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NOVEL CLINDAMYCIN LOADED TRANSFERSOMES FORMULATION FOR EFFECTIVE MANAGEMENT OF ACNE

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ABSTRACT

Acne vulgaris is a chronic inflammatory disease affecting many millions people in the world. The aim of the present study was to improve the therapeutic effectiveness of clindamycin via encapsulation it into a nanocarrier namely transfersome. total eight formulations prepared for optimization of Clindamycin loaded transfersomes (TE1-TE8) using varying amount of span 80, and phosphatidylcholine, the prepared transfersomes were evaluated for vesicle size determination, entrapment efficiency and surface Morphology. In all formulations formulation TE4 which contain smallest vesicle size and increase in entrapment efficiency which was selected as optimized formulation for topical gel preparation. The clindamycin encapsulation was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy.

KEYWORDS: Clindamycin, transfersomes, topical gel.

INTRODUCTION

Acne vulgaris is a disease of the pilosebaceous follicle characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules, and nodules) (Adityan *et al.,* 2009; Bhate and Williams, 2013). In such situation transdermal drug delivery remains the most preferential mode of administration. But, stratum corneum forms the most formidable barrier for the penetration of drug through skin. To overcome the stratum

corneum barrier, the use of lipid vesicles like ethosomes and transfersomes in delivery systems has involved increasing attention in recent years (Asbill *et al.*, 2000).

Clindamycin Phosphate is an antibiotic widely used for the treatment of acne (Orwa *et al.*, 1999). In oral dosage forms it produces pseudomonas colitis while in topical dosage forms and side effects like irritation, skin rash, itching etc. it's topical bioavailability is also less. So, to overcome these limitations an attempt has been made to prepare transfersomes of clindamycin phosphate and optimize it for enhanced delivery through the skin.

MATERIAL AND METHODS

Instruments

UV Vis Double beam Spectrophotometer - Labindia 3000+. FT IR instrument - Bruker alpha. Electronic weighing balance-Wensar. Digital pH meter - Khera, Brook Field Viscometer-Precision Electro Instrumentation India Private Limited, Thane, Zeta Sizer- Malvern Instruments.

MATERIAL

Clindamycin gift sample obtained from Euphoria Healthcare Pvt. Ltd. Mumbai, Soya Phosphetidyl Choline purchased from Ash Chemie India, Thane, Disodium Hydrogen Phosphate, Di potassium Hydrogen Orthophosphate, Di potassium Hydrogen Orthophosphate, Sodium Chloride, Carbopol 934P, Methyl Paraben, Propyl Paraben, Propylene Glycol purchased from S. D. Fine Chem. Ltd., Mumbai, Methanol, Ethanol, Chloroform from Qualigens Fine Chemicals, Mumbai.

METHODS

Formulation development of Clindamycin loaded transfersomes

Soya-phosphatidylcholine, Span 80 and Clindamycin were dissolved alcohol. Then solution was put in a round bottom flask. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotator vaccum evaporator at 40^0C. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the appropriate buffer by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated at room temperature. This thin film was then hydrated by phosphate buffer saline to get the transferosome.

Table 1: Formulation code and variable used in preparation of transferosome

S.NO	Formulation code	PC:S (mg)	Drug (mg)
1	**TE1**	95:05	100
2	**TE2**	90:10	100
3	**TE3**	85:15	100
4	**TE4**	80:20	100
5	**TE5**	80:20	100
6	**TE6**	85:15	100
7	**TE7**	90:10	100
8	**TE8**	95:05	100

