Development And Evaluation of Transferosomal Gel Using Cephalexin.

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Abstract

Background: In the present research work transferosomal gel was developed for an effective treatment of skin infection using cephalexin as model drug. The Transferosomes were prepared by thin film hydration method using Phospholipon 90H as a Lipid, Sodium deoxycholate as a surfactant and the vesicles were characterized on the basis of entrapment efficiency, particle size, Zeta potential, shape, morphology, x-ray diffraction analysis and in-vitro drug release. The transfersomal gel was prepared by dispersion method using Carbopol 934 as a gelling agent and evaluated for spreadability, viscosity, pH, drug release, antimicrobial study and stability.

Results: The entrapment efficiency of the optimized transferosomes was found to be 90.62% and the % Cumulative release of gel across the dialysis membrane was found to be 91.26% at the end of 24h.

Conclusion: Vesicular system can be of great help in decreasing the burden of Bacterial infections and other infections around the globe.

1 Introduction

Skin Infection is the most common condition in the ambulatory care. [1] Skin is the largest organ of the body, comprising up to 15% of the entire body weight. It performs various essential functions, including protection against external, physical, chemical, and biological agents, as well as prevention of excess water loss from the body and a role in thermoregulation. It is made up of three layers i.e., Epidermis, Dermis, Subcutaneous layer. [2]

Skin infection is caused due to microbial invasion of the skin and underlying tissues and depending on the severity of infection it can range from a from mild to serious life-threatening infections by just a tiny spot on the skin to the redness, swelling, pain, erythema onto the entire skin surface. The incidence of skin infections is increased due to rapidly growth in the number of aged populations, critically ill patients, immuno-compromised patients and also due to the emergence of multi-drug resistant pathogen. [3]

Often Drugs (topical, oral or parenteral antibiotics) are prescribed on the basis of condition of the skin infection. In the Literature survey it was seen, in case of mild impetigo and follicutis Topical mupirocin is given. While remaining infections are usually managed by either incision or oral and intravenous antibiotics. [4] Patients with some sign of gastric problems are treated ith parenteral therapy. Whereas patients with nomal health conditions are treated with oral antibiotics. [5]

Due to lack of permeation of most of the antibiotic agents from the topical formulations into sub dermal tissues and deeper skin layers therefore infections are mainly treated using high doses of oral or parenteral dosage form and because of this high dose of the antibiotic classes, there are various disadvantages which are as follow [5, 6]

1. Diarrhea- Most common in β lactams e.g., penicillin derived antibiotic, Cephalexin.

2. Nausea, vomiting- Seen in almost every antibiotic therapy.
3. Nephrotoxicity- Very common in Polypeptides e.g., Vancomycin.
4. Myotoxicity- Seen in cyclic lipopeptide e.g., Daptomycin.
5. Myelosuppression- Common in Linezolid.
6. Acute pancreatitis – Seen after Glycylcyclines e.g., aztreonam, tigecycline.
7. Mild thrombocytopenia- common in Oxazolidinones.
8. Drug Resistant- Very common in antibiotic therapy e.g. Cephalexin, Linezolid etc.

To improve patient compliance and its efficacy drug delivery system with modified Various drug delivery system modifies drug release profile, absorption, distribution and elimination are used. Conventional dosage form was unable to meet any of these needs. Most common routes of administration include oral, topical (skin), transmucosal (nasal, buccal, sublingual, vaginal, ocular and rectal) and inhalation route.

