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IN VITRO PERMEATION AND PHARMACO-DYNAMIC PROPERTIES OF GEL FORMULATIONS CONTAINING FLUOCINONIDE ENTRAPPED NIOSOMES

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ABSTRACT

Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages of liposomes, such as chemical instability, variable purity of phospholipids and high cost. The aim of this work is to formulate and evaluate niosomes as carriers for topical delivery of Fluocinonide, as an anti-inflammatory drug, test their stability and improve their anti-inflammatory effect through niosomal encapsulation with objectives of prolonging its action and avoiding its most side effects. Incorporation of Fluocinonide – entrapped niosomes into gelling agents and increasing their concentrations resulted in a marked decrease in amount of drug permeated from the gel through cellulose membrane and rabbit skin. Polymers used as gelling agents are Carbopol 934 and Carboxy Methyl Cellulose sodium (CMC Na). It was found that permeated amount of Fluocinonide decreased with increasing either concentration of Carbopol 934 from 1% to 2% or concentration of CMC Na from 2% to 4%. Amount of Fluocinonide permeated from cellulose membrane are significantly high compared to the amounts permeated across the skin ($p<0.01$) from the same formulation. On the other hand, there is an increase in the amount of drug permeated from niosomal gels through rabbit skin, compared to that permeated from control drug gels prepared from the same gelling agents (enhancement effect). Stability study was carried out to detect effect of temperature on leakage of drug from the niosomal vesicles and evaluate the percentage of drug retained in the niosomal vesicles and niosomal gel formulations, respectively at different storage temperature (4 ° C, 25 °C and 37 °C). The data obtained were compared statistically using one-way analysis of variance (ANOVA), using Tukey-Krammer Multiple Comparison Test. The anti-inflammatory activity (pharmaco-dynamic properties) of Fluocinonide in its selected formulae was studied using the rat hind paw edema technique and compared with control untreated group. Niosomal gel formula, (F13), containing Span 40: Tween 40: Cholesterol in ratios of 25: 25: 50, consequently and 3% CMC Na is proved to be the most stable and efficient formula for anti - inflammatory activity.

Keywords: Niosomes, Cellulose Membrane; Dorsal Rabbit Skin; Pharmaco-dynamic Properties.

INTRODUCTION

Drug delivery systems using colloidal particulate carriers such as niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs. Modification of the particles composition or surface can adjust the affinity for the target site and/or the drug release rate. Slowing the drug release rate may reduce the toxicity of drug, so, these carriers play an increasingly important role in drug delivery [1]. The self-assembly of non-ionic amphiphiles in aqueous medium resulting in closed bi-layer structures leads to the formation of niosomal vesicles or niosomes. Niosomal vesicles are analogous to liposomes and serve as drug carriers as they can incorporate both hydrophilic and lipophilic drugs. Niosomes exhibit flexibility in their structural characteristics includes composition, fluidity and size, so it can be designed according to the desired situation. Niosomes can improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of many drugs[2]. Among all routes of administration, the enhanced transdermal drug delivery of niosome encapsulated drugs was considered. The vesicular systems can overcome the permeation barrier of the skin and act as penetration enhancers for the drug [3]. Fluocinonide is a non-steroidal anti-inflammatory agent, used in the treatment of inflammatory and degenerative disorders of the musculoskeletal system. It is widely

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prescribed for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, extra-articular disorders, bursitis, tendonitis, and non-articular rheumatic condition [4]. Administration of Fluocinonide via the skin could be suitable to people who can't use the oral route due to vomiting or unconsciousness. Also, transdermal route can also avoid gastrointestinal incompatibility and side effects of the drug [5]. The goal of this work was to formulate and evaluate niosomes as carriers for topical delivery of Fluocinonide via gel preparations. Stability as well as in – vitro permeation were also studied. The anti-inflammatory activity of Fluocinonide from its selected formulae was investigated using the rat hind paw edema technique.

MATERIALS AND METHODS

Preparation of Niosomes by Shaking Method

Multilamellar vesicles were prepared by standardized mechanical shaking of the various lipid dispersions in the presence of hydrating fluid without subsequent sonication [5]. A mixture of Span 40, Cholesterol in a molar ratio of 50:50 and Span 40, Tween 40: Cholesterol in a molar ratio of 25: 25: 50 were dissolved in 15 ml chloroform in a 100 ml rounded-bottom flask. The organic solvent was removed at a temperature of 60 °C under reduced pressure on a rotary evaporator, to form a thin film on the flask wall. The excess organic solvent was then removed by leaving the flask in a desiccator under vacuum overnight. The obtained dried lipid film was hydrated with 5 ml of PBS (pH 7.4) containing Fluocinonide (2mg/ml) by shaking using a mechanical shaker (oscillating thermostatically controlled water bath shaker, at 60° C for about 1 hr [6,7]. It was essential to prepare the vesicles at a temperature above the gel –liquid transition temperature of the non-ionic surfactants; 50° C is the highest phase transition temperature, therefore, all vesicles' preparations were carried out at about 60° C.