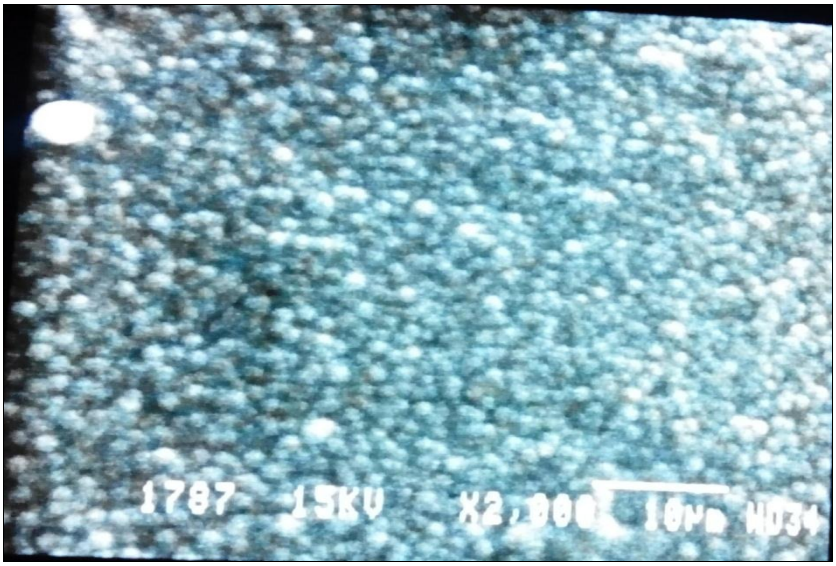
SP=span 80, PC=Phosphatidylcholine.

Evaluation of Clindamycin loaded transfersomes

Vesicle size determination: Vesicle size was determined using the particle size analyzer (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) (Pandey *et al.*, 2009).

Entrapment efficiency: Clindamycin was estimated in transfersomes by ultra centrifugation method and in liposomes by protamine aggregation method followed by ultra centrifugation. The total volume of the transfersomes suspension was measured and 2 ml of this formulation was transferred to 10 ml centrifuge tube. The suspension was diluted with distilled water upto 5 ml and centrifuged at 2000 rpm for 20 minutes to separate out undissolved drug in the formulation. Ethosomes were separated by ultra centrifugation at 20,000 rpm for 30 minutes. Supernatent and sediment was recovered and their volume was measured. Sediment was diluted with distilled water upto 5ml. The unentrapped drug contents were analyzed by estimating drug in supernatant by spectroscopic method (Nathji and Patni, 2012).

Transmission Electron Microscopy: Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).



TEM image of transfersomes

Preparation of ethosomal Gels

Preparation of carbopol gel base: 0.5 g Carbopol 934 was weighed and dispersed in water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to for gel consistency. Similarly 1 and 2% carbopol gels were prepared.

Table 2: Composition of different gel base

Formulation	Carbopol (%)
TF1	0.5
TF2	1.0
TF3	2.0

Preparation of transfersomes gels: 1g of transfersomes formulation was dissolved in 10ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the unentrapped drug. The supernant was decanted and sediment was incorporated into the gel vehicle.

The incorporation of the transfersomes into gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5, 1 and 2% w/w.

Evaluation of Gels

Determination of pH: Weighed 50 gm of gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 3–9 to treat the skin infections.

Spreadability: A modified apparatus suggested was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels. The modified apparatus was fabricated and consisted of two glass slides, the lower one was fixed to a wooden plate and the upper one was attached by a hook to a balance. The spreadability was determined by using the formula: S=ml/t, where S, is spread ability, m is weight in the pan tied to upper slide and t is the time taken to travel a specific distance and l is the distance traveled. For the practical purpose the mass, length was kept constant and‘t’ was determined.

The measurement of spreadability of each formulation was in triplicate and the average values are presented.

Measurement of viscosity: The viscosity of gels was determined by using a Brook Field viscometer DV-II model. A T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings.

Drug content: 1 gm. of the prepared gel was mixed with 100 ml. of ethyl alcohol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 296 nm. Drug content was calculated by linear regression analysis of the calibration curve (Patel and Patel, 2013).

***In-vitro* diffusion study:** An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. ethosomal and liposomal gel equivalent to 500 mg of Clindamycin was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer (Perumal, 2001).

RESULTS AND DISCUSSION

Evaluation of transfersomes

Table includes the value of vesicle size, and entrapment efficiency. The vesicle size of all transfersomes varied between 165.30 and 552.82 nm where as entrapment efficiency was found between 65.55 to 76.70%.

Table 3: Evaluations of transfersomes for Ve sicle size and Entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
TE1	165.30±3.302	65.55±1.311
TE2	256.71±2.303	72.83±1.241
TE3	478.32±2.456	70.62±1.112
TE4	269.00±3.214	76.70±0.995
TE5	552.82±1.254	74.76±1.021
TE6	345.91±1.268	66.62±1.854
TE7	319.35±2.548	73.62±1.119
TE8	312.93±1.327	64.08±1.213

Results showed that in formulation TE4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation TE4 Selected as optimized formulation for further evaluation.

Evaluation of transfersomes gel formulations

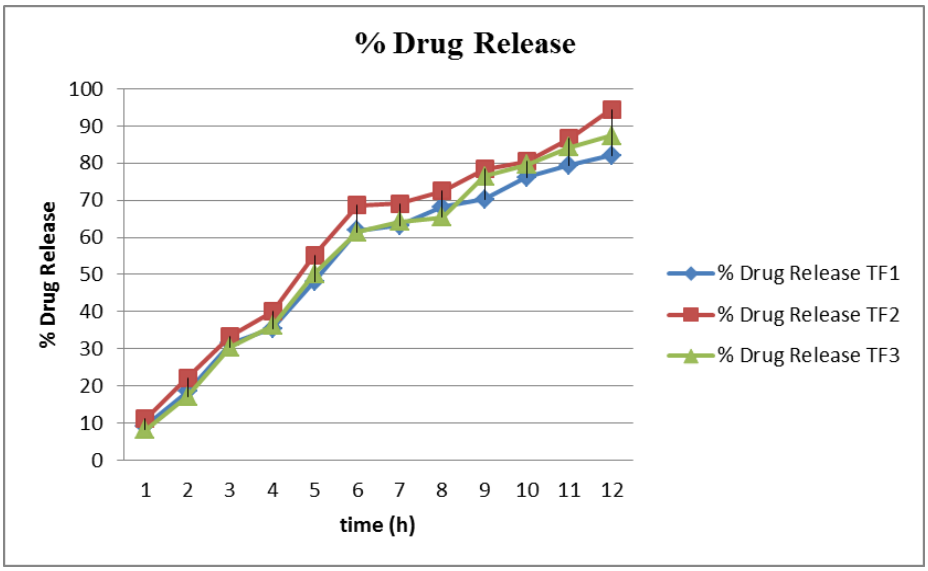
Table 4: Results of transfersomes gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
TF1	98.75 ±0.027	6.9±0.021	10.15±0.075	3450±1.70
TF2	98.95 ± 0.021	7.2±0.040	11.45±0.042	3570±0.86
TF3	97.51 ± 0.017	7.3±0.060	12.75±0.059	3840±1.90

Table 5: % Percentage Drug Release of Transfersomes gel

Time (h)	% Drug Release of Transfersomes gel					
	% Drug Release TF1	Cumulative* % Drug Release TF1	% Drug Release TF2	Cumulative* % Drug Release TF2	% Drug Release TF3	Cumulative* % Drug Release TF3
1	9.25	9.25	11.15	11.15	8.26	8.26
2	18.45	27.7	22.25	33.4	17.25	25.51
3	31.45	59.15	33.45	66.85	30.46	55.97
4	35.48	94.63	40.12	106.97	36.25	97.22
5	48.11	142.74	55.27	162.21	50.22	142.44
6	61.94	204.68	68.58	230.79	61.38	203.82
7	63.22	267.9	69.14	299.93	64.25	268.07
8	68.15	336.05	72.45	372.38	65.45	333.52
9	70.33	406.38	78.38	450.76	76.44	409.96
10	76.12	482.5	80.44	531.2	79.55	489.51
11	79.36	561.86	86.66	617.86	84.22	573.73
12	82.11	643.97	94.41	712.27	87.41	661.14

