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Formulation and Evaluation of Topical Gel Containing Azithromycin and Prednisolone Vesicles for Treating Psoriasis

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Subject: Pharmaceutics

Abstract
Psoriasis is a chronic, autoimmune systemic inflammatory disease, associated with metabolic syndrome, cerebrovascular disease, diabetes and many other diseases. There is various type of psoriasis but most common type of psoriasis is caused by Psoriasis vulgaris. It is characterized by rigid of skin due to increase in the level of cholesterol and fall in the level of ceramide. Apart from that it is associated with an immune system of the body means movement of immune cells from dermis to the epidermis, where they stimulate skin cells (keratinocytes) to proliferate. Various type of drug delivery system are used for the treatment of psoriasis including topical, oral or systemic but gels prepration of azithromycin and prednisolone are more effective in reduction of purities, scaling and hyperkeratosis of psoriasis plaque. Due to high entrapment efficiency and stability, gel preprations (Azithromycin & Prednisolone) reduce the scaly patches and suppression of humoral immunity.

Keywords: *Psoriasis, Drug delivery, Gel, Immunity*

Introduction
Psoriasis is recognized as a complex, chronic skin condition that can have a significant impact on patient's physical and mental health. It occurs when the immune system sends out faulty signals that speed up the growth cycle of skin. It is characterized by rigid of skin due to increase in the level of cholesterol and fall in the level of ceramide (Surver C et al 2002). The main causes of the disease is not understand till now date but researchers correlate this disease with an immune system of the body means movement of immune cells from dermis to the epidermis, where they stimulate skin cells (keratinocytes) to proliferate (Franlk et al; 2009) and trigger the release of cytokinin (tumour necrosis factor- alpha TNF-α) which causes inflammation and the rapid production of skin cells (Asadullah et al; 1999). For the correction of psoriasis, a number of non drugs or salt are used like lithium, β-blockers and antimalarial (cholroquinine) drugs (James et al; 2005). Various Drug delivery systems are used for the treatment of psoriasis such as oral (Willms et al; 2010), systemic (Bremmer et al; 2010) & topically includes creams (Mesa et al; 2010), ointment (Gold et al; 2009), shampoo (Kircik et al;2010) & gel (Iraji et al; 2010). On the basis of effectivity in reduction of purities, scaling and hyperkeratosis of psoriasis plaques we select gels. Niosomes are composed of bilayer non-ionic surface active agents (Patel et al; 2007) consist of cholesterol and non ionic surfactant (Arora et al; 2007). They can be prepared by various types of methods like Ether injection method, Hand shaking method (thin film hydration techniques), Sonication, Microfluidization, Multiple membarane extrusion method ,Reverse phase evaporation technique (REV),

Trans membrane ^{PH} gradient (inside acidic) drug uptake process, The bubble method, Formation of niosomes from proniosomes. Gel containing prednisolone & azithromycin are used for the treatment of psoriasis. As azithromycin as an antibiotic act against bacterial action which is one of the cause for the occurance for psoriasis & is also having keratolytic action to remove the scales of diseased skin. Prednisolone inhibit leukocyte infiltration at the site of inflammation, interfere in the function of mediators of inflammatory response, leads to suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of corticosteroids are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes. Niosomal gel provide the rapid penetration of drug through skin, restrict the action to target cell & act as a promising vehicle for the drug delivery by improving therapeutic performance of drug molecule, provide protection to drug from biological environment. Gel contain niosomes of

prednisolone & azithromycin as sustained release of drugs are required. As half- life of azithromycin is too high of 68 hours to give a sustain release so there is no need to incorporate it in niosomes, hence directly incorporated in gel & prednisolone with short half life of 1 hour need to incorporate in noisome.

Materials and Method

Material

Chemical: Chloroform, Diethyl ether, Glycerin, Hydrochloric acid, Pottasium di hydrogen phosphate, sodium hydroxide, potassium chloride were purchased from Loba Chemic Pvt. Ltd., Central Drug House Pvt. Ltd.,

Surfactants: Cholesterol, Span 60, Span 40, Triton X 100 were purchased from S.D Fine Chem Ltd

Niosomes were prepared using ether injection method (Karki et al; 2008) by slowly introducing a solution of cholesterol and surfactant dissolved in diethyl ether into a warm phosphate buffer maintained at 60^0C. The surfactant mixture in ether was injected through guage needle 14 into an aqueous solution of drug. Vaporization of ether leads to formation of vesicles. Formulae for various niosomal formulations on the basis of varying concentration of surfactant and cholesterol are shown in table1

Evaluation of Niosomes

Microscopy

Optical microscopy **:**The microscopy of prepared niosomes was done by optical microscope. The niosomal suspension was placed over glass slide & observed for the formation of niosomes. The photomicrographs of the preparation were obtained with the help of photomicroscope (Kyowa Gatner 10390). TEM (Transmission electron microscophy):Transmission electron microscopy was done to examine the size & morphology of prepared niosomes .A drop of dispersion was stratified into a carbon coated copper grid & left to adhere on the carbon substrate for about 1 minute. The dispersion in excess was removed by a piece of filter paper. The sample was air dried and observed under Hitachi transmission electron microscope at a voltage of 80 Kv (Muzzalupo et al; 2008).