Determination of Entrapment Efficiency of Niosomes Containing Fluocinonide

The Fluocinonide-entrapped niosomes were separated from the un-entrapped free drug by freeze thawing/centrifugation method using Centrifuge[8]. The entrapment efficiency (EE) of Fluocinonide [9] is defined as follows:

EE of Fluocinonide = (Amount entrapped / Total amount of Fluocinonide) X 100

Preparation of Gels Containing Fluocinonide

Preparation of Carbopol 934 Gels Containing Fluocinonide

The weighed amount of Carbopol 934 was dispersed in 100 ml distilled water and stirred using magnetic stirrer at a high speed (500 rpm). Stirring was continued until a thin dispersion, without lumps, as obtained then the stirring speed was reduced to allow foam to break and to maintain a good liquid turnover while adding the calculated amount of tri-ethanol-amine (0.01%-0.02%) required to form the gel. Carbopol 934 gel bases were prepared at a concentration of 1%, 1.5% and 2% containing different concentration of tri-ethanolamine. Tri-Ethanolamine neutralizes the carbonyl groups present on the polymer backbone, which usually can affect its gelling potency. To each of the above gel bases, Fluocinonide was added as 1% concentration and dispersed thoroughly using magnetic stirrer.

Preparation of Carbopol 934 Niosomal Gels Containing Fluocinonide

Topical niosomal gels were prepared by the same method [10]. An equivalent amount of niosomes separated from un-entrapped drug containing one gram of Fluocinonide was incorporated into the structured gels instead of drug. Three concentrations of Carbopol 934 (1%, 1.5% and 2%w/w) were used to investigate the effect of gelling agent concentration on the release of Fluocinonide from the prepared niosomal gels.

Preparation of CMC Na gels Containing Fluocinonide

The weighed amount of CMC Na powder was sprinkled gently on 100 ml boiling distilled water and stirred magnetically at a high speed (500 rpm). Stirring was continued until a thin hazy dispersion, without lumps, was formed. Leaving overnight in the refrigerator may be necessary for complete gel dispersion. CMC Na gel bases were prepared with a concentration of 2%, 3% and 4%. To each of the above gel bases, Fluocinonide was added as 1% concentration and dispersed thoroughly using magnetic stirrer.

Preparation of CMC Na Niosomal Gels Containing Fluocinonide

Topical niosomal gels were prepared by the same previous method. Three concentrations of CMC Na (2%, 3% and 4% w/w) were used to investigate the effect of gelling agent concentration on the release of Fluocinonide from the prepared niosomal gels.

In vitro Permeation Study Through Cellulose Membrane

Half gram of each formulation containing 5 mg of Fluocinonide was transferred to a dissolution cell to which a cellulose membrane was attached to one side and the other side of the glass tube was attached to the stem of the dissolution apparatus (USP standard, Scientific, DA-6D,Bombay, India) and immersed in a beaker of the dissolution apparatus containing 100 ml of PBS (pH 7.4). The temperature of the medium was maintained at 37 ± 0.5°C. At each specified time intervals for 24 hr, 4 ml sample was withdrawn and replaced by an equal volume of PBS (Ph 7.4). The drug was determined spectrophotometrically at λmax 368 nm [10].

Through Rabbit Skin

Dorsal full-thickness skins of white male rabbits weighing 3-4 kg were used. The skin was carefully removed from animals and prepared for experiment. The excised full thickness rabbit skin sample were equilibrated by soaking in PBS (pH 7.4) for about one hour before beginning of each experiment [10]. The prepared skin samples were mounted on the receptor compartment of the permeation cell with the stratum corneum facing upward and the dermal side facing downward. The donor compartment was kept on the receptor compartment and secured tightly with the help of clamps. The receptor compartment was then filled with 100 ml of PBS (pH 7.4). The temperature of media was maintained at 37 ± 0.5°C. Samples were analyzed as before for 24 hrs. Each experiment was performed in triplicate and the average result was calculated. The cumulative drug permeated (Qn) corresponding to the time of the n th sample was calculated from the following Equation:

$$Q_n = V_R C_n + \sum_{i=0}^{n-1} Vs\ Ci \qquad (1)$$

Where Cn and Ci are the drug concentrations of the receptor solution at the time of the n th sample and the i (the first) sample, respectively. VR and VS are the volumes of the receptor solution and the sample, respectively. The cumulative amount of drug permeated through cellulose membrane and rabbit skin per unit area (µ g/cm2) was calculated by dividing the amount of drug permeated (µ g) by the area of the diffusion cell (5 cm2). The permeation profiles were constructed by plotting the cumulative amount of drug permeated trough cellulose membrane and rabbit skin per unit area (µ g/cm2) versus time, hours[14].