***In-vitro* drug release data of transfersomes gel formulation**



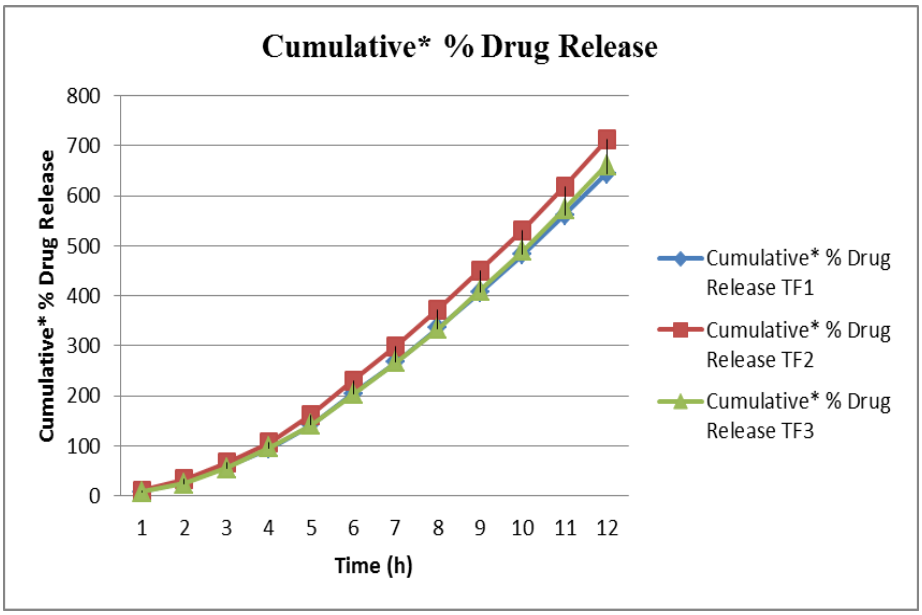


Table 6: *In-vitro* drug release data for TF2.

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	% Drug Release	Log % Drug Release	% Drug Remaining	Log % Drug Remaining
1	1	0	11.15	1.047	88.85	1.949
2	1.414	0.301	22.25	1.347	77.75	1.891
3	1.732	0.477	33.45	1.524	66.55	1.823
4	2	0.602	40.12	1.603	59.88	1.777
6	2.449	0.778	68.58	1.836	31.42	1.497
8	2.828	0.903	72.45	1.860	27.55	1.440
12	3.464	1.079	94.41	1.975	5.59	0.747

*Average of three readings.

Table 6: Regression analysis data of transfersomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
EF2	0.951	0.953	0.981	0.984

Result of stability studies: Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 28 ± 0.5ºC for a period of four weeks. There was no significant variation found in appearance, average particle size and % drug content of the transfersomes gel.

CONCLUSION

Clindamycin Phosphate is an antibiotic widely used for the treatment of acne. In oral dosage forms it produces pseudomonas colitis while in topical dosage forms it has side effects like irritation, skin rash, itching etc. it's topical bioavailability is also less. So, to overcome these limitations an attempt has been made to prepare transfersomes of Clindamycin Phosphate and optimize it for enhanced delivery through the skin.

Acne is a common condition that is often accompanied by physical and psychological morbidity. current acne treatments target at least 1 of the known pathophysiologic mechanisms involved in acne development. Combining these therapies can often lead to significantly better improvement and/or inhibit the development of antibiotic-resistant bacteria. With proper treatment of this condition, the negative effects of acne can be minimized. transfersomes improve the site specificity, overall drug safety and lower the dose several times then the currently available formulations for the treatment skin diseases because of their good penetration power and flexibility. Transferosome formulation is used for effective delivery of nonsteriodal anti inflammatory agents like ibuprofen and diclofenac. So that the drug will be choose salicylic acid which is NSAID category.

In this study an attempt has been made to formulate a supplement dermal therapy of Clindamycin. The clindamycin encapsulation was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy.

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