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FORMULATION AND EVALUATION OF ELASTIC LIPOSOMES OF STREPTOMYCIN SULFATE

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ABSTRACT

In the past few decades, skin conditions have become more common, placing a heavy load on healthcare systems all around the world. The creation of novel medications has traditionally been a standard procedure in the search for safe and effective treatments. Thus, this study aims at formulation and evaluation of Elastic Liposomes of Streptomycin sulfate. The formulation & evaluation of liposomal gel was performed according to standard protocol. Results showed that. At first five formulations of liposomes were created. The vesicle size was found to be ranged from 169.98±2.85 nm in case of F5 to 232.26±3.32nm in case of F3. While the entrapment efficiency ranged from 64.58±0.23 % for F2 to maximum of 74.65±0.14 % in F5. The zeta potential was found to be - 25.4 mv for the same F5 formulation. Further by using F5 formulation the three different formulation of liposomal gel were made. The pH of gel LG 2 was also close to skin pH which is 6.81. The spreadibility for LG2 was found to be 10.26. The in vitro drug release study indicated in 10 hours the % cummulative drug release was found to be 98.45 %. The high R^2 values of 0.993 for Korsmeyer-Peppas equation suggest that these models provide a good description of the drug release behavior of the elastic liposome gel formulation. 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 physical appearance, average particle size and % drug content of the elastic liposomes gel. Thus from the results it can be interpreted that liposomal gel of Streptomycin sulfate could be used for treatment of bacterial skin infection.

Keywords: Skin infection, Novel drug delivery system, Liposomes, Lioposomal gel, Streptomycin sulfate

INTRODUCTION

Skin is the biggest organ in the body. It protects and covers your body among its many other functions. It helps keep infections at bay. However, the microorganisms can infrequently cause a skin condition. It frequently happens when the germs get into your body through a cut, crack, or wound on your skin. Other skin infections may form where skin scrapes on skin, particularly in wet regions. Additionally, infections can happen if your immune system is weakened as a result of another sickness or medical operation or if there is insufficient blood flow to a particular area of your body. The 28th most frequent diagnosis among patients admitted to hospitals is bacterial skin infections. The three bacterial skin infections that doctors see the most frequently are cellulitis, impetigo, and

folliculitis (Marques and Abbade, 2020; Ibrahim *et al*., 2015).

The creation of novel medications has traditionally been a standard procedure in the search for safe and effective treatments. However, it required a lot of time, work, and money throughout the extended gestation period. Later, it was discovered that the distribution of the medication within the biological system has a significant impact on concerns relating to efficacy and safety because there is noticeable departure from the target site, which is the planned site of action (Flohr and Hay, 2021; Seth *et al*., 2017).

Almost all administrative channels have been used to exploit the novel carriers. However, it has been determined that the topical method is one of the most pertinent for treating dermatological illnesses more successfully. These innovative dermatological systems are distinct in their composition and construction, including their exterior and internal design, from the usual formulations based on creams and ointments. The selection of the system depends on a number of pharmaceutical and dermatological factors as well as the need for the treatment and the ailment (Bueno *et al*., 2017; Vyas *et al*., 2021).

Due to their great biocompatibility, biodegradability, and minimal immunogenicity, liposomes are now the most widely employed nanocarriers for a variety of hydrophobic and hydrophilic compounds that may be biologically active. Additionally, liposomes have demonstrated improved drug solubility and regulated drug delivery, as well as the ability to modify their surface for targeted, extended, and sustained release. Liposomes can be thought of as having developed from traditional, long-circulating, targeted, and immunological liposomes to stimuli-responsive and actively targeted liposomes based on their composition. More liposomes have advanced stages in clinical trials, and many liposomal-based drug delivery systems are currently clinically licensed to treat a number of disorders, including cancer, fungal infections, and viral infections (Carita *et al*., 2018; Ternullo *et al*., 2019).

