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PREPARATION AND EVALUATION OF LIPOSOMAL GEL OF LINCOMYCIN HCL

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ABSTRACT

The aim of the present study was to develop liposomal gel containing lincomycin. Lincomycin is used in the treatment of bacterial infections. It is used in infections of the skin such as acne (Pimples), abdomen, bone, skin, heart and soft tissues. It also used in post-operative infections. lincomycin is a drug which are known to have high solublity but low permeability. Lincomycin HCL. These drugs are available in the form of oral dosage that required a long period of time for treatment. The formulation was further subjected to their characteristic such as, viscosity, ph, conductivity, clarity, Invitro drug release, particle size, zeta potential, entrapment efficiency, % cumulative drug release etc. Overall, these results indicated that developed liposomal gel of lincomycin could have great potential.

KEYWORD: Lincomycin, Liposomal Gel, Entrapment.

1. INTRODUCTION

Most favourable therapeutic outcomes necessitate not only appropriate drug selection but also successful drug delivery. In human body, the skin is a best available space for drug delivery. Developing suitable drug delivery has become increasingly important in the pharmaceutical industry over the past three decades. The pharmacological response including the needed therapeutic effect and undesired adverse effect of a drug is dependent relatively, on the concentration of the drug at the site of action which depends upon the dosage form and the amount of absorption of the drug at the site of action.

In human body skin covers an area of about $2m^2$ and this multilayered organ receives

approximately one-third of all blood circulating throughout the body. Skin contains an uppermost layer, epidermis which has morphologically distinct regions basal layer, spiny layer, stratum granulosum and stratum corneum. It consists of highly cornified (dead) cells a constant medium of lipid membranous area. These extracellular membranes are unique in their compositions and are poised of ceramides, cholesterol and free fatty acid.

In total skin surface consist of 1/1000 of hair follicles and 200-250 worry ducts on every tetragon centimetres of the skin area. It is one of the most gladly reachable organs of the human body. The possible area of the whole skin as the harbour of drug administration to the human body has been recognized for some decades, but skin is an extremely difficult barrier to the access of materials allowing only small quantities of a drug to go through over a period of time. Transdermal drug delivery the delivery of drugs crosswise the skin at a controlled rate to the systemic circulation is distinct from topical drug penetration which intention narrow areas. Transdermal drug delivery takes benefit of the relative accessibility of the skin.

Liposomes as vesicular drug delivery system

Liposomes are small man-made vesicles of spherical shape that can be created from cholesterol and natural in toxic phospholipids. Liposomes are assured systems for drug delivery due to their shape, size along with hydrophobic and hydrophilic quality (besides biocompatibility). Liposomal properties change significantly with lipid composition, surface charge, size, and the method by which they are prepared. Furthermore, the choices of bilayers mechanism decide the,, rigidity/fluidity‟ and type of the bilayers. For example, unsaturated phosphatidylcholine classes from natural sources provide much additional permeability and less stable bilayers, whereas the saturated phospholipids with long acyl chains form a rigid as well as impermeable bilayer structure**.**

It is shown that phospholipids precipitate and form clogged structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can convey aqueous or lipid drugs, depending on the nature of the drugs used. Usually, liposomes are defined as spherical vesicles with particle sizes ranging from 30 nm to numerous micrometers. They consist of one or more lipid bilayers surrounding with aqueous units, where the polar skull groups are aimed at the pathway which is at the center and exterior aqueous phases. On the other hand, self-aggregation of polar lipids is not limited to conservative bilayers structures which rely on molecular shape, temperature and surrounding environment but may self-assemble into a variety of colloidal particles.

Liposomes are amiable and are used as transporter for numerous molecules in cosmetic as well as pharmaceutical industry. Food and farming industries also have extensively studied the use of liposome encapsulation to produce as delivery systems that can deceive the compounds that are unbalanced (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield its functioning. Liposomes can entrap both hydrophobic and hydrophilic complexes together, avoiding decomposition of entrapped substances, and release the entrapped substances at designated targets**.**

Because of their biocompatibility, biodegradability, low toxicity and aptitude to entrap both hydrophilic and lipophilic drugs together and short site-specific drug delivery to tumor tissues liposomes have enhanced rate both as an investigational scheme and commercial purpose.

Liposomal encapsulation technology (LET) is the latest drug delivery method used by medical investigators to administer drugs that work as curative promoters to specific body organs. This application of delivery system is targeted for delivery of vital combinations to the body. LET is a technique of generating sub-microscopical foams called liposomes, which frequently encapsulate equipments. These liposomes form a barrier around their contents, which oppose the enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The chemical substances encapsulated in liposomes are, therefore, protected from oxidation and degradation. These protective phospholipids shield/barrier remains undamaged until the contents of the liposome are delivered to some specific targeted glands, organ, or system where the contents would be utilized Also, drugs with different lipophilic values can be encapsulated into liposomes: strongly lipophilic drugs are entrapped totally in the lipid bilayers, powerful hydrophilic drugs are situated completely in the aqueous section and drugs with intermediary log divides between the lipid and aqueous phases, both in the bilayers and in the aqueous core**.**

2. EXPERIMENTAL WORK

2.1 Preparation of lincomycin HCL loaded liposomes

Liposomes were prepared by rotator evaporation method given by Touitou *et al.*, 2000 with slight modification in which drug was dissolved in methanol to give a concentration of 1.0% w/v of drug solution. The accurately weighed amounts (10% w/v) of phospholipids and surfactant (7:3 ratio) were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in minimum quantity of methanol and chloroform mixture in ratio of 2:1. The

round bottom flask was rotated at 45º angle using rotator evaporator at 40°C in order to make uniform lipid layer. The organic solvent was removed by rotary evaporation under reduced pressure at the same temperature (40°C). Final traces of solvents were removed under vacuum overnight. The prepared lipid film in the inner wall of round bottom was hydrated with 10% w/v of drug solution in water followed by rotating the flask containing mixture of drug by rotation at speed of 60rev/min for 1 hr. After complete hydration of film, the prepared formulation of liposomes was subjected to sonication at 4^0 C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The prepared formulation was stored at 4°C in closed container till further use for analysis.

2.2 Optimization of liposomes

Optimization of lipid: surfactant ratio

In the liposomal formulation, the lipid: surfactant ratio was optimized by taking their different ratio such as 5:5, 6:4, 7:3 and 8:2 ratio and all other parameters were kept remain constant. The prepared formulations were optimized on the basis of average particle size and entrapment efficiency.

Table 2.1: Optimization of lipid: surfactant concentration.

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F-1	5:5	1.0	361.28±0.62	50.48±0.37
F-2	6:4	1.0	319.31±0.84	57.26±0.71
F-3	7:3	1.0	288.47±0.33	70.37±0.52
F-4	8:2	1.0	249.94±0.58	77.82±0.63

2.3 Optimization of drug concentration

Drug concentration was optimized by taking different concentration of drug and prepared their formulation and all other parameter such as Soya PC: Span 80, sonication time kept remain constant. The formulation optimized on the basis of entrapment efficiency and average vesicle size.

Table 2.2: Optimization of drug concentration.

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Average particle size (nm)	% Entrapment efficiency
F-5	8:2	1.0	261.78±0.37	69.72±0.32
F-6	8:2	1.5	245.61±0.62	74.85±0.39
F-7	8:2	2.0	283.83±0.92	65.27±0.85

2.4 Optimization of sonication time

Sonication time was optimized by sonicating the formulation for different time i.e 30, 60 and 90 sec at 4^0C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The optimization was done on the basis of average particle size and % Entrapment efficiency.

Table 2.3: Optimization of sonication time.

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Sonication time (Sec)	Average vesicle size (nm)	% Entrapment efficiency
F-8	8:2	1.5	30	219.73±0.83	74.65±0.29
F-9	8:2	1.5	60	189.86±0.46	69.92±0.45
F-10	8:2	1.5	90	141.27±0.21	61.28±0.76

Table 2.4: Optimized formulation liposomes.

Formulation code F-8	
Phospholipid: Surfactant (10 % w/v)	8:2
Drug (% w/v)	1.5
Sonication time (sec)	30

2.5 Characterization of liposomes

2.5.1 Vesicle size

The average diameter was calculated using the flowing formula.

$$Average\ Diameter = \frac{\Sigma n.d}{\Sigma n}$$

Where n = number of vesicles; d = diameter of the vesicles

2.5.2 Surface Charge and Vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method. Zeta potential measurement of the liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility.

Table 2.5: Characterization of Optimized formulation of liposomes.

Characterization.	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-8	219.73±0.83	74.65±0.29	-31.80±0.25

2.5.3 Entrapment efficiency

Entrapment efficiency of Lincomycin HCL liposomal formulation was determined using the Sephadex G-50 column.

$$\% \, Entrapment \, Efficiency = \frac{Therotical \, drug \, content - Practical \, drug \, content}{\text{Therotical drug content}} \times 100$$

2.5.4 pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter.

2.6 Preparation of gel base

Carbopol 940 (1-3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. Gel was also prepared with plain drug by adding 10 mg of drug and dispersed properly by following same procedure given above. The same procedure was used to formulate noisome containing gel in which previously prepared liposomal cake was added in place of plain drug. liposomes preparation corresponding to 1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

2.7 Evaluation of gel

A. Determination of pH

Digital pH meter had calculated the pH of the anti-acne gels.

B. Spreadability

The Spreadability of liposomal gel formulation was measured on the basis of slip and drag characteristics of the gels. The measurement of Spreadability of each formulation was done in triplicate and the average values are presented.

C. Viscosity

The viscosity of the prepared gel was determined by a Brookfield digital viscometer. The viscosity was assessed using spindle no. 6 at 10 rpm at ambient room temperature of 25-30 ^{0}C.

D. Drug release kinetic study of formulation

The release kinetic was studied by various kinetic models as zero order plot, first orderplot, higuchi plot and korsmeyer-peppas.

E. Stability study

The stability study was performed as per ICH guidelines. The formulated gel were filled in

the collapsible tubes and stored at different temperatures and humidity conditions, viz.25^0 C ± 2^{0}C/ 60% ± 5% RH, 30^0 C ± 2^{0}C/65% ± 5% RH, 40^{0}C ± 2^{0}C/ 75% ± 5% RH for a period of six months and studied for appearance, pH, viscosity and spreadability.

3. RESULT OF PREFORMULATION STUDIES

3.1 Physiochemical Properties of Lincomycin HCL

A) Physical evaluation

It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

Table 3.1: List of Sensory characters.

S. No.	Sensory characters	Result
1.	Color	White powder
2.	Odor	Odorless or faint *odor*
3.	Taste	Bitter Taste

B) Solubility

Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (water, ethanol, methanol, 0.1N HCL, and 7.4 pH buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature). Soluble in methanol and ethanol, butanol, isopropanol, ethyl acetate, n-butyl acetate, amyl acetate.

C) Identification test

Sample of pure Lincomycin HCL

The IR spectrum of sample drug shows the peak values which are characteristics of the drug and the graph.

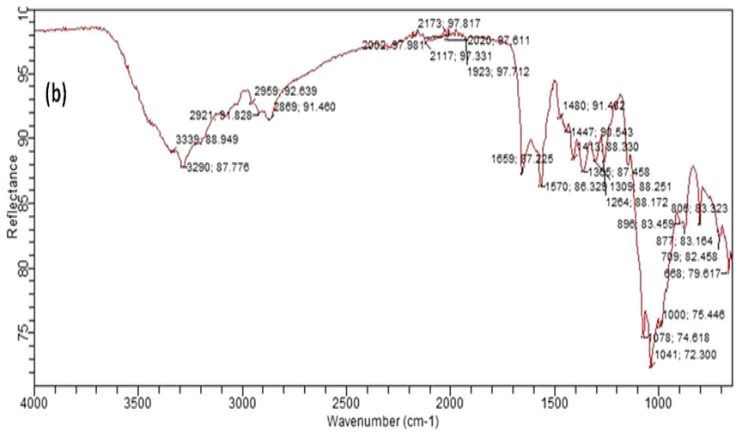

Figure 3.1: FT-IR spectrum of Lincomycin HCL.