Drug entrapment efficiency of niosomes

Entrapment efficiency of niosomes was determined by ultracentrifugation method. The dispersion was each ultracentrifuged at 12,000 rpm for 1 hr at 0^0 C. The supernatant was removed and formed niosomal pellet were resuspended in phosphate buffer solution pH (6.5) to ensure complete removal of all free Prednisolone. The supernatant (free Prednisolone) was collected and measured spectrometrically at λ_{max} 245 using PBS as blank. The concentration of entrapped drug was determined spectrophotometrically after lysis of niosomal pellet with alcohol at 245 nm. Diethyl ethercontaining surfactant & cholesterol in the same ratio is used as blank in niosomal formulation. The entrapped concentration was expressed as % age entrapment efficiency which can be defined as the % age fraction of the total input drug encapsulated in the surfactant bilayer & aqueous compartment in the niosomes. It was calculated using following formulae

% Entrapped efficiency

$$= \frac{\text{Entrapped Prednisolone}}{\text{Total drug (supernatant + sediment)}} \times 100$$

In vitro release study

The study was done by using membrane diffusion technique. In this 10 mg of niosomal formulation was placed in glass tube having diameter of 2.5 cm with an effective length of 8 cm that was previously covered with soaked osmosis cellulose membrane, which act as donar compartment. The glass tube was placed in a beaker containing 100 ml of phosphate buffer saline (pH 6.5). The receptor compartment containing suspension was just touching the surface of diffusion medium. The temperature of medium was maintained at 37 ± 0.5^0 C and agitated at 100 rpm speed using magnetic stirrer. Aliquots of 5 ml of was withdrawn at different interval of time 0.5,1,2,3 up to 24 hours & same volume of medium was replaced with fresh medium. The collected samples were analyzed at 245 nm in double beam UV-Visible spectrometer using phosphate buffer (pH 6.5) as blank.

Analysis of release mechanism

To ascertain the drug release mechanism, release rate of the niosomal formulations, data obtained from release studies of various niosomal formulation was fitted into various release models. The model selected were Zero order, First order, Higuchi, Korsemayer peppas model (Attia et al; 2007). The standard data for the interpretation of diffusional release mechanism is shown in table 2

Zeta potential analysis

Zeta potential of niosomal preparation is related to the stability of niosomes. Zeta potential indicate the degree of repulsion between adjacent similarly charged particles for small molecules. High value of zeta potential confirms stability i.e the solution or dispersion will resist aggregation. Zeta potential for niosomal formulation was performed using zeta

sizer. (Beckman coulter instrument) (Muuzzalupo *et al.*,2008).

Formulation of gel

Niosomal formulations exhibiting maximum in vitro release rate and high entrapment efficiency was selected for the formulation of gel containing different concentration of carbopol. AZI was added directly into gel base. Ingredients along with their quantities used in formulation of gel are shown in table 4

Table1. Different formulation of noisome with varying concentration of surfactant

Formulation	Surfactant (Span 60mg)	Cholesterol (mg)	Drug (mg)	Formulation	Surfactant (Span 60mg)	Cholesterol	Drug (mg)
F1	10	5	25	F6	17	15	25
F2	12	7	25	F7	20	17	25
F3	12	12	25	F8	20	20	25
F4	15	12	25	F9	25	25	25
F5	15	15	25	10	25	25	25

Table2. Interpretation of diffusional release mechanism

Release exponent	Drug transport mechanism	Rate as a function of time
n=0	Fickian diffusion	$t^{0.5}$
0.5>n>1	Anomalous transport	t^{n-1}
n>1	Case 2^{nd} transport	Zero order release t^{n-1}

Table3. Zeta potential value & corresponding stability

Zeta- potential	Stability behavior
0 to ± 5	Rapid coagulation
±10 to ± 30	Incipient stability
±30 to ± 40	Moderate stability
±40 to ±60	Good stability
±61	Excellent stability

Table 4: Ingredients & quantities used in formulation of carbopol gel

Ingredients	Quantity(F1) (50 gm)	Quantity(F2) (50 gm)	Quantity(F3) (50 gm)
Carbopol 934	500mg	550mg	450mg
Triethanolamine	5 ml	5ml	5ml
Propylene glycol	15ml	15ml	15ml
Glycerin	0.845 ml	0.845ml	0.845ml
Prednisolone (niosomal pellet)	25mg	25mg	25mg
Azithromycin	100 mg	100mg	100mg
Methyl paraben	25 mg	25mg	25mg
Propyl paraben	250 mg	250mg	250 mg
Distilled water	Make up to 50 ml	Make up to 50 ml	Make up to 50 ml

Parabens were dissolved in 40 ml of water with the aid of heat and allowed to cool. Carbopol 934 was added in small amount to the solution using high speed mixer until a smooth dispersion was obtained. To the dispersion glycerin, propylene glycol and known amount of drugs (PRE niosomal pellet, AZI) were added. Then neutralizing agent triethanolamine was added very slowly to avoid entrapped air. Finally, remaining water was added along with continous trituration to make a transparent gel.

Evaluation of Gel

Physiochemical evaluation of gel

Homogenity: All developed gel was tested for homogeneity by visual inspection after the gel has been set in the container. They were tested for their appearance and presence of aggregate.

Grittness: All the formulation was evaluated microscopically for the presence of particles if any.

