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Formulation and Evaluation of Clobetasol Propionate Loaded Transferosomes for Psoriasis

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Keywords: Transferosomes, in-vitro drug release, psoriasis, clobetasol propionate, thin film hydration technique, vesicular drug delivery system.

ABSTRACT

The invention of the project was to develop and evaluate Clobetasol propionate loaded transferosomes for an antipsoriatic activity to increase bioavailability and to increase absorption. It provides prolonged drug delivery and enhances bioavailability. The intention is to design and develop antipsoriatic transferosomes and characterize the same by applicable and critical parameters. Clobetasol Propionate is a synthetic antipsoriatic drug. Clobetasol Propionate drug was evaluated by the preliminary tests as colour, odour, appearance, melting point, solubility, infrared spectra, Differential scanning calorimetry (DSC), and determination of λ max and calibration curve. Clobetasol Propionate λ max was estimated to be at 238 nm. Then the Transferosomes were prepared with the help of trial and error method by taking drug ratio 1:1, 1:1severaling thin film hydration process, different formulations of transferosome were prepared. Three preparations (F1-F3) were prepared by varying soya lecithin and tween 80 concentrations. The formulations were subjected to characterization as UV, IR, DSC, particle size, Zeta potential, entrapment efficiency, percent release of drugs and were also characterized by physicochemical characteristics such as SEM. The results show that the concentration of the F2 transferosome is the best based on the entrapment efficiency range of 88.73%. And the approximate average particle size distribution is 266 nm. The Zeta potential for optimized batch F2 was -21.3mV and the drug release of optimized batch F2 was 80%. For stability analysis and the In-vitro anti-Psoriatic test, an optimized batch was used. The optimized batch showed appropriate particle size, stable, good release rate and effective entrapment efficiency and therefore F2 batch was suitable, elegant, palatable and acceptable.

INTRODUCTION:

Psoriasis is a common, persistent, non-communicable skin condition with no known cause or treatment. This illness has the potential to have a severe detrimental influence on people's life. People of all ages and from all nations are affected by psoriasis. At least 100 million individuals worldwide suffer from psoriasis, which is a severe global problem with reported prevalence rates of 0.09 percent to 11.4 per cent. [1,2,3]. Psoriasis has an unpredictable symptom course, a number of external triggers, and significant comorbidities, including arthritis, cardiovascular diseases, metabolic syndrome, inflammatory intestinal disease, and depression. Psoriasis is a disabling, painful, non-communicable, chronic condition that has a significant negative influence on sufferers' quality of life. It is also incurable. Psoriasis can be brought on by both internal and external causes such as stress, systemic medications, infections, mild trauma, sunburn, and skin cancer. Psoriasis affects the skin and nails, and it is linked to a number of comorbid conditions.[4,5,6] Psoriasis treatment is still based on control of symptoms. Vesicles have become the vehicle of choice in drug delivery systems called Vesicular Drug Delivery System." Bingham discovered the biological origin of these vesicles for the first time in 1965, giving them the name Bingham bodies. If selective absorption can be achieved, it is possible to predict that encapsulating a medication in vesicular structures will prolong its time in systemic circulation and, possibly, lessen its toxicity. Vesicular drug delivery systems like liposomes, niosomes, transfersomes, herbosomes etc. are much more effective in releasing the drug at target site and for better availability of drug. Transferosomes are a novel drug delivery system (NDDS), which consists of hydrated bilayers that form spontaneously when phospholipids are dispersed in water. They are simple microscopic vesicles, with a membrane composed of lipid bilayers enclosing an aqueous volume. Since the 1990s, various patents and research papers have been published on a novel class of liposomes known as transfersomes, which was first introduced by Cevc. These belong to the category that is variously termed deformable, highly deformable, elastic or ultra-flexible liposomes or vesicles [7,8]. One amphiphilic component, such as phosphatidylcholine, is present in transfersomes and can form a bilayer as a result of lipid rearrangement in the aqueous solvent. Additionally, it contains a bilayer softening component, such as Tween-80, Span-85, Span-80, sodium cholate, sodium-deoxycholate, etc., that contributes to the vesicles' deformable and adaptable nature. Transferosomes resemble lipid vesicles, liposomes, in morphology but, functionally, transfersomes are sufficiently deformable to penetrate pores much smaller than their own size. [9,10,11]

MATERIALS AND METHODS:

Materials:

Clobetasol Propionate was received as a gift sample from symbiotec pharma lab pvt. limited. Span 80 and soya lecithin, chloroform, ethanol, methanol were obtained from research –lab fine chem, indusries, Islampur. All other chemicals used in this study were of analytical grade.