Despite the fact that liposomes showed promise for transdermal drug administration, there is limited actual use for these formulations on the skin. These, however, can be included in the gels that are applied to the skin. Liposomes put into the gels have been shown to be stable. Clinically appropriate hydrogels provide a number of benefits, including excellent rheological characteristics, good tissue compatibility, ease of handling, and ease of application. Pharmaceutical usage of carbopol gels is permitted through a number of various methods of administration. These gels have good rheological qualities that result in long residual durations at the site of administration and higher and maintained skin concentrations of medications as compared to ordinary gels and creams. This makes them ideal for usage topically (Madan *et al*., 2019; Paavola *et al*., 2000). Thus, this study aims at formulation and evaluation of Elastic Liposomes of Streptomycin sulfate.

MATERIALS & METHODS

Chemicals

Soya Phosphetidyl Choline, Disodium Hydrogen Phosphate, Di potassium Hydrogen Orthophosphate, Sodium Chloride, Methanol, Ethanol, Chloroform, Carbopol 934p, Methyl Paraben, Propyl Paraben, Propylene Glycol

was obtained from S.D Fine chemicals & Qualigens fine chemicals.

Preparation of Streptomycin sulfate loaded elastic liposomes

Elastic 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 of phospholipids and surfactant were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in minimum quantity of ethanol (5ml). 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 2% w/v of drug solution ethanol in 7% distilled water v/v, followed by rotating the flask containing mixture of drug by rotation at speed of 60 rev/min for 1 hr. After complete hydration of film, the prepared formulation of elastic liposomes was subjected to sonication at 4°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 (Hussain *et al.*, 2016).

Preparation of Gel Base

Carbopol 934 (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. 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 liposome containing gel, Elastic liposomes preparation corresponding to 0.75% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base (Sharma *et al.*, 2012).

Table 1: Optimization of lipid: surfactant concentration

Formulation code	Soya PC (% w/v)	Span 80 (% w/v)	Drug (mg)	Ethanol (ml)
F1	4	2	4	5
F2	5	3	6	5
F3	6	4	8	5
F4	7	5	10	5
F5	8	6	12	5
F6	9	7	14	5

Characterization of elastic liposomes

Microscopic observation of prepared elastic liposomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared elastic liposomes formulation.

Vesicle size

Microscopic analysis was performed to determine the average size of prepared elastic Liposomes. Formulation was diluted with distilled water and one drop was taken on a

glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer (Maurya *et al.,* 2010).

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the elastic liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Utreja *et al.,* 2011).

Entrapment efficiency

Streptomycin sulfate entrapped within the elastic liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the elastic liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai) at 18000 rpm at a temperature of 4°C for 45 minutes, where upon the pellets of liposomes and the supernatant containing free drug were obtained. The elastic liposomes pellets were washed again with phosphate buffer to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with phosphate buffer solution by measuring absorbance at 210 nm using Labindia 3000+ spectrophotometer (Nava *et al.,* 2011).

Characterization of Elastic Liposomes Containing Gel

Measurement of viscosity

Viscosity measurements of prepared topical liposomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm (Mitkari *et al.,* 2010).

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Patel *et al.,* 2001).

Drug content

Accurately weighed equivalent to 100 mg of topical liposome gel was taken in 10 ml volumetric flask, add 5 ml of methanol and sonicate it for 10 min and after sonication volume was made upto 10 ml with methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 0.1mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 210 nm. (Seth *et al.,* 2005).

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel

filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.,* (1956). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 20g of weight was noted, good spreadibility show lesser time to spread.

***In vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment.

A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength 210nm of drug

Stability Studies

Stability study was carried out for drug loaded elastic 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.

RESULTS AND DISCUSSION

The vesicle size was found to be ranged from 169.98±2.85 nm in case of F5 to 232.26±3.32nm in case of F3. While the entrapment efficiency ranged from 64.58±0.23 % for F2 to maximum of 74.65±0.14 % in F5. The zeta potential was found to be - 25.4 mv for the same F5 formulation. Further by using F5 formulation the three different formulation of liposomal gel were made. Three formulation namely LG1, LG2 & LG 3 were formulated. The % drug content was found to be maximum 99.65% in LG 2 formulation. The pH of gel LG 2 was also close to skin pH which is 6.81. The spreadibility for LG2 was found to be 10.26. While for LG1 & LG-3 the spreadibility was found to be 11.12 & 9.65 Gm.cm/sec respectively. Further the viscosity of LG1, LG2 & LG3 was observed to be 3658, 3545 & 3421cps. In the first 2 hours (0.5 to 2 hours), there is a relatively rapid drug release, with approximately 42.25% of the drug released by the 2-hour mark.