Calculation of Permeation Parameters across Cellulose Membrane and Rabbit Skin

The permeation parameters of Fluocinonide as steady state transdermal fluxes (Jss), permeability coefficient (Kp) through the cellulose membrane or rabbit skin, Diffusion coefficient (D) within the cellulose membrane or rabbit skin and lag time (tL) were calculated from the penetration data. The steady state transdermal fluxes (Jss) of Fluocinonide were calculated from the slope of linear portion of the cumulative amount of drug permeated through unit area of the cellulose membrane or rabbit skin versus time plot [14, 15]. The permeability coefficient through the cellulose membrane or rabbit skin and lag time were calculated according to the following Equations using specific computer program,

$$Jss = KpCd \qquad (2)$$

$$Kp = Jss/Cd \qquad (3)$$

$$tL = h2/6D \qquad (4)$$

Where:Jss= steady state transdermal fluxes (µ g/cm2.hour), Kp=permeability coefficient (cm/ hour), Cd= the initial drug concentration in the donor compartment (5 mg), D=diffusion coefficient (cm2/ hour), h= thickness of the cellulose membrane (0.009) and skin (0.099 cm), tL= lag time (hr). Lag time is the x-intercept of the extrapolated linear portion of the cumulative amount of drug permeated through unit area of the cellulose membrane or rabbit skin.

Statistical Analysis

Data were expressed as mean of three experiments ± the standard deviation (SD). The obtained data were compared statistically using one-way analysis of variance (ANOVA), using Tukey-Krammer Multiple Comparison Test. A p-value of 0.05 or less was considered to be significant.

Stability Studies

Niosomal formulations of F1, F2, F3, F6, and F10 were sealed in glass vials and stored at 4oC, 25oC and 37oC. The temperature selection was done on the basis of likely temperatures with which the vesicles would come in contact either during storage or in administration. Samples from each formula at each temperature were analyzed for the free drug to determine the leakage rate.

The Pharmacodynamic Activity

The carrageenan induced rat paw edema method was used to compare the efficacy of the niosomal gel formulations, with the control drug formulations. Adult male albino rats, weighing (180± 20 g), were used in this study. They were housed in groups and allowed free access to food and water prior to the experiments. The animals were divided into 7 groups, each consisting of six animals. The drug was given according to the following schedule:

Group 1: control untreated, received carrageenan (as1% conc.) Only.
Group 2: standard treated with F15.
Group 3: treated with F3.
Group 4: treated with F6.
Group 5: standard treated with F16.
Group 6: treated with F10
Group 7: treated with F13

Carrageenan-induced Rat Hind Paw Edema

Niosomal gels of Fluocinonide and Fluocinonide gels (0.5 g) containing 5 mg of drug [4] were applied to the planter surface of the left hind paw by gently rubbing 50 times with the index finger. The area of application was occluded with bandages and it was left in place for two hours. The dressing was then removed and the gel remaining on the surface of the skin was wiped off with a piece of cotton. Acute inflammation (paw edema) was induced in rats by injection of 0.1ml of 1% carrageenan solution in normal saline sub-cutaneously into sub- plantar region of the left hind paw, two hours after topical administration of the drug [4]. The thickness of the injected paw was measured

immediately after Carrageenan injection and after 1, 2, 4, 6, 8 and 24 hours using a micrometer (Model 120-1206 BATY and Co. Ltd., Sussex, England). The mean percentage inhibition of edema thickness at each time intervals was calculated from the mean effect in control and treated animals according to the Equation:

% inhibition in edema thickness= {1-(V t / VC)} x100 (5)

Where: Vt = mean increase in thickness of carrageenan paw edema of treated groups. VC = mean increase in thickness of carrageenan paw edema of control groups.

Statistical Analysis

Data were expressed as mean ± SD. The results were statistically analyzed as for mentioned under the *In vitro* permeation study.

RESULTS AND DISCUSSION

UV scanning showed that the maximum absorption of Tnoxicam (λmax) was found to be at 368 nm in PBS, pH 7.4.

A.In Vitro Permeation Study To justify the proper concentration of Carbopol 934 required for producing gel with suitable consistency for topical application, permeation of Fluocinonide from niosomal gel across cellulose membrane was studied. Fig. 1 and 2 showed the permeation of Fluocinonide from F3, F4, F5, F6, F7 and F8 through cellulose membrane. It was obvious that the permeated amount of Fluocinonide decreased with increasing concentration of Carbopol 934 from 1% to 2%. This decrease of permeated amount may be related to the higher resistance to drug diffusion by increasing the viscosity of the prepared gel from 1% to 2% [20, 21]. It was observed that the F3 and F6 produced gel with a consistency suitable for topical application, so it was chosen to prepare all the gel formulations in this study.