Preparation of Clobetasol Propionate loaded transferosome:

Preparation of clobetasol propionate loaded transfersomes by technique of thin film hydration by using rotary evaporator:

Drug loaded Transferosomes were prepared by using technique of thin film hydration with three different concentrations (1:1), (1:1.5), (1:2) and drug kept constant for all three concentration. Required amounts of soya lecithin and surfactant were poured into a RBF and dissolved by shaking in a mixture of 2:1 combination of chloroform and methanol. The thin film was developed by rotary evaporation utilizing rotary evaporator at a pressure of 25 ^{0C}, 600 mm / hg and 100 rpm for 15 minutes. Vacuum is used to dry the film for an hour. Clobetasol Propionate was dissolved in a phosphate buffer of 10 ml 7.4 pH. Next, Rotary evaporator hydrated the film with the buffer for half an hour. Mixture was stirred in magnetic stirrer for a half hour. Then, they studied the transferosomes under a microscope. Transferosomal suspension was store in a 4^{0C} fridge [12, 13,14].

Table no. 1 Composition of Clobetasol Propionate loaded transferosomes

Batch	Clobetasol propionate	Soya lecithin	Tween 80	CHCL3:CH3OH
F1	500	500	5	2:1
F2	500	750	10	2:1
F3	500	1000	15	2:1

Characterization of the prepared nano-transfersomes and liposomes:

Particle Size-

By Using Particle size analyzer (Horiba scientific SZ100), the vesicle particle size of Clobetasol Propionate loaded transferosomes were calculated. For particle size calculation sample was diluted using double distilled water and kept for sonication for ten minute. The cuvette was used for particle size analysis, prepared sample was injected into cuvette then cuvette was placed in sample holder. [15,16,17]

Zeta Potential-

The significance of Zeta Potential is that its value can be correlated with the colloidal dispersion is stable. The Zeta Potential denotes the strength of repulsiveness in dispersion amongst adjacent, similarly charged substances. For the calculation of Clobetasol Propionate loaded transferosomes zeta potential, one ml of the transferosomal suspension diluted up to 10 ml with distilled water, 5 ml of diluted transferosomal sample was transferred to cuvette and zeta potential was calculated[18,19].

Drug Entrapment efficiency:

10 ml of prepared formulation was taken in centrifuged tube and centrifuged for 1 hr. to separate the Supernant from transferosomes. The supernant was then collected and diluted properly with distilled water. By using UV spectrometer at 238 nm against the Phosphate.

$$\frac{\text{Total Drug Concentration- Non entrapped drug concentration} \quad X\ 100}{\text{Total Drug Concentration}}$$

buffer saline (PH 7.4) [20,21]. The following equation used to determine quantity of entrapped Clobetasol Propionate transferosomes.

Scanning electron microscopy

The SEM was performed on VEGA 3 TESCAN SEM. The sample was attached using double-sided adhesive tape on a SEM-stub. The sample was covered with a very thin vacuum

coating of gold. Sample operated at 10kv in the SEM chamber [22,23].

***In-vitro* drug release study** [24, 25,26]

Franz diffusion cell was used for the determination of %DR. The Franz Cell device has two major chambers that are divided by a membrane. Nevertheless, the membrane can also be made of egg membrane. Through the top chamber, the sample was put to the membrane. The percent drug release of transferosomes loaded with clobetasol propionate was calculated using the equation below. Bottom chamber contains 20ml of PBS 7.4 Stirrer with a magnet for maintaining RPM and temperature of medium. 100 rpm and 37^{o}C chosen for this PBS medium and the sample (5ml) was collected in between different interval of time. And fresh medium also replaced with the removed sample medium. The process carried out for 90 min and analysis of sample done by UV at 238nm.

$$\% \text{ Drug Release} = \frac{\text{Amt.of Drug release}}{\text{Dose}} \times 100$$

Kinetic release model:

The in-vitro drug release data were fitted to the Higuchi release model and Korsemeyer-Peppas model using DD Solver software to study the mechanism of drug release from prepared transferosomal system and the model with the highest correlation coefficient was considered to be the best model.