Drug content studies

1.0 gm of each gel formulation were taken in 100 ml of volumetric flask containing 20 ml of phosphate buffer saline (pH 6.5) and stirred for 30 minute. The volume was made upto 100 ml & 1ml of the above solution was further diluted to 50 ml with phosphate buffer saline (pH 6.5). The resultant solution was filtered through membrane filter. The absorbance was recorded by using UV spectrophotometer at respective absorption maxima of AZI & PRE i.e 298 nm & 245 respectively. Drug content was determined from the calibration curve.

pH determination

2.5 gm of gel was accurately weighed & dispersed in 25 ml of purified water. The pH of the dispersion was measured using pH meter.

Spreadibility

For the determination of spreadibility excess of sample was applied in between 2 glass slide & was compressed to uniform thickness by placing 1000 gm wt for 5 minute. Weight 50 gm was added to pan (Aejeaz et al; 2010). Time required toseparate the two slides i.e the time in which the upper glass slide move over the lower plate was taken as measure of spreadibility.

$S = (m \times l)/t$

S= Spreadibility

m = Weight tied to upper slide.

l = Length moved on upper glass slide

t = Time taken

Viscosity measurement

Viscosity of prepared formulation was determined using Brook field viscometer with spindle no 64 at 10 -50 rpm at temperature 37 ± 0.5^0C (Martinez et al; 2007). Spindle was lowered perpendicularly into gel placed in a beaker taking care that the spindle does not touch the bottom of beaker . The spindle was rotated at different speed & reading were recorded after 30sec when the gel level stabilized.

In vitro release permeation study of prepared gel

In vitro release studies was carried out using franz diffusion cell with receptor compartment volume of 25 ml & an effective area of 2.54 cm^2. Receptor solution composed of distilled water.

Phosphate buffer saline (pH 6.5) was added to the cell & temperature was maintained at 37 ± 0.5^0 C and solution was stirred continuously at 600 rpm(Bhatia et al; 2004). Wister rat skin was mounted on the receptor compartment with stratum corneum slide facing donar compartment. The donar compartment was filled with 200 mg of prepared gel. At appropriate time intervals of 0.5, 1, 2, 3 & upto 24 hrs,5 ml of sample were withdrawn & immediately replaced by equal amount of receptor solution. The sample were analysed by UV spectrophotometer at respective absorbance maxima of 298 nm & 245 nm.

Release kinetic studies of gel

In order to study the exact mechanism of drug release from the gels, drug release data was analyzed according to zero, first order, Higuchi square root and Korsemeyer- Peppas equations. Most appropriate model was chosen on the basis of goodness of fit test (Schmolka et l; 1972).

In vitro skin retenation study

The amount of drug retained in the skin was determined by using skin samples employed in permeation studies. After completion of permeation experiment, skin mounted on the diffusion cell was removed. The skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove the adhering formuation. Then, the skin sample was homogenized with 20 ml of chlorofoam:methanol (2:1 v/v) for the extraction of rug **(**Bhatia et al; 2004). The solution thus obtained was filtered and absorbance was measured using UV spectrophotometer at respective absorption maxima of AZI (298nm) & PRE (245nm).

Stability studies of gel

The prepared gel formulation were packed in air tight container at three different temperature conditions i.e refrigeration temperature ($4\text{-}8^0$C), room temperature (25 ± 2^0C) & oven (45 ± 2^0C). The sample were

withdrawn at different time interval over a period of one month & evaluated for physical appearance & drug content. (Gupta *et al;*2007, Amriutiya *et al*;2009).

Results:

Preparation and evaluation of niosome

Prednisolone loaded niosomes were prepared by ether injection method with varying concentration of surfactant & cholestrol. Niosome prepared were spherical in shape & found to be unilamillar (single layer vesicle) as observed under optical microscope (Fig 1)

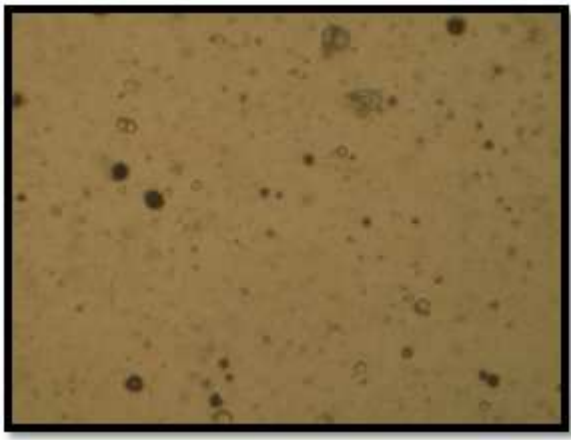
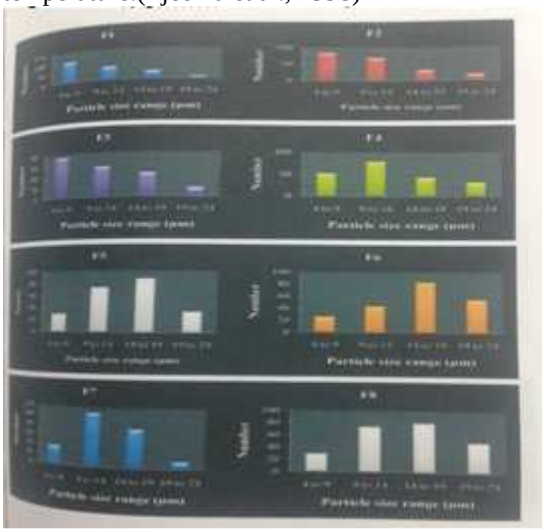