It indicated that the incorporation of Fluocinonide-entrapped niosomes into 1% w/w Carbopol 934 as a gelling agent to prepare topical formulation resulted in a significant decrease in the amount of drug permeated from the gel through cellulose membrane, compared to that permeated from drug gel prepared from the same gelling agent. The obtained data were compared statistically using one-way analysis of variance (ANOVA), using Tukey-Krammer Multiple Comparison Test ($p<0.001$). The slower release of the drug from niosomal gel formulations may be due to the encapsulation of drug into vesicles (micro reservoirs) providing prolonged drug release rate. The effect of CMC Na concentration on the permeation of Fluocinonide from niosomal gel of F1 and F2 across cellulose membrane was studied for the selection of suitable oncentration of CMC Na required for producing gel with suitable consistency for topical application. Figs. 4 and 5 showed the permeation of Fluocinonide from niosomal gel of F9-F14 across cellulose membrane prepared with different CMC Na concentrations (2%, 3% and 4% w/w). Results indicated that the permeated amount of Fluocinonide decreased with increasing concentration of CMC Na from 2% to 4%. This decrease in the released amounts of drug may be related to the increased micro viscosity of the gel by increasing CMC Na concentration[12]. It was observed that the formulations prepared using 3% w/w CMC Na produced gel with a consistency suitable for topical application, while the other formulations prepared with 2 and 4% w/w produced gels with low and high consistency, respectively. Therefore, 3% w/w CMC Na was used to prepare all the gel formulations for further study.

Drug permeation profiles of niosomal gel formulations of F10, F13 and F16 were presented comparatively in Fig. 6. It revealed that the incorporation of Fluocinonide- entrapped niosomes into 3% w/w CMC Na as a gelling agent to prepare topical formulation resulted in a marked decrease in the amount of drug permeated from the gel through cellulose membrane, compared to that permeated from drug gel prepared from the same gelling agent. The cumulative amounts of drug permeated after 6 hrs were 269.2± 3.5, 416.5± 4.2 and 483± 4.6 (µ g/cm2) from F10, F13 and F16, respectively. The obtained data were compared statistically using one-way analysis of variance (ANOVA), using Tukey-Krammer Multiple Comparison Test ($p<0.001$). The slower release of the drug from niosomal gels may be due to the encapsulation of drug into vesicles (micro reservoirs) providing prolonged drug release rate[22].

B. Permeation Parameters

Permeation results of Fluocinonide from F3-F16 were treated according to Fick's law. Table 2 demonstrated the steady-state transdermal fluxes (Jss), permeability coefficient (Kp), diffusion coefficient (D) and lag time (tL) values of Fluocinonide permeation through cellulose membrane from different gel formulations. The lower values of niosomal gel compared to drug gel are suggestive of prolonged drug release from niosomal gel formulations. The steady-state transdermal fluxes (Jss), decreased as a function of gelling agent concentration (% w/w) i.e. as the concentration of gelling agent increases, the steady state transdermal flux (Jss) will decrease. This might be due to that, this increase may reduce the available drug molecules for permeation through cellulose membrane. Statistical analysis showed that there is significant change between the steady-state transdermal fluxes (Jss) of niosomal gel preparations compared to plain Figs. 7 and 8 showed that the incorporation of Fluocinonide-entrapped niosomes into 1% w/w Carbopol 934 and 3% CMC Na as gelling agents to prepare topical formulations resulted in an increase in the amount of Fluocinonide permeated from gels through the dorsal rabbit skin, compared to that permeated from control drug gel prepared from same gelling agents. This increase in the amount of drug permeated may be

related to that the vesicular system can overcome the permeation barrier of the skin and act as penetration enhancer for the drug, thus destabilizing the packaging order of the stratum corneum lipids [3, 23], as well as the nonionic surfactant that used in the preparation of niosomes can solubilize lipids within the stratum corneum.

Table 3 showed different parameters of permeation. The enhancement of permeation of niosomal gel compared to control drug gel may be due to the topically applied surfactant vesicles may extract the order within and between the corneocyte upon binding to the keratin filament, hence increase drug permeability across skin. The enhancement ratios of different niosomal gel formulations were listed in Table 3. Statistical analysis shows significant change between different niosomal gel formulations compared to control drug gels and no significant change between niosomal gels of the same gels base.