Statistical data analysis:

For this kind of data analysis, a single factor ANOVA with filled input and output ranges was chosen. We obtained an ANOVA Single Factor Sheet. ANOVA is a statistical approach for comparing means and the estimating variables that go along with them (such the "variation" within and between groups). ANOVA was created by statistician Ronald Fisher. The idea of total variance, on which the ANOVA is founded, separates the observed variance of a particular variable into components associated with distinct sources of variation. ANOVA expands the t-test beyond two means by offering a statistical test to determine if two or more population means are equal. The ANOVA is used, in other words, to discover whether two or more means are different from one another.

Short term stability study:

Stability tests were conducted during storage to check the drug's leakage from the transferosomes and short-term stability test was carried out by using a stability chamber (Remi programmable environmental test chamber). The suspensions of clobetasol propionate formulations were closed in 20 ml glass vials and stored for a while of 60 days at refrigeration temperature (4^{oC}) and room temperature (25±2 °C/60±5 per cent RH) as per ICH guideline [27]. Samples from each transferosomal formulation which are kept for examination were they transferred at given time intervals.The samples carried out were studied for organoleptic properties, UV, IR, drug entrapment efficiency, drug release at 238 nm.

***In vitro* antipsoriatic activity**

NCCS provided the keratinocytes cell line (B16F10), was kept in Dulbecco's Modified Eagles medium (DMEM) from the National Centre for Cell Sciences (NCCS), Pune, India, The cell line was grown in DMEM supplemented containing sodium bicarbonate, L-glutamine, & 10% FBS an antibiotic solution comprising Penicillin (100U/ml), Streptomycin (100g/ml), and Amphotericin B (2.5g/ml) in a flask for tissue culturing. In a humidified 5 percent CO2 incubator, cultured cell lines were incubated at 37°C (NBS Eppendorf, Germany). The vitality of the cells was determined by using an inverted phase-contrast microscope to observe the cells directly, followed by the MTT assay method.

RESULTS AND DISCUSSION:

Particle Size:

The mean particle size of prepared F1 to F3 batches of Clobetasol Propionate Transferosomes was found to be between 250 and 300 nm. When concentration of soya lecithin and surfactant increases the particle size decreases. Optimized formulation batch F2 have concentration 1:1.5 was show average particle size 266 nm and having PI 0.316.

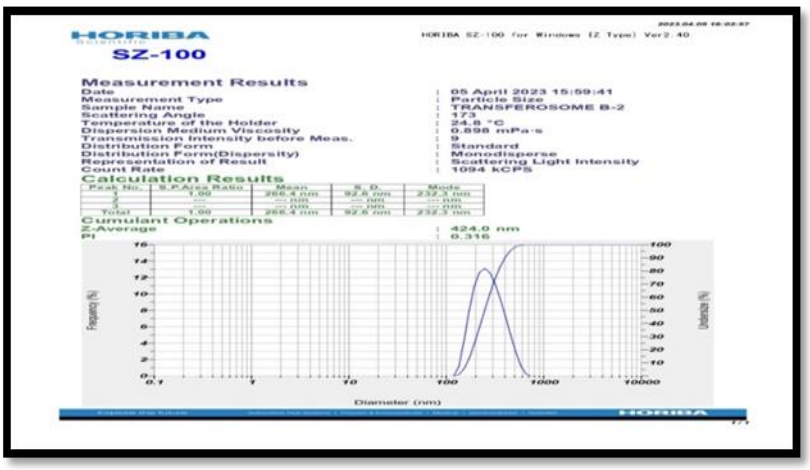

Fig no. 1 Partial size of optimized

Zeta potential measurement:

The value of the zeta potential of the transferosomes of all batches F1-F3 was measured in the series of -1.0 to -25.9 mV and the result suggests that the transferosomes have good stability. The resulting zeta potential value is directly related to the stability of transferosomes in an aqueous suspension and the result suggests that the transferosomes having good stability. Fig. 2 shows zeta potential of the optimized batch –21.3mV.

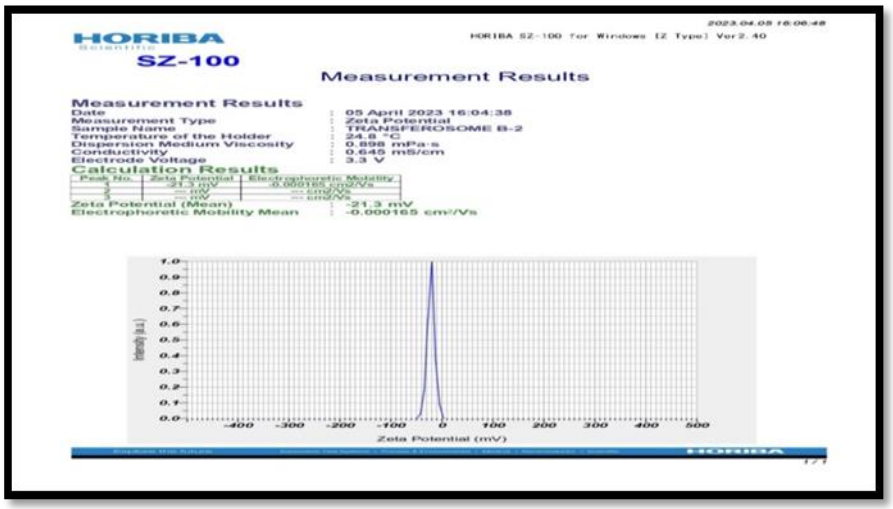

Fig.2. Zeta Potential of optimized batch F2

Scanning Electron Microscopy (SEM):

The surface morphology of transferosomes vesicles of Clobetasol Propionate loaded

transferosomes is shown in Fig no. 3 indicated the presence of the spherical shape with smooth surface. Optimized batch F2 for particle size shows 266 µm size. By using scanning electron microscopy we can determine the morphology, size, shape of the transferosomes and the images of transferosomes were captured by SEM.

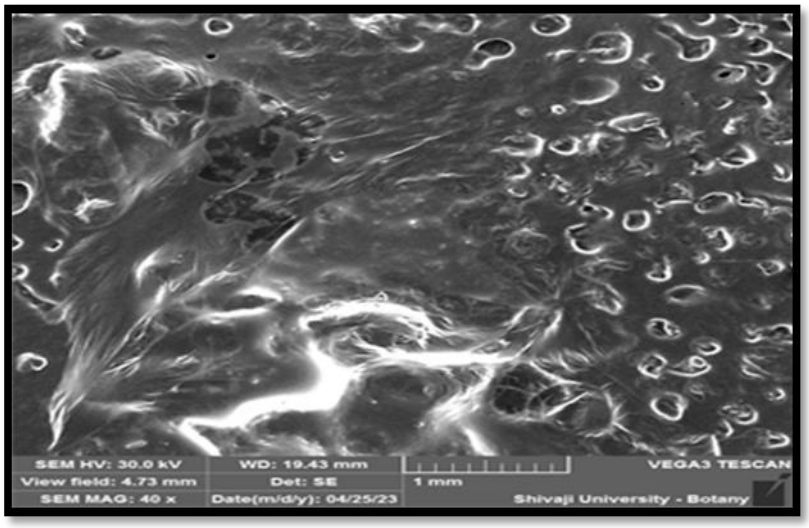

Fig No. 3 SEM Photograph of Optimized batch

Entrapment efficiency:

By using the centrifugation method the % EE of prepared Clobetasol Propionate transferosomes was performed. Use in a proportion of (1:1, 1.5:1, 2:1) Clobetasol Propionate, soya lecithin, tween 80 demonstrates optimum %EE. Batch F2 transferosomal formulation Shows maximum %EE. Batch F2 shows a maximum entrapment efficiency 88.73%. The F1 to F3 Clobetasol Propionate %EE was found 58.11% to 88.73% using various concentrations of soya lecithin and tween 80. Drug entrapment efficiency of optimized batch 88.73% which means good capacity of polymer to entrap the drug and it is successful when the concentrations of polymer 1:1.5 fold by that of drug concentration. Good entrapment indicates the formulation is with a stand to applicability on skin membrane for certain period.

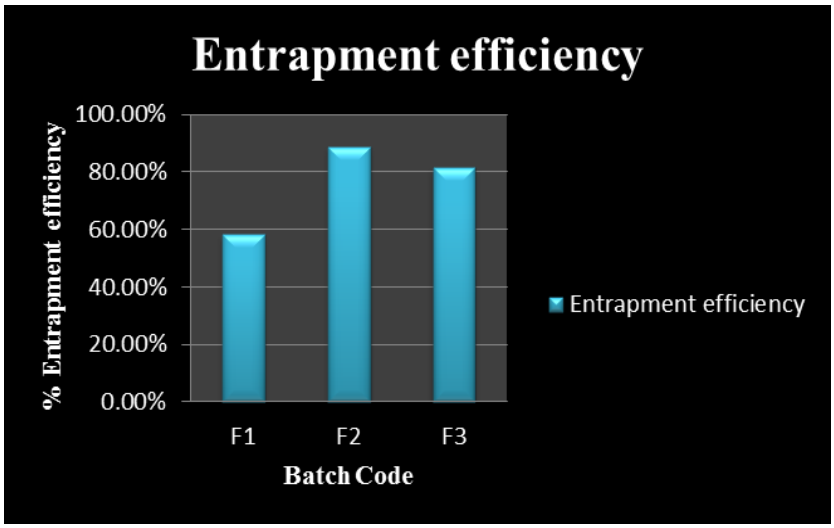

Fig No.4 % entrapment efficiency of Clobetasol Propionate loaded transfersomes from F1 to F3 formulation

In-vitro drug release study:

The % DR of Clobetasol Propionate was performed with the help of Franz diffusion cell by using PH 7.4 buffer. The % DR was carried out for 90 min and drug release percentage was measured at various time intervals. Batch F2 shows maximum drug release at minimum time interval it was found to be 80%. When the Soya lecithin concentration increases the drug release also increases. It might be due to tween 80, soya lecithin or equal amount of tween 80, soya lecithin used in formulation.

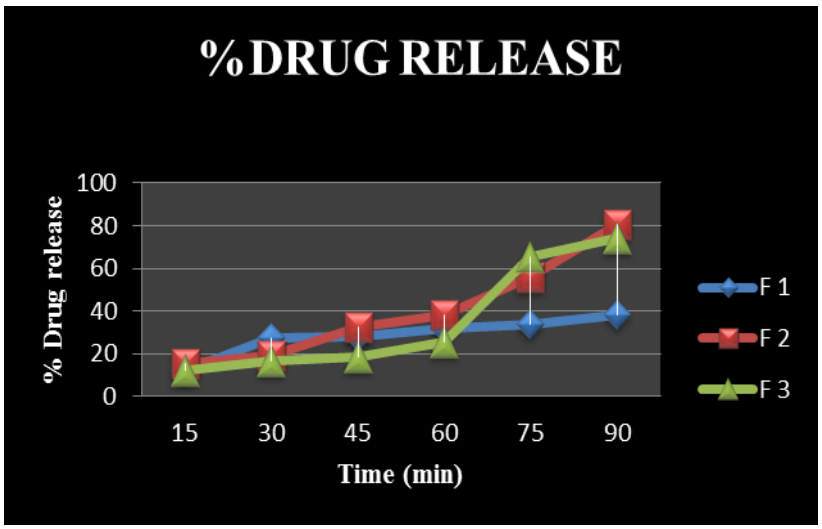

Fig No.5 % Drug Release from F1 to F3 batches

Kinetic release model:

The in-vitro diffusion kinetic release study was performed using two mathematical model Higuchi and Korsmeyer-pepps. If the concentration of polymer increases by 1:1.5 fold concerning drug concentration then this will be the suitable proportion of polymer of drug which fit Korsmeyer-peeps equation that indicates a good release rate within 90 min. The higher regression coefficient values in Table No.2 for batch F2 formulation suggested that the formulations F2 follow Korsmeyer-peeps drug release.

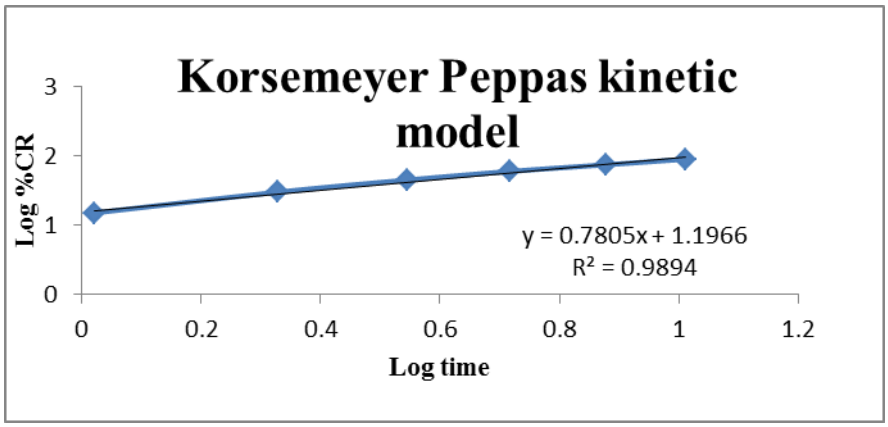

Fig No.6 Korsemeyer Peppas Kinetic model

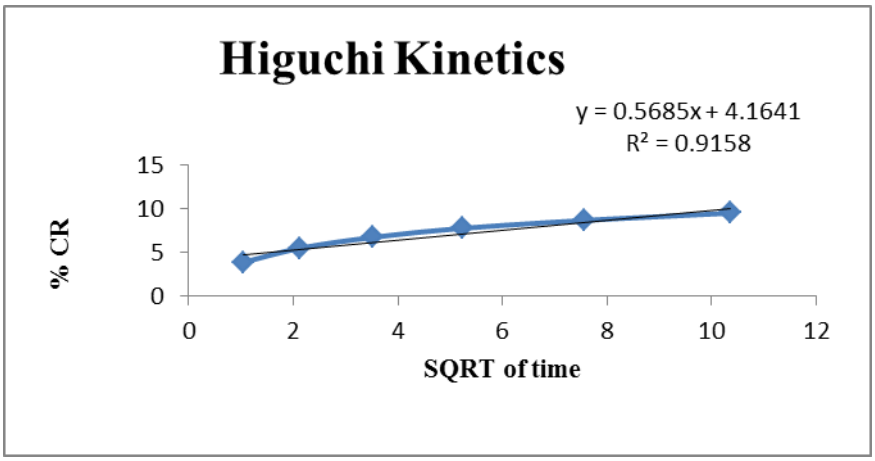

Fig No.7 Higuchi Kinetics

Table No. 2 Release Kinetic Model Fitting

Model	Higuchi	korsmeyer-pepps
(R^2)	0.9198	0.9894

Short-term stability studies of optimized formulation:

The stability studies of the Clobetasol Propionate loaded transfersomes were evaluated for optimized formulation of F2 after storage at 4ºC and room temperature for 60 days as per ICH guidelines. The sample was withdrawn periodically after 30 days. The per cent drug entrapment efficiency of samples was determined as a function of the storage time. After performing the stability study, it was observed that at intermediate stability conditions there was no change in colour, odour, %EE, and %DR of prepared clobetasol propionate loaded transferosomes. From this research, it is concluded that the prepared clobetasol propionate loaded transfersomes formulation was stable during storage. Observed data of stability studies of optimized formulation shown in Table No.3.

Table no 3 Stability study of optimized clobetasol propionate loaded transpersonal

Formulation	Test	Storage condition	After 30 days	After60 days
Batch F2	Entrapment efficiency	25±2 °C/60±5 per cent RH	90.3%	89.1%
	Drug release	25±2 °C/60±5 per cent RH	80.3%	79%

batch F2

In-vitro antipsoriatic activity:

The in-vitro antipsoriatic activity of prepared transferosome batch F2 was performed. The study was carried out for determination of given sample was either antipsoriatic. It was found that sample containing a concentration of 1000 µg/ml in showed 60.84%% inhibition. Therefore comparing these values to the standard, we can state that the given sample acted as an antipsoriatic.

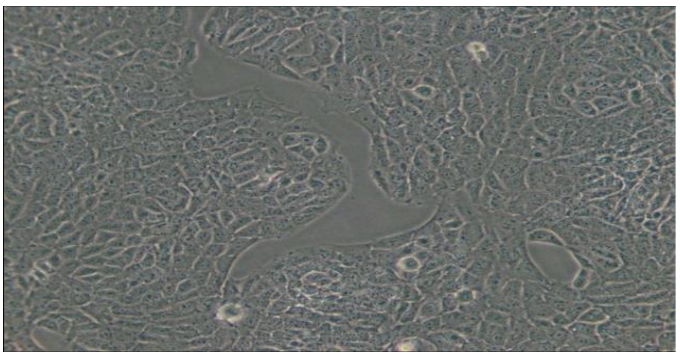

Fig 8 Standard: measuring the present of inhibition against keratinocytes cells

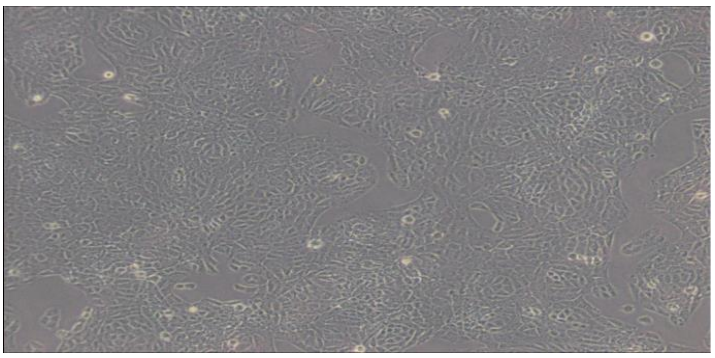

Fig 9 Test: Compound exhibited good antipsoriatic activity against keratinocytes cells

Statistical Analysis:

The primary technique for single-factor analysis of variance is also referred to as one-way ANOVA because it only considers one factor. The significance level was found $P \leq 0.05$ shown in table no. 4 for entrapment efficiency table no.5 for drug release, table no.6 for particle size. This data accept alternative hypothesis and reject null hypothesis and the data was very significant data.

Table no.4 Statistical analysis: Entrapment efficiency

Anova: Single Factor						
Entrapment efficiency						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
F 1	3	174.33	58.11	100		
F 2	3	266.19	88.73	100		
F 3	3	244.68	81.56	100		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1538.896	2	769.4479	7.694479	0.022074	5.143253
Within Groups	600	6	100			
Total	2138.896	8				

Table no.5 Statistical analysis: drug release

Anova: Single Factor						
Drug release						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
F 1	6	220	36.6667	30.2667		
F 2	6	239	39.8333	591.767		
F 3	6	365	60.8333	142.967		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2070.11	2	1035.06	4.05904	0.039	3.68232
Within Groups	3825	15	255			
Total	5895.11	17				

Table no. 6 Statistical analysis: particle size

Anova: Single Factor						
Particle size						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
F 1	3	888	296	16		
F 2	3	798	266	16		
F 3	3	756	252	16		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3032	2	1516	94.75	2.89077E-05	5.14325
Within Groups	96	6	16			
Total	3128	8				

CONCLUSION:

In the present research containing Clobetasol Propionate used for antipsoriatic activity, Transferosomes were formulated by thin film hydration method and evaluated by parameter such as particle size, zeta potential, %EE, %drug release , SEM . Thin film hydration process used for preparation of Clobetasol Propionate transferosomes. Drug concentration was also kept constant in all formulating batches F1-F3 and statistically analyzed data collected from experiment research. ANOVA was used to establish the statistical validity of the polynomials. Model for particle size, entrapment efficiency and release of drugs in vitro was found to be significant based on the probability (P- value). Concentration of polymer increases by 1:1.5 fold with respect to drug concentration then this will be the suitable proportion of polymer of drug which fit the korsmeyer-pepps equation that indicate a good drug release rate within 90 min. When we framed all the observation into graph then it is observed that % cumulative drug release is increased with square root of time as per Higuchi kinetic model and as per Korsemeyer Peppas model. Clobetasol Propionate is water insoluble and hence Higuchi kinetic model cannot be applicable and thus our drug release kinetic fit to Korsemeyer Peppas kinetic model with regression coefficient 0.9894. Optimized batch of transferosomes F2 show antipsoriatic activity with 60.84% percent inhibition. It can also be concluded that the particle size, entrapping efficiency and in vitro drug release depend on the concentration of non-ionic surfactant and Soya lecithin used in the formulation. When

concentration of soya lecithin and surfactant increases the particlesize decreases, and the drugs release increases. Optimized formulation batch F2 have concentration 1:1.5 was show particle size 266 nm, drug release 80 percent within 90 min with maximum drug entrapment efficiency as 88.73 per cent and zeta potential -21.3mV showing formulation stability. An optimized F2 formulation is acceptable and palatable with better absorptivity and stability.

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