This initial burst release can be advantageous for achieving an immediate therapeutic effect. After the initial burst, the drug release continues at a slower rate. By the 4-hour mark, about 62.21% of the drug has been released, indicating a sustained release profile. This sustained release phase (up to 10 hours) suggests that the formulation may be suitable for achieving a prolonged therapeutic effect while minimizing the frequency of dosing. Thus, in 10 hours the % cummulative drug release was found to be 98.45 within 10 hours

The Regression analysis data of elastic liposomes gel formulation for Zero Order, First Order, Higuchi's Model and Korsmeyers Peppas Equation suggested the R^2 value of 0.973, 0.796, 0.991 & 0.993 respectively.

The high R^2 values for Higuchi's model and the Korsmeyer-Peppas equation suggest that these models provide a good description of the drug release behavior of the elastic liposome gel formulation (Batch LG2).

The presence of a sustained and controlled release mechanism, as suggested by these models, can be advantageous in pharmaceutical formulations, especially for drugs that require prolonged therapeutic effects with minimized side effects.

These regression analysis results are valuable for understanding the drug release kinetics of the formulation and can aid in further optimizing the formulation for specific therapeutic objectives.

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 physical appearance, average particle size and % drug content of the elastic liposomes gel.

Table 2: Evaluations of elastic liposomes for vesicle size and entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
F1	225.65±2.15	67.78±0.45
F2	215.62±1.65	64.58±0.23
F3	232.26±3.32	69.98±0.36
F4	210.14±4.85	72.25±0.25
F5	169.98±2.85	74.65±0.14
F6	220.14±2.14	70.32±0.23

Table 2: Evaluations of elastic liposomes for vesicle size and entrapment efficiency

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F1	225.65±2.15	67.78±0.45
F2	215.62±1.65	64.58±0.23
F3	232.26±3.32	69.98±0.36
F4	210.14±4.85	72.25±0.25
F5	169.98±2.85	74.65±0.14
F6	220.14±2.14	70.32±0.23

Table 3: Vesicle size and entrapment efficiency of optimized formulation

Formulation Code	Vesicle Size (nm)	Entrapment Efficiency (%)	Zeta potential (mV)
F5	169.98±2.85	74.65±0.14	- 25.4

Table 4: Results of elastic liposomes gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
LG-1	97.85	6.72	11.12	3658
LG-2	99.65	6.81	10.26	3545
LG-3	96.65	7.41	9.65	3421

Table 5: *In-vitro* drug release data for LG2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.70711	-0.301	18.85	1.275	81.15	1.909
1	1	0	29.95	1.476	70.05	1.845
2	1.41421	0.301	42.25	1.626	57.75	1.762
4	2	0.602	62.21	1.794	37.79	1.577
6	2.44949	0.778	73.36	1.865	26.64	1.426
8	2.82843	0.903	86.65	1.938	13.35	1.125
10	3.16228	1	98.45	1.993	1.55	0.190

Table 6: Regression analysis data of elastic liposomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
LG2	0.973	0.796	0.991	0.993

CONCLUSION

Elastic liposomes are specially designed particles or vesicles that can offer a fresh approach to issues with transport. three different Streptomycin sulfate elastic liposomal gel formulations were created and tested for several characteristics, including elasticity, yield, zeta potential, entrapment efficiency, and particle size. The batch LG-2 formulations were determined to be the best on the basis of many parameters. For a 21-day research, the chosen elastic liposomal gel formulations from batch LG-2 displayed good stability without significantly degrading their attributes at various temperatures. Accordingly, the study's findings show that