Out of the various drug delivery system, vesicles as a drug carrier system have become the vehicle of choice. Lipid vesicles were mostly used in immune therapy, biology of membrane and diagnostic technique and also in genetic engineering. [7] It provides an efficient method for delivery of drug to infection site, leading to reduce drug toxicity with lesser adverse effects. It reduces therapy cost by improving bioavailability of medication, especially in case of poorly soluble drugs and also by reducing the dose and dosing frequency thereby improving patient compliance. In vesicles both hydrophilic and lipophilic drugs can be incorporated. [8]

Transferosomes is commonly known as “Ultra Deformable Vesicles” and it contain a lipid vesicle made up of Phospholipids and an edge activator. Transferosomes passes stratum corneum layer by squeezing themselves many times smaller than its size owing to its elasticity nature which is achieved by mixing suitable surface-active components and lipids [9]

Drug carrier can be engineered to slowly degrade, react to certain stimuli (pH, Temperature) and be site-specific. The ultimate aim is to control degradation of drug and loss, prevention of harmful side effects and increase the availability of the drug at the disease site. [10] Encapsulation of a drug in vesicular structures can be designed to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity. [11, 12]

Topical route is the most preferred route for mild skin infection but due to poor permeability of most antibiotic agents into sub dermal tissues and deeper skin layers these routes becomes ineffective for most drugs. Transferosomes or ultra-deformable vesicles are one of the most superior drug delivery system for topic and transdermal drug delivery. It is said to pass through the intact skin very efficiently and move deep enough through the various layers of the skin and also get absorbed into the systemic circulation. [13] Transferosomes in the size range of 200–300 nm are reported to easily penetrate through the skin. [14] Therefore by making Transferosomes of the drug and loading it into topical formulations

many side effects can be avoided and the drug can reach under the layers of the skin as well as systemic circulation.

2 Results

2.1 Optimization and formulation of Transferosomes

2.1.1 Optimization of Transferosomes

Optimization of Transferosomes was done by selecting the ratio of Lipid and Surfactant, Temperature, method of preparation, type of hydration method, and also selection of amount of drug.

- **Selection of ratio of Lipid and Surfactant**

Ratio of Lipid and Surfactant plays a key role in the film forming ability of the Transferosomes. Therefore, it was optimized on the basis of the film forming ability (Table 1)

Table 1
Selection of Ratio of Lipid and Surfactant

Ratio	Amount of Lipid (mg)	Amount of Surfactant (mg)	Temperature	Observation
4 : 2	550	275	**50°C**	No film
4 : 1	**550**	**137.5**		**Film formed with good sediment**
4 : 0.5	550	68.75		Film formed but no sediment

- **Selection of Temperature**

In thin-film hydration method, at particular temperature organic solvents gets evaporated to form a thin-film. When higher temperature is used it leads to over drying of the thin-film whereas at lower temperature thin-film is not formed. Therefore, temperature was optimized on the basis of film forming ability (Table 2).

Table 2
Selection of Temperature

Temperature (°C)	Observation
45	No film
50	**Film formed**
60	No film

- **Selection of type of Hydration Method**

As drug was soluble in both organic solvent (Methanol) and Phosphate Buffer, therefore while preparing Transferosomes different batches was prepared and was optimized on the basis of amount of sediment and entrapment efficiency (%). And it was found that when the drug was dissolved in the Phosphate buffer it yields higher entrapment efficiency.

- **Selection of amount of Drug**

Amount of drug was selected on the basis of entrapment efficiency. Batches were prepared by keeping the concentration of Lipid 90H and Surfactant i.e., Sodium Deoxycholate constant and changing the drug concentration. 100 mg of the drug was selected as a final concentration as it had shown highest entrapment efficiency (Table 3).

Table 3
Selection of concentration of Drug

Amount of Drug (mg)	Amount of Lipid (mg)	Amount of Surfactant (mg)	Temperature (°C)	Entrapment efficiency (%)
25	550	137.5	50	15.23
50	550	137.5	50	31.346
100	**550**	**137.5**	**50**	**90**

2.1.2 Formulation of optimized Transferosomes

After optimization of various parameters, the optimized Transferosomes was prepared by using thin-film hydration method, using 550 mg of Lipid 90H, 137.5 mg of Sodium Deoxycholate. The organic solvent i.e., Methanol was evaporated at 50^{o}C using Rota vacuum evaporator until uniform thin-film is formed. The 100 mg of cephalexin was dissolved in hydration media i.e., Phosphate buffer (pH 7.4) and it was used as a hydration media to hydrate the thin-film for 30 minutes. After rehydration, milky white suspension was formed which was ultra-centrifuged using cooling centrifuge at 12000 rpm and 4^{o}C for

30 mins. Due to centrifugation final Transferosomes were obtained in the form of jelly like substance which was evaluated further.

2.2 Evaluation of optimized Transferosomes

The prepared Transferosomes were evaluated for the following parameters:

- **Entrapment efficiency**

The developed optimized formulations showed the highest entrapment efficiency i.e., 90.62%.

- **Vesicle size, distribution and zeta potential**

Vesicle size and size distribution are important parameters for drug permeation. The size and size distribution of optimized drug loaded formulation were determined by dynamic light scattering method (DLS) using a computerized inspection system by Malvern zetasizer. The vesicle size of Liposomes and Transferosomes were in the range of 175-178 nm with the polydispersity index of 0.526 (Table 4), indicating that the Transferosomes were homogeneously dispersed. [21] Zeta potential was found to be -32.6 mV (Table 4). Negative value of zeta potential might be due to the lipid composition in the formula. Phosphatidylcholine is a zwitterionic compound with the isoelectric point between 6 and 7. During the process of formulation phosphate buffer saline at pH 7.4 was used as the hydrating medium, where the pH was a little bit higher than the isoelectric point of phosphatidylcholine, causing phosphatidylcholine carried a negative charge. [22]

Table 4
Results of Particle Size, Distribution and Zeta Potential

Batch	Particle Size	Polydispersity Index	Zeta Potential
Optimized Batch	176.7 nm	0.526	-32.6 mV

- In-vitro **diffusion study**

Results revealed that the transferosomal suspension is having the highest cumulative amount of drug release (92.962%) up to 24 h (Figure 1). The release studies clearly indicated that transferosomal gel releases the drug in sustained manner. The highest release was observed in suspension, because of association of surfactant molecule with the phospholipid bilayer leading to better partitioning of the drug, and ultimately increase the drug release from the vesicles.

- **Scanning electron microscope (SEM)**

The surface morphology and three-dimensional nature of the Transferosomes were further visualized by SEM (Figure 2).

- **X-ray diffraction (XRD)**

The X-ray diffraction studies were carried out to identify the presence or absence of crystalline state of cephalexin in Transferosomes. XRD patterns with distinctive crystalline peaks of CEX are shown in (Figure 3). As seen in figure the XRD spectrum of CEX displayed sharp and intense peaks of crystallinity, which suggested a highly crystalline nature. The XRD spectra of the Transferosomes of Cephalexin showed a reduction of peak intensity (Figure 4), as compared to the CEX, which indicated decreased crystallinity or changes into an amorphous phase of the drug.

2.3 Optimization and formulation of Transferosomal Gel

2.3.1 Optimization of Transferosomal Gel

Optimization of Transferosomal gel was done on the basis of concentration of Carbopol 934 (0.25%, 0.5%, 1%, 1.5% and 2%) as described in the table below. Based on the various evaluation parameters such as appearance, pH, spreadability, viscosity and drug content, formulation 1 (F1) was selected as an optimized batch (Figure 5 & Table 5).

Table 5
Optimization of Transferosomal Gel

Ingredients	Quantity			
	F1	F2	F3	F4
Cephalexin	1%	1%	1%	**1%**
Carbopol 934	0.5%	1%	1.5%	**2%**
Propylene Glycol	0.1%	0.1%	0.1%	**0.1%**
Methyl Paraben	0.02%	0.02%	0.02%	**0.02%**
Triethanolamine	0.1%	0.1%	0.1%	**0.1%**
Distilled Water	q.s. 100g	q.s. 100g	q.s. 100g	**q.s. 100 g**

2.4 Evaluation of Transferosomal gel

- **Spreadability**

The spreadability of the prepared Transferosomal gel was found to be 46.15 g.cm/sec. This indicates that the gel can get easily spread out when applied on skin.

- **Viscosity**

Viscosity of the optimized Transferosomal gel was found to be 64,500 cps, which indicates that the gel is neither too viscose nor too soft.

- **pH**

The pH of the Transferosomal gel formulations was in the range of 7.2 ± 0.2 to 7.4± 0.2, which lies in the normal pH range of the skin and therefore it will not induce any skin irritation.

- In-vitro **diffusion study**

Results revealed that the Transferosomal gel is having the highest cumulative amount of drug release (91.26%) up to 24 h (Figure 6). The release studies clearly indicated that Transferosomal gel releases the drug in sustained manner.

2.4.1 Drug Content

Drug content of the formulation was found to be 96.52% which indicates that the drug is efficiently entrapped in the Transferosomal gel.

2.4.2 Microbial study

The microbial study of the Transferosomal gel formulation was carried out by zone of inhibition method on three different organisms i.e., *Escherichia coli, Staphylococcus aureus and Klebsiella pneumonaie* and the results are in below mentioned Table 6 & Figure 7 & 8.

The result showed that the formulation had exceptional anti-bacterial activity against the bacteria's.

Table 6
Microbial Study of Transferosomal Gel

Micro organism	Sample	Zone of Inhibition (mm)
E. Coli	0.8 %	35.33
	1 %	38.33
	Blank	No inhibition
S. Aureus	0.8 %	34.66
	1 %	37.33
	Blank	No inhibition
K. Pneumoniae	0.8 %	30
	1 %	32
	Blank	No inhibition

2.5 Stability Study of the Transferosomal Gel

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of environmental factors such as temperature, humidity and light. The stability of developed Transferosomal gel was established over a period of one month at 30 days, 60 days and 90 days' time intervals, the optimized formulation was kept at 40°C ± 2/RH70 % ± 5 and was evaluated for organoleptic properties, pH and drug content and the results are mentioned in the Table 7. Results indicates that the formulation was stable and no significant changes were observed.

Table 7
Stability study of Transferosomal Gel

Parameter	Initial	30 Days	60 Days	90 Days
Appearance	Light Yellow in color	No change	No change	No change
pH	7.3	7.26	7.29	7.23
Drug Content (%)	96.52	96.43	96.26	95.94

3 Discussion

The goal of this research was to increase the permeability of the poorly permeable drug, so as to increase its efficiency in treating the minor skin infection and also decrease the side effects/ adverse effects of the antibiotics. By referring the results, and also referring the previous authors work, it is safe to say that Transferosomes are effective in achieving the objectives of the research work. It was effective in increasing the permeability of the Cephalexin drug and it can also release the drug in controlled manner, thereby decreasing the antibiotic load which in turn decreases the possible side effects of the antibiotics. By interpreting the results of Anti-microbial test, it was also effective in showing the anti-bacterial activity.

4 Methods

4.1 Chemical and Reagents

Cephalexin was procured from local market, Mumbai, India. Phospholipon 90H was procured from Lipoid GmbH, Sodium deoxycholate from Vishal Chemical, India. Carbopol 934 was procured from Lubrizol Pvt Ltd., India. All the chemicals and reagents used were of analytical reagent grade.

4.2 Formulation and Development of Transferosomes

4.2.1 Optimization of Transferosomes

- **Selection of Lipid**

A particular amount of lipid such as 90G, 90H, S75 and S100 was dissolved in methanol and a desired concentration solution was prepared and absorbance was taken at 260 nm. On the basis of low absorbance value S100 and 90H was selected.

- **Selection of Surfactant**

A particular amount of surfactant such as SLS, Span 60, Span 40, sodium cholate and sodium deoxycholate was dissolved in methanol and a desired concentration solution was prepared and absorbance was taken at 260 nm. On the basis of low absorbance value sodium deoxycholate was selected.

- **Selection of Ratio of Lipid and Surfactant**

The batches of various ratios of Lipid: Surfactant was made and was selected on the basis of tranferosomal film formation.

- **Selection of Temperature**

The batches at various temperatures were made and selected on the basis of tranferosomal film formation.

- **Selection of Method of preparation**

Batches were prepared using various methods of preparation and final method was selected on the basis of entrapment efficiency.

- **Selection of type of hydration method**

Batches were made by dissolving drug in organic solvent and water, and the method was selected on the basis of film forming capacity.

- **Selection of amount of drug**

Amount of drug was selected on the basis of entrapment efficiency and antimicrobial study.

4.2.2 Formulation of Transferosomes

Transferosomes were prepared using thin-film hydration method. Thin-film hydration method involves two steps i.e., formation of thin-film and hydration of the thin-film. So, to form thin- film Lipoid 90H and Sodium deoxycholate was dissolved in required amount of organic solvent i.e., Methanol in RBF and using Rotary vacuum evaporator at 50^{o}C the organic solvent was evaporated. In a separate beaker, drug (CEX) was dissolved in 10ml Phosphate buffer pH 7.4. Using this solution hydration of thin-film was done for 30mins. Due to hydration milky white suspension gets formed which was ultra-centrifuged using cooling centrifuge at 12000 rpm and 4^{o}C for 30 min. Due to centrifugation final Transferosomes are obtained in the form of jelly like substance. [12, 15]

Evaluation of Transferosomes

The prepared Transferosomes were evaluated for the following parameters:

- **Entrapment efficiency**

For the determination of entrapment efficiency, the unentrapped drug was separated using centrifugation method. The resulting solution was then separated, and supernatant liquid was collected. The collected supernatant was then diluted as specified and assayed for calculating unentrapped drug concentration by UV spectrophotometer at 260 nm. [8]

The entrapment efficiency (EE %) was calculated as follows:

$$EE\% = \frac{(TotalDrug - UnentrappedDrug)}{TotalDrug} \times 100$$

- **Vesicle size, distribution and zeta potential**

Vesicle size, size distribution and zeta potential of the pure hydrated solution was measured using zeta-sizer.

- In-vitro **diffusion study**

The *in-vitro* diffusion study was carried out using Franz diffusion cell. The receptor compartment was filled with 20 ml of Phosphate buffer pH 7.4. Calculated quantity of sediment was kept in donor compartment over a dialysis membrane. The aliquot of 1 ml was taken in suitable interval of time for 18 h and analyzed using UV spectrophotometer at 260 nm. [10]

- **Scanning electron microscope (SEM)**

Surface characteristics such as uniformity in diameter, smoothness of surface etc. are important parameters to evaluate the Transferosomes. Surface morphology of the Transferosomes was analyzed using Scanning electron microscope.

- **X-ray diffraction (XRD)**

X-ray diffraction (XRD) analysis is used to illustrate the crystalline structures of CEX powders and prepared Transferosomes.

4.3 Optimization and formulation of Transferosomal Gel.

Transferosomal gel was prepared using sediment i.e. Transferosomes, 0.25% of Carbopol 934, 0.5% of propylene glycol and 0.02% of methyl paraben. Optimization of the prepared gel was done based on concentration of Carbopol 934 (0.25%, 0.5%, 1%, 1.5% and 2%). In this, Carbopol 934 was allowed to disperse in sufficient quantity of Distilled water for 1 h. In separate beaker methyl paraben was dissolved

in another part of water. After 1 h of soaking of Carbopol 934, sediment, methyl paraben solution and propylene glycol was mixed together using mechanical stirrer and was stirred for 10 min till Transferosomal gel was formed and the pH was adjusted using Triethanolamine. [11]

Evaluation of Transferosomal gel

Evaluation of gel was done based on following parameters:

1. Spreadability

Spreadability of the formulation was determined by an apparatus consisting of a wooden block, which was provided by a pulley at one end. Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (About 2 g) was placed on the ground slide. The gel was sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. 100 g weight was placed on top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scraped off from the edges. The top plate was then subjected to pull of 100 g with the help of string attached to the hook and the time (in seconds) required by the top slide to cover distance of 30 cm was noted. A shorter interval indicates better spreadability. Spreadability was calculated using the formula: [16]

Where,

L = Length of Glass Slide (cm),

W = Weight Applied (g)

T = Time required for separation of Slide (sec)

$$Spreadability = \frac{L \times W}{T}$$

2. Viscosity

Viscosity was measured on Brookfield Viscometer Instrument using Spindle no. 6 at 10 rpm for about 2 min. [16]

3. Ph

pH of the formulated gel was determined using Calomel glass electrode (pH meter) at room temperature. The pH meter was calibrated using standard buffer solutions of pH 4 and pH 7 before use. 1 g of gel was

dissolved in about 10 ml of water and stirred until it forms dispersion, kept it aside for 2 hours. The volume was made up to 100 ml and pH of the dispersion was measured with the help of calibrated pH meter. [17]

4. *In-vitro* diffusion study

The *in-vitro* diffusion study was carried out using Franz diffusion cell. The receptor compartment was filled with 20 ml of Phosphate buffer pH 7.4. Calculated quantity of gel was kept in donor compartment over a dialysis membrane. The aliquot of 1 ml was taken in suitable interval of time for 24 h and analyzed using UV spectrophotometer at 260 nm. [18]

5. Drug Content

Transferosomal gel equivalent to 0.1 mg drug was mixed vigorously in 10 ml Methanol till maximum gel gets dissolved. The solution was then filtered using Whatman filter paper and 1 ml of the filtered solution was again diluted using 10 ml Methanol. The absorbance of the final solution was taken in UV Spectrophotometer at 260 nm against blank gel solution prepared in the similar manner. [19]

6. Microbial Study

The microbial study of the Transferosomal gel formulation was carried out by zone inhibition method by using nutrient broth medium on three different organisms i.e., *Escherichia coli, Staphylococcus aureus and Klebsiella pneumonaie*. [20]

7. Stability Study Of The Transferosomal Gel

The stability of developed formulation was established over a period of 1 month as per ICH conditions. The Transferosomal gel was stored at 40^{o}C ± 2^{o}C/75% RH for a period of 1 month. The Transferosomal gel was then evaluated at 7, 15 days and 1 month. Following parameters were evaluated.

5 Conclusions

A recent approach to TDDS (Transdermal drug delivery system) is to deliver the drug via Transferosomal gel into systemic circulation at predetermined rate using skin as a site of application. Transferosomes- a type of vesicular drug delivery system has ability of increasing penetration of the drugs and it can sustain the drug for longer period thereby reducing the dose and dosing frequency of the drugs so as to decrease the side effects and improves the patient compliance. [5] The result of present study indicate that the Transferosomal Gel formulated by using Lipid 90H and Sodium deoxycholate can be used to enhance transdermal drug delivery of CEX because of excellent release and permeation of the drug. The

microscopic study indicated that the Transferosomes were uniform in diameter with smooth surface. The Transferosomal Gel showed a good antibacterial activity. Hence, the Transfersomal gel can be effectively utilized for transdermal drug delivery in various skin condition.

6 List Of Abbreviations

Sr. No.	ABBREVIATIONS	FULL FORM
1	*S. epidermidis*	*Staphylococcus epidermidis*
2	*S. aureus*	*Staphylococcus aureus*
3	*S. pyogenes*	Streptococcus pyogenes
4	rpm	Rotation per minute
5	MLV	Multilameller vesicles
6	ULV	Unilamellar vesicles
7	LUV	Large unilamellar vesicles
8	w/o	Water in oil
9	o/w	Oil in water
10	% EE	Entrapment Efficiency
11	mV	Millivolt
12	DLS	Dynamic light scattering
13	PVA	Poly vinyl alcohol
14	g	Gram
15	mg	Miligram
16	^{o}C	Degree Celsius
17	µg	Microgram
18	ml	Millilitre
19	nm	Nanometre
20	cm	Centimetre
21	µS	Microsiemens
22	BCS	Bio-pharmaceutical classification system
23	IR	Infrared spectrometer
24	FTIR	Fourier-transfer Infrared spectrometer
25	UV	Ultra-visible
26	SEM	Scanning electron microscope
27	XRD	X-ray diffraction
28	SD	Standard Deviation

29	RSD	Relative Standard Deviation
30	CEX	Cephalexin
31	mM	milimolar
32	RH	Relative Humidity
33	TDDS	Transdermal Drug Delivery System
34	q.s	Quantity Sufficient
35	sec	Seconds
36	mg	Miligram
37	e.g.	Example

Declarations

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References

1. Hersh, A. L., Chambers, H. F., Maselli, J. H. & Gonzales R. National Trends in Ambulatory Visits and Antibiotic Prescribing for Skin and Soft-Tissue Infections. *Arch. Intern. Med*, **168** (14), 1585–1591 (2008).
2. Dryden, M. S. 2009, Skin and Soft Tissue Infection: Microbiology and Epidemiology. *Int. J. Antimicrob. Agents, 34* (SUPPL. 1), S2–S7.
3. Ki, V. & Rotstein, C. Bacterial Skin and Soft Tissue Infections in Adults: A Review of Their Epidemiology, Pathogenesis, Diagnosis, Treatment and Site of Care. *Can. J. Infect. Dis. Med. Microbiol*, **19** (2), 173–184 (2008).
4. Sukumaran, V. & Senanayake, S. Bacterial Skin and Soft Tissue Infections. *Aust. Prescr*, **39** (5), 159–163 (2016).
5. Eron, L. J. *et al.* ; 2003, Managing Skin and Soft Tissue Infections: Expert Panel Recommendations on Key Decision Points. *J. Antimicrob. Chemother, 52* (SUPPL. 1), 342-420.
6. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition.* CLSI document M02-A11 (ISBN 1-56238-781-2 [Print]; ISBN 1-56238-782-0 [Electronic]).

7. Ogihara-Umeda, I. *et al.* Rapid Diagnostic Imaging of Cancer Using Radiolabeled Liposomes. *Cancer Detect. Prev*, **21** (6), 490–496 (1997).
8. Ceve, G. Lipids vesicles and other colloids as drug carrier on the skin. *Adv Drug Del Rev*, **56**, 675–711 (2004).
9. Bansal, S., Kashyab, C., Aggarwal, G. & Harikumar, S. L. A comparative review on Vesicular Drug delivery system and stability issues. *International journal of research in pharmacy and chemistry;*, **2** (3), 704–713 (2012).
10. Patel, H. M., Stevenson, R. W., Parsons, J. A. & Ryman, B. E. Use of Liposomes to Aid Intestinal Absorption of Entrapped Insulin in Normal and Diabetic Dogs. *Biochim Biophys Acta- Gen. Subj*, **716** (2), 188–193 (1982).
11. Todd, J. A., Modest, E. J., Rossow, P. W. & T. Z Liposome Encapsulation Enhancement of Methotrexate Sensitivity in a Transport Resistant Human Leukemic Cell Line. *Biochem. Pharmacol;*, **34** (4), 541–546 (1982).
12. Heather, A. E. B. Transfersomes for Transdermal Drug Delivery. *Expert Opin. Drug Delivery*, **3** (6), 727–737 (2006).
13. Zhang, J. P., Wei, Y. H., Zhou, Y., Li, Y. Q. & Wu, X. A. Ethosomes, Binary Ethosomes and Transfersomes of Terbinafine Hydrochloride: A Comparative Study. *Arch. Pharm. Res*, **35** (1), 109–117 (2012).
14. Murthy, S. N., Shivakumar, H. N. & Vitthal, S. 2010, Chapter 1 - Topical and Transdermal Drug Delivery, First Edit.;Kulkarni,23–32
15. Biju, S. S., Talegaonkar, S., Mishra, P. R. & Khar, R. K. ; 2006, Vesicular System: An Overview; Indian Journal of Pharmaceutical science; 68(2), 141-153.
16. