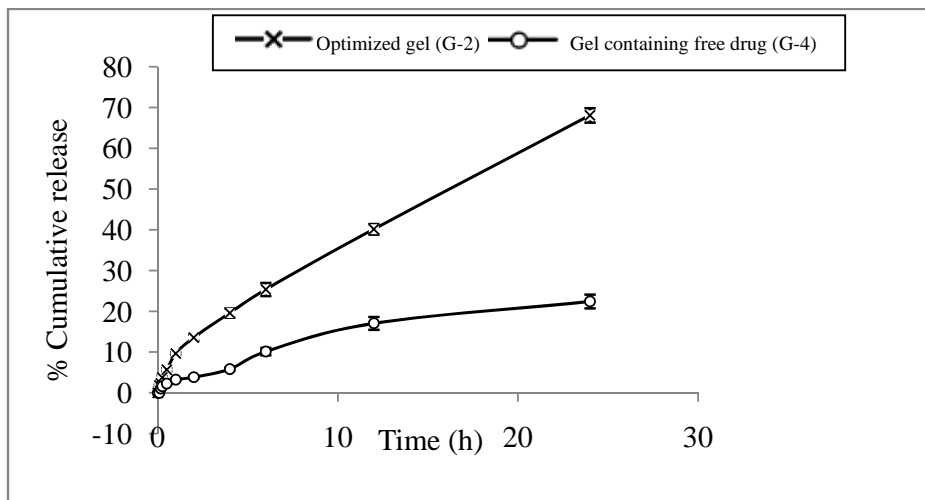


Fig. 8: Comparative release profile (rat skin) of optimized gel formulation and free drug gel (n=3).

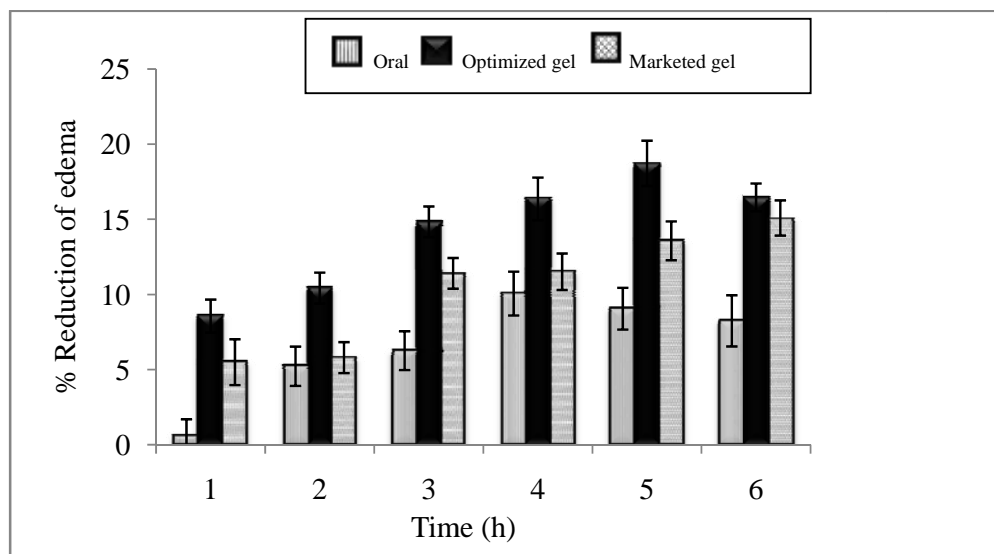


Fig. 9: Comparative pharmacodynamic analysis of Aceclofenac ethosomal vesicles G-2, Aceclofenac oral suspension in distilled water, and marketed Aceclofenac gel (Hifenac®) formulation by carrageenan-induced rat paw edema model.

***In-vivo* anti-inflammatory study**

The *in-vivo* performance of selected optimized ethosome containing formulation (G-2) was compared with oral Aceclofenac as well as marketed Aceclofenac gel (Hifenac®) by carrageenan induced paw edema model (Fig. 9). The ethosomal vesicle gel was found to have shown highest inhibition of edema (18.731±1.508%) as compared to marketed Aceclofenac gel (15.092±1.169%) and oral Aceclofenac (10.063±1.458%).

Stability studies

Stability studies have shown that appearance and content uniformity was found to be good during three month testing at both the temperatures. However, total percentage *in-vitro* release for 24 h has dropped significantly (from 73.332±0.538 at zero month to 59.433±0.552 at third month). Therefore, it was found that this formulation is not stable at higher temperature. The reason behind this finding might be the degradation of the lecithin at higher temperature and hence it is recommended to store this formulation at lower temperature.

CONCLUSIONS

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

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