C. Effect of Membrane Type on Fluocinonide Permeability

Fig 9 showed the amount of Fluocinonide permeated across cellulose membrane and rabbit skin from F3, F6, F10, F13, F15 and F16 after 6 hours. The results showed that the amounts of Fluocinonide permeated from cellulose membrane are significantly high compared to the amounts permeated across the skin ($p<0.01$) from the same formulation. This indicates the barrier properties of the skin to the drug. It is clear that there is a decrease in the amount of drug permeated from the niosomal gels through cellulose membrane, compared to that permeated from control drug gel prepared from the same gelling agent. On the other hand, there is an increase in the amount of drug permeated from niosomal gels through rabbit skin, compared to that permeated from control drug gels prepared from the same gelling agents (enhancement effect). This may be explained on the basis that there are two types of interaction between the skin and vesicles which may facilitate transdermal drug delivery. The first is the adsorption and fusion of the loaded vesicles onto the surface of skin which lead to high thermodynamic activity gradient of the drug-stratum corneum surface. The second is the effect of lipid vesicles on stratum corneum which may change the drug permeation kinetics due to an impaired barrier function of stratum corneum[14, 25].

D. Stability Studies

The percentages of drug retained in the niosomal vesicles and niosomal gel formulations were evaluated at different storage temperatures (4± 1, 25± 1 and 37± 1oC) and at definite time intervals for total period of 60 days. One of the major problems that may limit the widespread of noisome is its physical instability [26, 27]. Depending on their composition, the final formulations may have shorter shelf-lives partly due to physical instability. The results are recorded in Table 4. The results indicate that there is a direct relationship between the rate of leakage of drug out of the vesicles and the temperature of storage, the leakage rate increased with increasing temperature[26-29]. This may be attributed to phase transition of surfactant and lipid causing vesicles' leakage at higher temperature during storage and also may be due to the fluidity of the vesicular membrane. The increased degree of drug leaching with time at all storage conditions indicate that there could be fusion of niosomes leading to formation of large vesicles which would be less stable and hence get disrupted releasing the entrapped drug[30]. There was no significant change in the release pattern of the drug from different niosomes and niosomal gel formulations at $p < 0.05$ using one-way analysis of variance(ANOVA), followed by Tukey-Krammer Multiple Comparison Test.

From results of accelerated stability testing of different niosomal and niosomal gel formulations of Fluocinonide stored at 4oC, 25oC and 37oC within 60 days, It was obvious that the degradation of Fluocinonide was found to be zero-order reaction based on the values of correlation coefficient (r). Niosomal gel of formula F13 containing 3% CMC Na, mixture of Span 40: Tween 40: Chol; (25 : 25 : 50) showed the best stability with t90 of 154.9 days.

E. Pharmaco- dynamic Activity

The anti-inflammatory activity of Fluocinonide from its selected formulae was studied using the rat hind paw edema technique as the model for inflammation and compared to the control untreated group. The results showed that all the different Fluocinonide formulae containing the calculated dose of the drug administrated to the rats produced significant inhibition in edema thickness, produced by carrageenan when compared with the non-treated group. This inhibition in edema is statistically significant at $P < 0.05$. Fig 10 and 11 showed the percent edema inhibition by topical application of standard group I (treated with F15), test 1 group (treated with F3), test 2 group (treated with F6), standard group II (treated with F16), test 3 group (treated with F10) and test 4 group (treated with F13). It was observed that the standard group I and standard group II produced maximum percent edema inhibition after 4 hrs (45.37% and 47.65%) and then the effect was reduced gradually with time up to 24 hrs, while test 1, test 2, test 3 and test 4 groups enhanced drug permeation and produced maximum percent edema inhibition after 8 hrs (65.18%, 68.88%, 72.44% and 75.4%), respectively, and continued for 24 hrs. This indicates that the prepared formulations exhibited a better efficacy than the standard preparation and this may be due to enhanced drug delivery from lipid vesicles.

Table 1. Formulations of Fluocinonide gel and their niosomal preparations

Formulation	Span 40 Molar Ratio	Tween 40 Molar Ratio	Cholesterol Molar Ratio	Carbopol 934 (w/w)	CMC Na (w/w)
F1	50	-	50		
F2	25	25	50		
F3	50	-	50	1 %	-
F4	50	-	50	1.5 %	-
F5	50	-	50	2 %	-
F6	25	25	50	1 %	
F7	25	25	50	1.5 %	
F8	25	25	50	2 %	
F9	50	-	50		2 %
F10	50	-	50		3%
F11	50	-	50		4 %
F12	25	25	50		2 %
F13	25	25	50		3%
F14	25	25	50		4 %
F15	-	-	-	1%	-
F16	-	-	-	-	3%

Table 2. Permeation Parameters of Fluocinonide From F3 - F16 Through Cellulose Membrane