Streptomycin sulfate can also be packaged as a liposomal carrier, which is optimal for topical delivery.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Marques, S.A. & Abbade, L.P.F. (2020) Severe bacterial skin infections. *Anais Brasileiros de Dermatologia*, 95, 407–417
- Ibrahim, F., Khan, T. & Pujalte, G.G. (2015) Bacterial skin infections. *Primary Care*, 42, 485–499
- Flohr, C. & Hay, R. (2021) Putting the burden of skin diseases on the global map. *British Journal of Dermatology*, 184, 189–190
- Seth, D., Cheldize, K., Brown, D. & Freeman, E.F. (2017) Global burden of skin disease: Inequities and innovations. *Current Dermatology Reports*, 6, 204–210
- Bueno, J., Demirci, F. & Baser, K.H. Antimicrobial strategies in novel drug delivery systems: Applications in the treatment of skin and soft tissue infections. *In The Microbiology of Skin, Soft Tissue, Bone and Joint Infections* (2017 1 Jan), (pp. 271–286). Academic Press: Cambridge, USA.
- Vyas, T., Rapalli, V.K., Chellappan, D.K., Dua, K., Dubey, S.K. & Singhvi, G. (2021) Bacterial biofilms associated skin disorders: Pathogenesis, advanced pharmacotherapy and nanotechnology-based drug delivery systems as a treatment approach. *Life Sciences*, 287, 120148
- Carita, A.C., Eloy, J.O., Chorilli, M., Lee, R.J. & Leonardi, G.R. (2018) Recent advances and perspectives in liposomes for cutaneous drug delivery. *Current Medicinal Chemistry*, 25, 606–635
- Ternullo, S., Gagnat, E., Julin, K., Johannessen, M., Basnet, P., Vanić, Ž. & Škalko-Basnet, N. (2019) Liposomes augment biological benefits of curcumin for multitargeted skin therapy. *European Journal of Pharmaceutics and Biopharmaceutics*, 144, 154–164
- Madan, S., Nehate, C., Barman, T.K., Rathore, A.S. & Koul, V. (2019) Design, preparation, and evaluation of liposomal gel formulations for treatment of acne: In vitro and in vivo studies. *Drug Development and Industrial Pharmacy*, 45, 395–404
- Paavola, A., Kilpeläinen, I., Yliruusi, J. & Rosenberg, P. (2000) Controlled release injectable liposomal gel of ibuprofen for epidural analgesia. *International Journal of Pharmaceutics*, 199, 85–93
- Maurya, S.D., Prajapati, S., Gupta, A., Saxena, G. & Dhakar, R.C. (2010) Formulation development and evaluation of ethosome of stavudine. *Int. J. Pharm. Edu Res.*, 13, 16.
- Utreja, P., Jain, S., Yadav, S., Khandhuja, K.L. & Tiwary, A.K. (2011) Efficacy and toxicological studies of cremophor EL free alternative paclitaxel formulation. *Current Drug Safety*, 6, 329–338
- Nava, G., Pinon, E., Mendoza, L., Mendoza, N., Quintanar, D. & Ganem, A. (2011) Formulation and in vitro, ex vivo and in vivo evaluation of elastic liposomes

for transdermal delivery of ketorolac tromethamine. *Pharmaceutics*, 3, 954–970

- Mitkari, B.V., Korde, S.A., Mahadik, K.R. & Kokare, C.R. (2010) Formulation and evaluation of topical liposomal gel for fluconazole. *Indian Journal of Pharmaceutical Education and Research*, 44, 324–333.
- Patel, V.B., Misra, A.N. & Marfatia, Y.S. (2001) Preparation and comparative clinical evaluation of liposomal gel of benzoyl peroxide for acne. *Drug Development and Industrial Pharmacy*, 27, 863–869
- Seth, A.K., Misra, A. & Umrigar, D. (2004) Topical liposomal gel of idoxuridine for the treatment of herpes simplex: Pharmaceutical and clinical implications. *Pharmaceutical Development and Technology*, 9, 277–289