Fig 1: Optical micrographs of niosomes

Histograms (Fig 2) were plotted between particle size distribution and no. of particles, representing the vesicle size of niosome in different formulation which were found to be in the range of 5-30µm, larger deviation in vesicle size can be explained on the basis of varying injection, speed of surfactant, cholestrol solution, height of injection due to aggregation of some vesicle & variation in temperature.(Ijeoma et al., 1998)



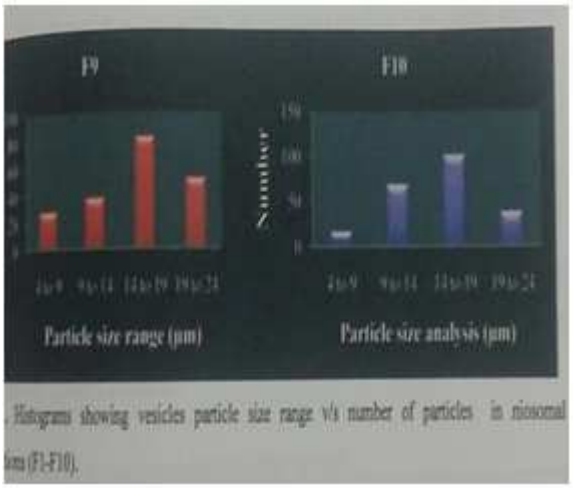


Fig 2: Histogram showing vesicles particle size range v/s number of particles.

Transmission electron microscopy of formulation F3 and F8 (fig 3) concluded that niosomes were discrete and spherical in shape (Mouzametal et al., 2011) with unilamillar structure

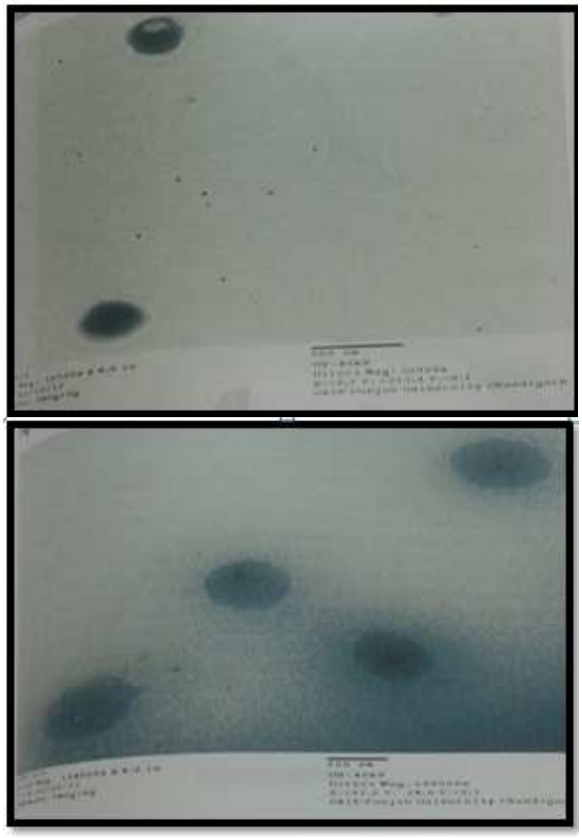


Fig 3: TEM of formulation F3 & F8 Entrapment Efficiency

Table 5: Entrapment efficiency of formulations

(F1-F10) Entrapment efficiency of different formulation (F1-,F10) were found to be 32%, 35%, 38%, 47%, 51%, 58%, 62%, 66.5%, 64%, 63.26% respectively.

Formulation	Surfactant quantity (mg)	Cholestrol (mg)	% Entrapment efficiency*
F1	10	5	32±1.324
F2	12	7	35±0.439
F3	12	12	38±0.180
F4	15	12	47±0.275
F5	17	15	51±0.362
F6	20	15	58±0.586
F7	20	17	62±0.975
F8	34	20	66.5±0.134
F9	50	25	64±0.694
F10	75	25	63.26±0.965

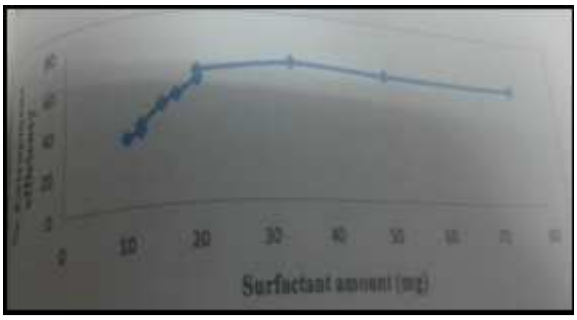


Fig 4: Entrapment efficiency v/s surfactant

From data it was concluded from fig 4 that with increase in amount of cholesterol & surfactant the entrapment efficiency goes on increasing up to surfactant & cholesterol ratio of 2:1 (F8) and again decrease with increase (F9 & F10).(Rangasamy et al .,2008).Decrease in amount of surfactant may be due to high viscous systems produced due to high concentration of surfactant which affect niosome structure (Ijeoma et al., 1998).

***In Vitro* Release**

Table 6: Cumulative %age release of formulation (F8-F10)

Time (hr)	% Cumulative release of F8	% Cumulative release of F9	% Cumulative release of F10
0	0	0	0
0.5	10.082±0.043	6.145±0.491	4.69±0.956
1	14.79±0.064	7.97±0.532	10.42±0.487
2	25.28±0.654	10.141±0.876	16.9±0.657
4	39.62±0.865	14.97±0.563	22.3±0.693
6	45.05±0.876	17.94±0.435	28.05±0.547
8	51.07±0.564	22.2±0.638	31.56±0.386
10	53.69±0.543	24.6±0.345	33.13±0.548
12	59.28±0.432	24.8±0.456	40.189±0.657
14	64.84±0.432	26.27±0.567	46.73±0.874
16	73.35±0.431	20.98±0.465	59.9±0.765
18	70.76±765	20.75±0.765	67.63±0.564
20	67.95±0.576	19.98±0.654	61.58±0.362
22	66.96±0.674	19.87±0.764	59.95±0.673
24	66.28±0.896	18.64±0.325	57.85±0.964

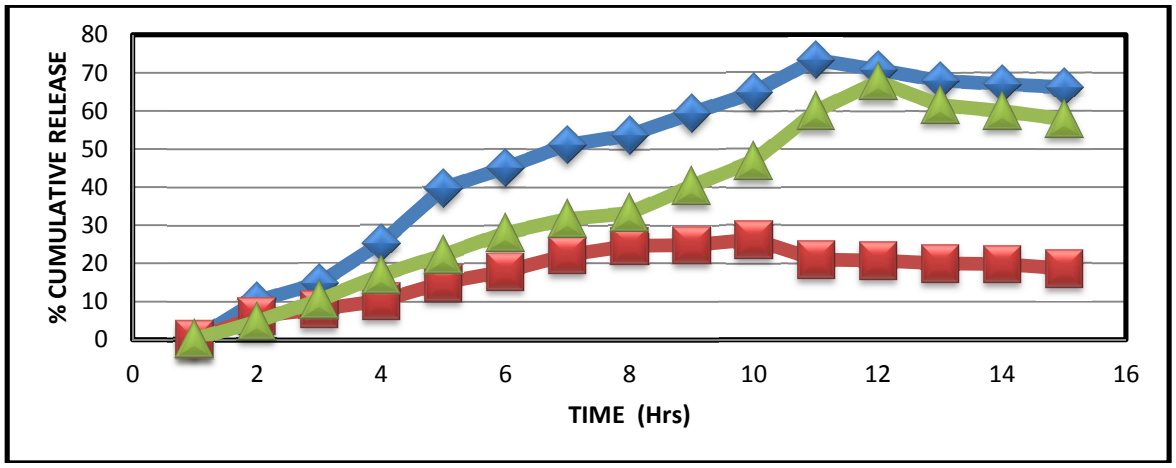


Fig 5: % Age cumulative release of formulation (F8-F10)

The result showed the consistent *in vitro* release in formulation F8 , then in formulation F9 and F10 due to presence of non-ionic surfactants in optimized concentration, which act as permeation enhancer (Arora et al., 2007) or due to small size of vesicles which enhances the effective surface area for penetration of drug via skin and due to greater concentration of prednisolone drug in formulation F8 vesicle in comparison to formulation F9 & F10 (Agarwal et al., 2001)

Analysis release mechanism

To ascertain drug release mechanism and release rate , *in vitro* of prepared formulation were fitted for various release models. The models selected were zero order, first order, Higuchi, Korsemeyer-Peppas model. All formulation are best fitted for Korsemeyer- Peppas equation. All formulation revealed non-fickian diffusion because slope of all formulations Korsemeyer- Peppas equation is near one.

Table 7: Data for analysis of release mechanism

%CPR (F8)	Log CPR(F8)	%CPR (F9)	Log CPR(F9)	%CPR (F10)	Log CPR (F10	T	√T	LogT
10.082±0.043	1.003	6.145 ± 0.491	0.788	4.69±0.956	0.671	0.5	0.707	-0.301
14.79 ± 0.064	1.169	7.97 ± 0.532	0.901	10.42±0.487	1.017	1	1	0
25.28 ± 0.654	1.402	10.141 ± 0.876	1.006	16.9±0.657	1.227	2	1.414	0.301
39.62 ± 0.865	1.597	14.97 ± 0.563	1.175	22.3±0.693	1.348	4	2.000	0.602
45.05 ± 0.876	1.653	17.94 ± 0.435	1.253	28.05±0.547	1.447	6	2.449	0.778
51.07 ± 0.564	1.708	22.2 ± 0.638	1.346	31.56±0.386	1.499	8	2.828	0.903
53.69 ± 0.543	1.729	24.6 ± 0.345	1.3909	33.13±0.548	1.552	10	3.162	1
59.28 ± 0.432	1.772	24.8 ± 0.456	1.394	40.189±0.657	1.604	12	3.464	1.079
64.84 ± 0.432	1.811	26.27 ± 0.567	1.419	46.73±0.874	1.669	14	3.741	1.146
73.35 ± 0.431	1.865	20.98 ±0.465	1.321	59.9±0.765	1.777	16	4	1.204
70.76 ± 0.765	1.849	20.75±0.765	1.317	67.63±0.564	1.83	18	4.242.	1.255
67.95 ± 0.576	1.832	19.98±0.654	1.300	61.58±0.362	1.789	20	4.472	1.301
66.96 ± 0.674	1.825	19.87±0.764	1.298	59.95±0.673	1.777	22	4.690	1.342
66.28 ± 0.896	1.821	18.64±0.325	1.270	57.85±0.964	1.762	24	4.898	1.380

Table 8: Kinetic release data of different model for formulations

Model	Slope(F8)	R^2 value (F8)	Slope (F9)	R^2 value (F9)	Slope (F10)	R^2 value (F10)
Zero order	16.16	0.938	1.261	-0.25	3.128	0.853
First order	0.634	0.463	0.027	0.385	0.049	0.637
Higuchi	19.76	0.923	5.531	0.577	12.86	0.937
Korsemeyer Peppas	0.503	0.964	0.369	0.812	39.06	0.847

From the kinetic release data (Table) it was concluded that formulation F8 follow korsemeyer peppas model & indicate anomalous diffusion mechanism diffusion coupled with erosion. Formulation F9 follow korsemeyer peppas model & indicate fickian diffusion mechanism. Formulation F9 follow korsemeyer peppas model & indicate fickian diffusion mechanism. . Formulation F10 follow Higuchi model & indicate diffusion controlled mechanism.

Zeta potential analysis

The zeta potential of niosomal suspension recorded by Beckman coulter delsa of formulation F8 was found to be 25.90mV. as shown in fig indicate thate suspension is stable, devoid of agglomeration and evenly distributed.

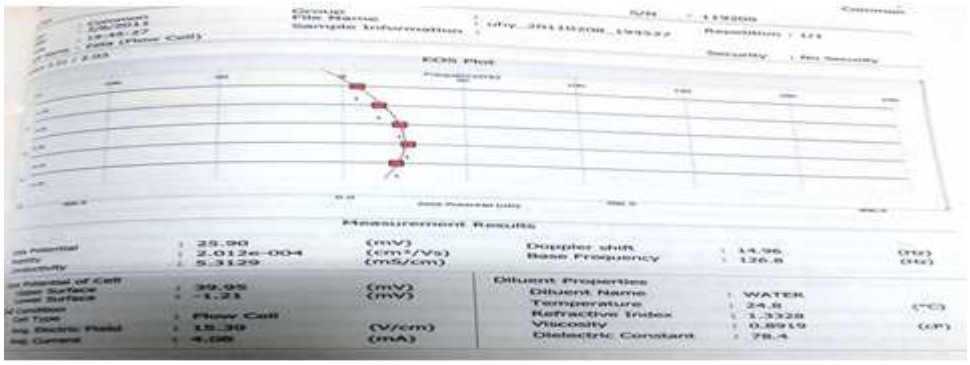


Fig 6: Zeta Potential of formulation F8

Formulation & evaluation of niosomal gel

Formulation F8 of niosome loaded with prednisolone having maximum in vitro release rate and highest entrapment efficiency was selected in combination with simple base azithromycin to formulate niosomal gel. The gel was formulated using carbopol and evaluated for following parameters

Physicochemical evaluation

The various physicochemical parameter (Table: 9) like grittiness, homogeneity was checked by observing formulation under microscope .

Table: 9 physicochemical properties of formulation (F1-F3)

Formulation	Homogeneity	Grittiness	%Drug Content (azithromycin)	%Drug Content (Prednisolone)	pH	Spreadibility (g.cm/s)
F1	+++	-	87.5±0.54	90±0.67	6.7±0.65	15.85±1.68
F2	++	-	92.4±0.65	90.5±0.98	7.1±0.51	14.65±2.42
F3	+++	-	89.7±0.43	88.9±0.46	7.3±0.32	11.25±1.56

Excellent +++, Good++, Satisfactory +, No grittiness-

Percent drug content in all three formulation was in range 87.5-92.5%, indicating homogeneity. pH of all formulations was found neutral (6.7-7.3). The spreadibility was found to be in the range of 11.25-15.61g cm/s. (Table:10) depicts the viscosity measurement at different angular velocity.

Table 10: Viscosity at different angular velocity

Angular velocity (Spindle 60)	Viscosity (cps)		
	F1	F2	F3
10 rpm	7800±7.63	8200±17.55	8600±10.40
20 rpm	6600±7.64	7700±20.20	7850±10.48
30 rpm	6170±9.78	7480±11.54	7650±5.46
40 rpm	6402±3.21	7210±7.61	7360±6.54
50 rpm	6180±8.95	6900±9.57	7125±10.57

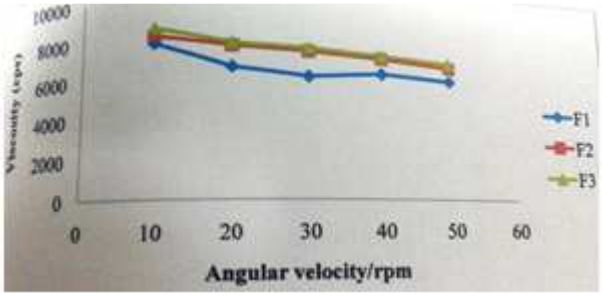


Fig: 7 Velocity at different angular velocity

Study depicts that formulation exhibited pseudoplastic rheology, as evidenced by shear thinning on increase in shear stress with increased angular velocity.The viscosities of formulations were in following orders F1<F2<F3. The viscosity increased with increasing concentration of sodium alginate and carbopol. F3 showed the maximum viscosity, this is due to change in polymeric concentration.

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***In vitro* permeation study**

Data on comparative *in vitro* permeation studies of prepared formulations at different time interval (Table:11) and amount of azithromycin & prednisolone were determined

Table 11: Cumulative % release of different formulation (F1-F3)

Time (hr)	%Cumulative drug release of F1		%Cumulative drug release of F2		%Cumulative drug release of F3	
	Azithromycin	Prednisolone	Azithromycin	Prednisolone	Azithromycin	Prednisolone
0	0	0	0	0	0	0
0.5	2.16±0.098	8.09±0.031	1.81±0.054	5.32±0.078	0.96±0.006	3.36±0.321
1	5.68±0.876	12.07±0.098	3.46±0.081	7.76±0.465	2.04±0.076	7.24±0.543
2	9.98±0.765	21.03±0.081	7.48±0.765	9.03±0.654	4.18±0.065	12.96±0.643
4	12.08±0.534	33.98±0.079	9.84±0.654	11.79±0.764	6.82±0.041	19.30±0.865
6	16.84±0.532	41.07±0.312	13.52±0.043	15.49±0.864	7.96±0.076	23.28±0.986
8	19.43±0.876	47.09±0.217	17.39±0.321	19.97±0.564	11.01±0.065	28.56±0.061
10	23.31±0.007	50.03±0.538	19.28±0.876	23.74±0.623	15.84±0.067	30.38±0.650
12	28.08±0.786	54.82±0.243	21.04±0.985	25.72±0.543	18.09±0.085	37.89±0.976
14	33.07±0.795	61.29±0.421	24.08±0.865	22.89±0.326	22.04±0.086	43.63±0.065
16	37.04±0.841	67.53±0.058	29.48±0.041	21.93±0.541	25.98±0.016	40.34±0.341
18	48.09±0.684	65.32±0.216	32.19±0.086	21.07±0.0.654	28.84±0.032	62.76±0.768
20	54.91±0.086	63.95±0.076	48.28±0.076	19.98±0.754	31.42±0.065	60.85±0.658
22	61.42±0.976	62.46±0.036	53.04±0.041	20.36±0.548	34.67±0.075	58.67±0.885
24	64.06±0.865	61.96±0.069	58.98±0.065	20.28±0.659	38.48±0.043	55.58±0.662

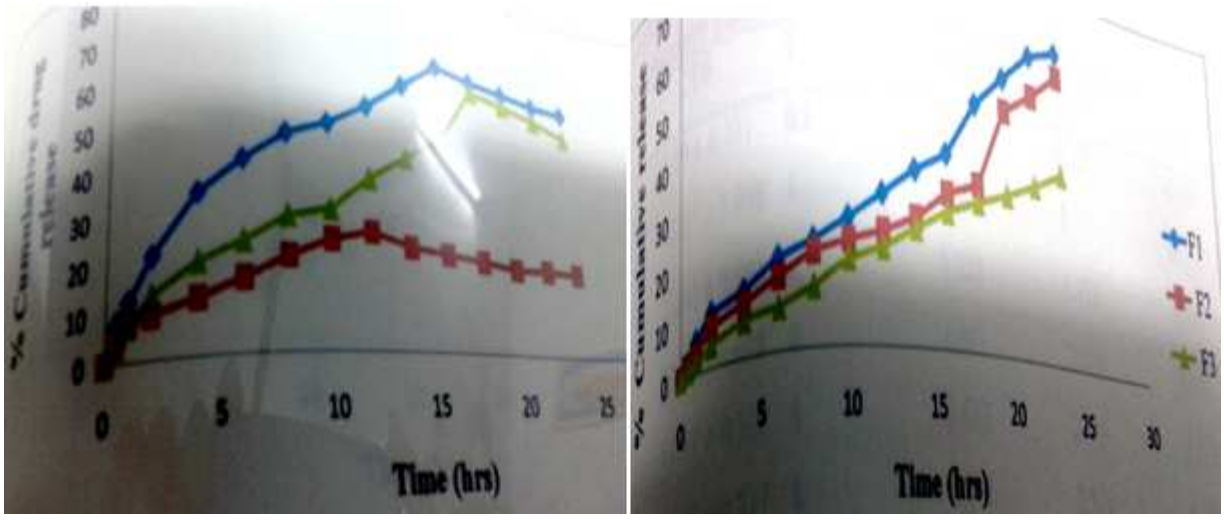

Fig8: *In vitro* release of (a) prednisolone **In vitro release of (b) azithromycin**

Release Kinetic studies of niosomal gel

The release mechanism of prednisolone in niosomal gel was diffusion controlled and was influenced by polymer added. It was verified with different kinetic model (Table 12) All three formulation follows korsmeyer's plot which indicate that there is anomalous diffusion or diffusion coupled with erosion.

Table 12: Release kinetics of azithromycin from gel

Model	Slope(F1)	R^2 value (F1)	Slope (F2)	R^2 value (F2)	Slope (F3)	R^2 value (F4)
Zero order	3.548	0.610	16.38	0.643	2.598	0.954
First order	0.03	0.670	0.039	0.605	0.052	0.693
Higuchi	14.96	0.949	6.69	0.931	13.77	0.956
Korsemeyer Peppas	0.548	0.964	0.483	0.962	0.717	0.984

Release in case of azithromycin all three formulation revealed non-fickian diffusion as the slope of all formulation for korsemeyer -peppas was near one and release mechanism was not influenced by variable added

Table 13: Release kinetics of Prednisolone from gel

Model	Slope(F1)	R^2 value (F1)	Slope (F2)	R^2 value (F2)	Slope (F3)	R^2 value (F4)
Zero order	2.575	0.982	2.207	0.947	1.579	0.996
First order	0.057	0.792	0.058	0.827	0.056	0.844
Higuchi	13.04	0.899	8.568	0.791	6.261	0.865
Korsemeyer Peppas	0.795	0.972	0.827	0.973	0.926	0.992

***In vitro* skin retention sudy**

The percentage drug content (azithromycin & prednisolone) in skin from various formulatio (Table 14) it was concluded that formulation FI was highly retained in skin as compared to other two solution

Table 14: %age of drug retained on skin

S. No.	Formulation	% drug retained (Azithromycin)	% drug retained (prednisolone)
1	F1	23±0.76	59±0.12
2	F2	11±0.57	26±0.76
3	F3	19±0.42	39±0.63

Stability studies

The stability studies were conducted on formulation F1 at different temperature & humidity condition. refrigeration and at room temperature up to one month showed that formulation was stable (Table15)

Table 15: Stability study of drus at different temperature at different time interval

Weeks	Refrigeration Azithromycin	Refrigeration Prednisolone	At 25° C Azithromycin	At 25° C Prednisolone	At 45° C Azithromycin	At 45° C Prednisolone
1	96.65±1.54	92.83±0.54	93.45±0.75	91.87±1.63	87.96±0.98	87.20±0.98
2	86.74±1.23	90.43±0.36	88.54±0.86	86.82±1.76	85.45±0.56	83.43±0.76
3	85.64±0.96	85.64±1.14	85.73±0.97	84.63±0.56	83.42±0.45	78.42±0.79
4	83.31±0.87	82.43±0.94	82.03±1.32	82.25±0.79	81.56±0.23	76.44±1.42
5	82.34±1.81	80.65±1.56	81.76±0.54	79.08±0.42	78.95±1.18	75.08±1.56

* Each value represnt the mean ±S.D. of 3 determination

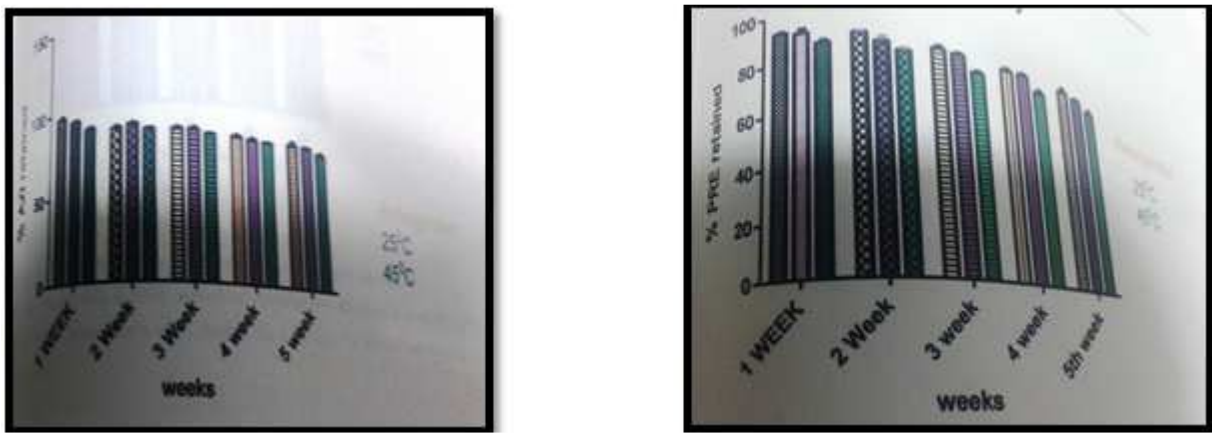

Fig 9: Histogram Showing % of Azithromycin and Prednisolone retained at various temperature

Conclusion

Niosomal/Vesicular gel, has been explored extensively for topical application to enhance skin penetration as well as skin retention. Prednisolone and azithroycin together provide effective results in the treatment of psoriasis. Prednisolone was incorporated in vesicle using span 60 (hydrophilic span form vesicle & entrap lipophilic drug) as surfactant, which give high entrapment efficiency and high stability to prepared vesicles. Prednisolone help in reduction of scar by suppression of humeral immune response. Azithromycin is used to remove scaly patches and to avoid the effects produced by prednisolone. Entrapment efficiency were checked by centrifugation method and was found maximum in formulation F8 having surfactant & cholesterol in ratio 2:1. Niosomal prednisolone formulation were evaluated for parmaters like microcopy, particle size, shape, drug content, in vitro release, zeta potential etc . All formulation size range of 50-100µm. Drug release mechanism were confirmed to be non-fickian diffusion by following kossermeyer peppas kinetic model. Azithromycin was incorporated into simple base gel along with dried niosomal pellets of prednisolone.All formulation tend tend to have confirmatory physicochhemical properties as pH tend to neutral, viscosity varies by adding amount of carbopol in different amount. Gel tend to be easily spreadable as its value come in range of 11.2-15.6g.cm/sec. F1 formulation provide highest in vitro release rate. Drug release mechanismfrom gel were confirmed to be non-fickian diffusion by following kossermeyer peppas kinetic model. Stability study showed no significant change when kept under different temperature & humidity conditions.

"Cite this Article"

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