Formulations	(J_{ss}) $(\mu g.cm^{-2}.hr^{-1}) \pm$ SD	(t_L) (hr) ± SD	(K_p) $(cm.hr^{-1}.10^{-3}) \pm$ SD	(D) $(cm^2.hr^{-1}.10^{-5}) \pm$ SD	Enhance m-nt ratio
F3	1.85±0.09	3.13±0.56	0.037±0.34	0.00052±0.91	1.22
F6	2.335±0.054	3.54±0.44	0.046±0.39	0.00046±0.32	1.54
F10	2.126±0.076	5.176±0.84	0.042±0.76	0.000315±0.92	1.013
F13	2.263±0.08	6.75±0.67	0.045±0.68	0.00019±0.76	1.08
F15	1.516±0.15	0.905±0.76	0.03±0.64	0.0018±0.45	1
F16	2.098±0.13	0.917±0.89	0.041±0.23	0.0017±0.76	1

Table 3. Permeation Parameters of Fluocinonide From Niosomal Gels and Control Drug Gel, Through Rabbit' Skin

Formulations	(J_{ss}) $(\mu g.cm^{-2}.hr^{-1}) \pm$ SD	(t_L) (hr) ± SD	(K_p) $(cm.hr^{-1}.10^{-3}) \pm$ SD	(D) $(cm^2.hr^{-1}.10^{-5}) \pm$ SD	Enhance m-nt ratio
F3	1.85±0.09	3.13±0.56	0.037±0.34	0.00052±0.91	1.22
F6	2.335±0.054	3.54±0.44	0.046±0.39	0.00046±0.32	1.54
F10	2.126±0.076	5.176±0.84	0.042±0.76	0.000315±0.92	1.013
F13	2.263±0.08	6.75±0.67	0.045±0.68	0.00019±0.76	1.08
F15	1.516±0.15	0.905±0.76	0.03±0.64	0.0018±0.45	1
F16	2.098±0.13	0.917±0.89	0.041±0.23	0.0017±0.76	1

Table 4. Stability Studies of F1, F2, F3, F6, F10 and F13 at Different Storage Temperature Conditions

Formulation	Temperature	Percent drug retained after the following time (days) intervals						
		0	7	14	21	30	45	60
F1	4℃	100	95.36	93.57	92.34	90.66	88.23	86.67
	25℃	100	90.85	89.23	88.32	85.74	82.48	77.65
	37℃	100	85.75	83.74	77.87	72.86	65.26	60.27
F2	4℃	100	97.56	96.87	95.98	92.56	91.76	89.53
	25℃	100	94.65	93.65	90.76	88.54	85.78	79.75
	37℃	100	88.65	85.76	79.34	75.76	69.86	62.87
F3	4℃	100	98.65	97.28	96.45	95.56	94.87	93.76
	25℃	100	95.87	95.23	94.23	92.87	91.23	88.87
	37℃	100	90.43	89.87	86.34	84.56	80.87	76.5
F6	4℃	100	99.7	99.2	98.65	98.32	97.54	96.81
	25℃	100	96.53	96.04	95.5	94.5	92.34	90.34
	37℃	100	92.54	90.53	88.45	85.34	82.56	79.52
F10	4℃	100	98.85	97.87	97.21	96.32	95.76	94.34
	25℃	100	96.74	95.72	93.78	91.52	89.45	87.56
	37℃	100	91.22	90.58	87.45	85.83	83.88	78.54
F13	4℃	100	99.8	99.4	98.88	98.67	98.22	97.6
	25℃	100	97.54	97.2	95.87	95.12	93.65	92.7
	37℃	100	94.32	92.78	91.76	89.43	87.54	81.76

Fig. 1. Effect of concentration of Carbopol 934 on the permeation of Fluocinonide from F3, F4 and F5 through cellulose membrane

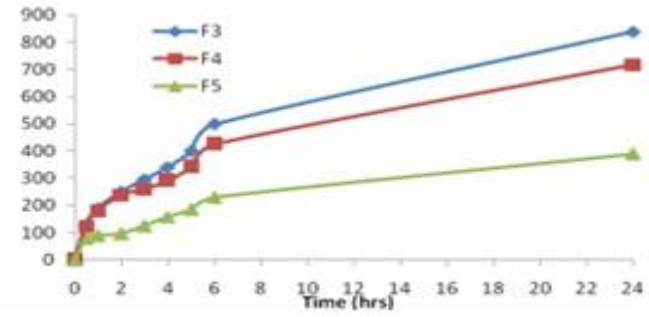


Fig 2. Effect of concentration of Carbopol 934 on the permeation of Fluocinonide from F6, F7 and F8 through cellulose membrane

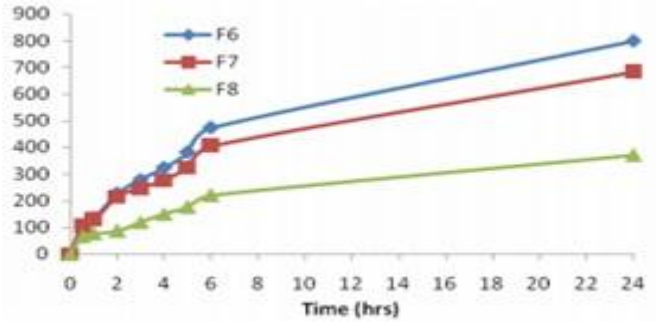


Fig 3. Effect of concentration of Carbopol 934 on the permeation of Fluocinonide from F3, F6 and F15 through cellulose membrane

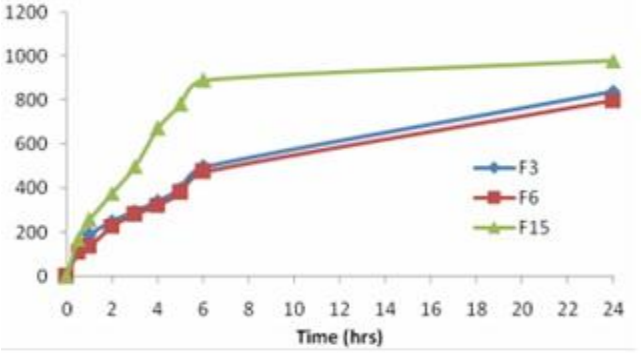


Fig 4. Effect of concentration of CMC Na on the permeation of Fluocinonide from F9, F10 and F11 through cellulose membrane

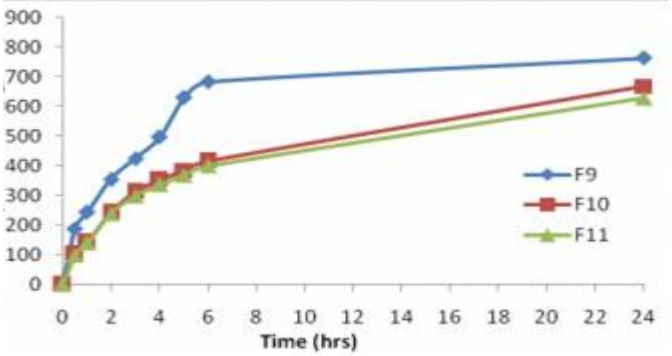


Fig 5. Effect of concentration of CMC Na on the permeation of Fluocinonide from F12, F13 and F14 through cellulose membrane

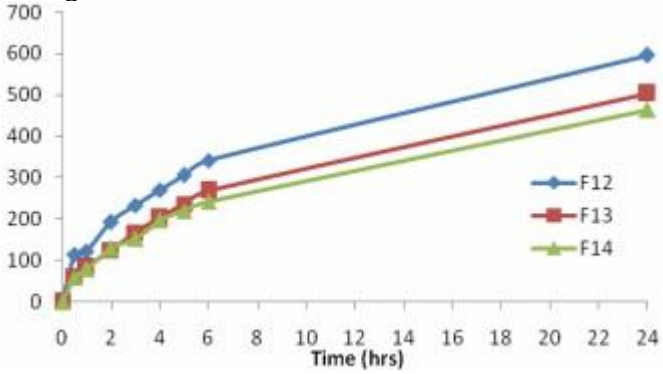


Fig 6. Permeation results of Fluocinonide from F10, F13 and F16 through cellulose membrane

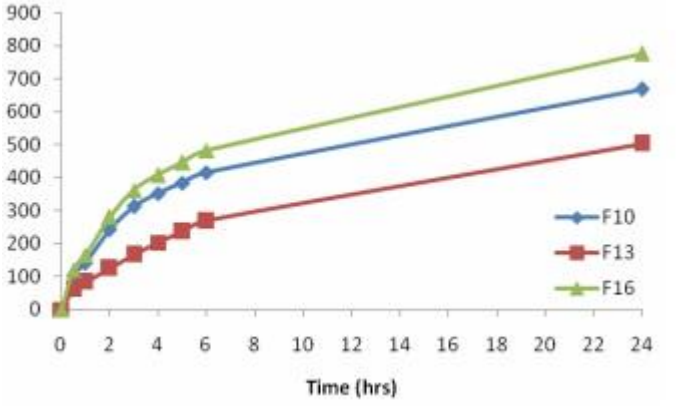


Fig 7. Permeation data of Fluocinonide from F3, F6 and F15 through rabbit skin

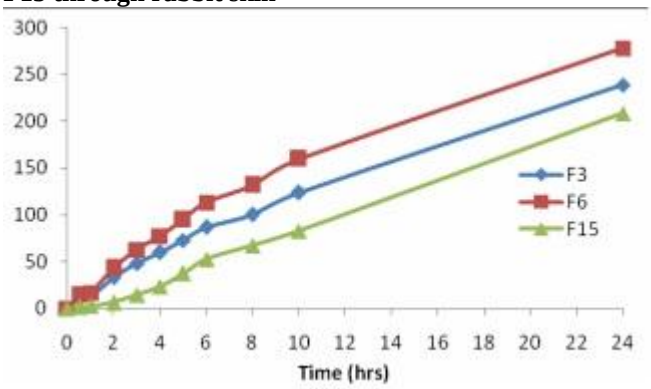


Fig 8. Permeation data of Fluocinonide from F10, F13and F16 through rabbit skin

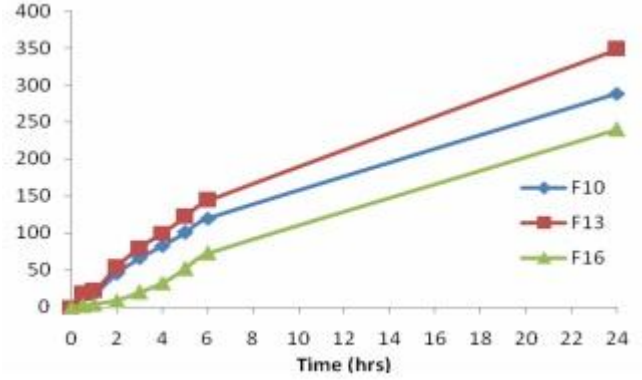


Fig 9. The amount of drug permeated across cellulose membrane and rabbit skin from F3, F6, F10, F13, F15 and F16 after 6 hours

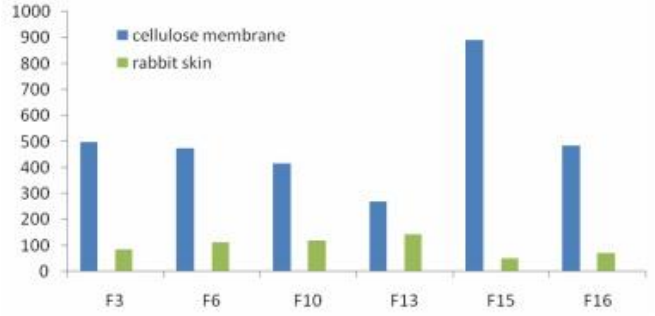


Fig 10. Percentage edema inhibition by topical application of different Fluocinonide formulations (Carbopol 934 gel)

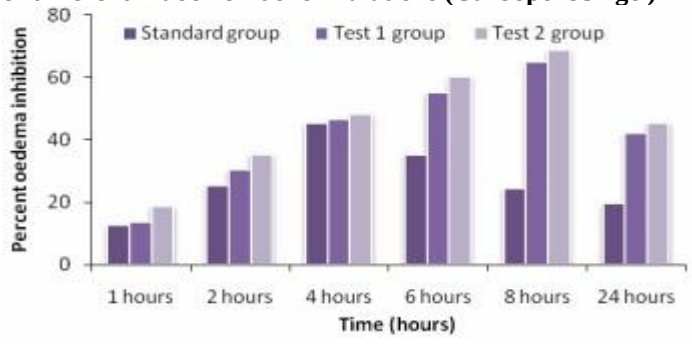
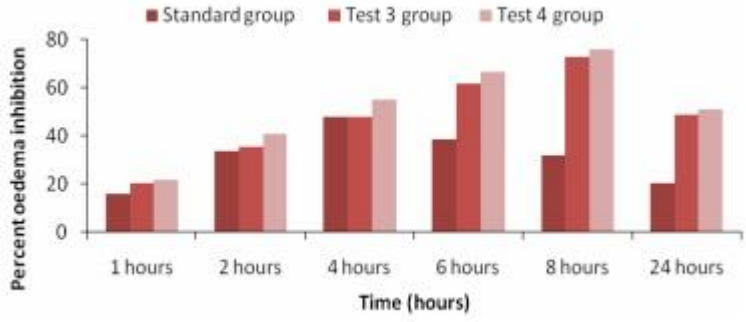


Fig 11. Percentage edema inhibition by topical application of different Fluocinonide formulations (CMC Na gel)



CONCLUSION

Incorporation of Fluocinonide–entrapped niosomes into gelling agents and increasing their concentrations resulted in a marked decrease in amount of drug permeated from the gel through cellulose membrane. While, incorporation of Fluocinonide – entrapped niosomes from gelling agents resulted in increase in permeated drug from gels through the dorsal rabbit skin compared to control drug gel. Niosomal gel of 3% CMC Na is the most stable formula. All tested Fluocinonide gel formulations produced a marked anti-inflammatory activity. Although the inhibition in thickness of rat paw edema in all niosomal gels of Fluocinonide is significantly different at $P < 0.05$ from carrageenan treated group (control) and from the standard group but the percent edema inhibition in test 4 group is more pronouncing than the other groups, thus niosomal gel (F13), containing Span 40: Tween 40: Cholesterol, 25: 25: 50, and 3% CMC Na is a promising formula for an anti-inflammatory topical application.

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