D) Loss on drying

Loss on drying is directly measured by IR moisture balance. Loss on drying value of Lincomycin HCL was found to be 0.69

E) Results of pH

Average pH of the solution was 7.6

F) Melting point

Average Melting point of the Lincomycin HCL was 188-190^{o}C.

G) Moisture content determination

Moisture content of Lincomycin HCL.

H) Determination of λ_{max} of Lincomycin HCL

The λ_{max} of Lincomycin HCL was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

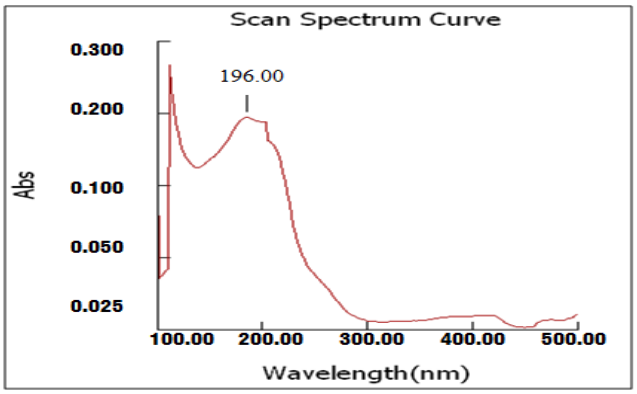

Figure 3.2: Wavelength maxima of Lincomycin HCL buffer pH 7.4.

3.2 Compatibility studies of Drug and Excipients

In the compatibility testing program, blends of drug and excipients are prepared by triturating the drug with Individual excipients. The λ_{max} were recorded for determination of Compatibility of Lincomycin HCL with other excipients.

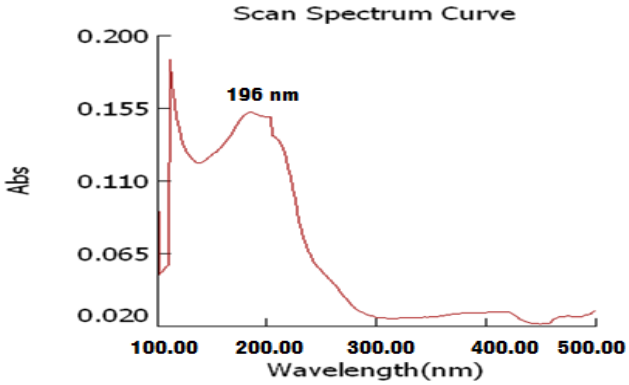

Figure 3.3: Wavelength maxima of Lincomycin HCL.

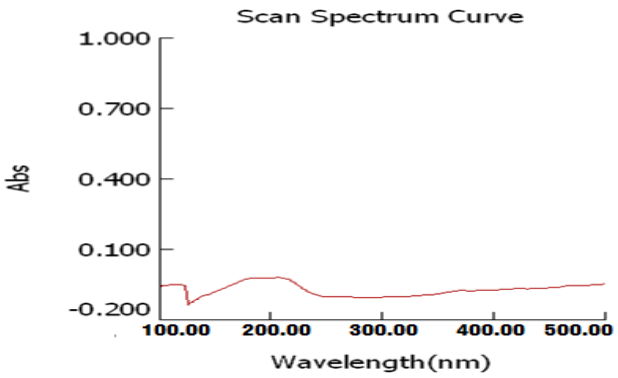

Figure 3.4: Wavelength maxima of Lincomycin HCL+Excipients.

3.3 Results of formulated Liposome development

3.3.1 Average vesicle size and zeta potential

Vesicle size of liposomes were examined under trinocular microscopic and also determined by light scattering method and found that average vesicle size of optimized formulation F-8 was 219.73±0.83 nm. Zeta potential was -31.80±0.25.

3.3.2 % Entrapment efficiency

% Entrapment efficiency of optimized liposomes formulation (F-8) was found 74.65±0.29%. It was clearly shown that when formulation was sonicate for 30 sec then the % EE was 74.65±0.29 (F-8) and when it sonicate for 60 and 90 sec then the % EE was found 69.92±0.45 (F-9) and 61.28±0.76 F-10) respectively. The 60 sec is selected as optimized time for sonication because it provided the required size of vesicle 219.73±0.83 nm and good % EE 74.65±0.29. The F-8 formulation was selected as optimized formulation.

3.3.3 Stability study

Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance. The average vesicle size of liposomes was found 223.61±0.83, 230.62±2.43 and 215.32±1.92nm after 1, 2 and 3 month of storage at 4.0 ±0. 2°C while at 25-28±2°C the average vesicle size was found 279.37±1.74, 321.86±2.34 and 409.78±3.24 nm after 1, 2 and 3 month of storage. % Entrapment efficiency in liposomal formulation was 63.56±2.72, 53.23±1.82 and 40.23±2.76 % after 1, 2 and 3 month of storage at 25-28±2°C while there was no significant changes in % drug remain and physical appearance in liposomal formulation was observed after 3 month of storage at 4ºC.

3.4 Evaluation of prepared liposomes gel

Table 3.2: Characterization of gel based formulation of liposomes.

Characterization	Viscosity (cps)	Release after 12hr	pH	Spreadibility (g.cm/sec)
Prepared Gel	2750±50	98.21±0.52	6.99-7.01	12.83±0.62

3.4.1 Stability studies

Stability study was carried out for drug loaded liposomes at two different temperatures i.e. refrigeration temperature (4.0 ±0. 2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

Table 3.3: Characterization of optimized formulation of liposomes.

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ± 0. 2°C	25-28± 2°C	4.0 ± 0. 2°C	25-28± 2°C	4.0 ± 0. 2°C	25-28± 2°C
Average particle size (nm)	223.61± 0.83	279.37± 1.74	230.62± 2.43	321.86± 2.34	215.32± 1.92	409.78± 3.24
% EE	74.65± 0.25	63.56± 2.72	72.23± 1.57	53.23± 1.82	69.24± 1.94	40.23± 2.76
Physical Appearance	Normal	High turbid	Normal	High turbid and agglomeration	Normal	High turbid and agglomeration

4. SUMMARY AND CONCLUSION

The preliminary study showed that Lincomycin HCL is white powder and Odorless or faint *odor*. It is Soluble in methanol and ethanol, butanol, isopropanol, ethyl acetate, n-butyl acetate, amyl acetate. The melting point was in the range of 140-142^{o}C which is in compliance with the standard value of 188-190^{o}C. From the FTIR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process drug and cholesterol has not reacted with the drug to give rise to reactant products. So there is no interaction between them which is in favor to proceed for formulation of vesicular drug delivery system. The U.V study shows that the drug and Excipient are compatible with each other.

Liposomes were prepared by rotator evaporation method. In the liposomal formulation, the lipid: surfactant ratio was optimized by taking their different ratio. The prepared formulations were optimized on the basis of average particle size and entrapment efficiency Drug concentration was optimized by taking different concentration of drug and prepared their formulation and all other parameter such as Soya PC: Span 80, sonication time kept remain constant. The formulation optimized on the basis of entrapment efficiency and average vesicle size.

Vesicle size of liposomes were examined under trinocular microscopic and also determined by light scattering method and found that average vesicle size of optimized formulation F-8 was 219.73±0.83 nm. Zeta potential was -31.80±0.25. % Entrapment efficiency of optimized liposomes formulation (F-8) was found 74.65±0.29%. Stability study data was revealed that the optimized formulation (F-8) stable after 3 month of storgae at 4ºC while at 25-28±3°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance. The pH was determined using digital pH meter. The pH values of the prepared gel were within acceptable limits of 6.99-7.01. Viscosity measurements of prepared topical liposome based gel was found to be 3550cps. The Spreadability of prepared liposomal gel was observed in all formulations at 12.83±0.62 g.cm/sec. *In vitro* drug release of prepared gel formulation after 12 hours 98.21±0.52.

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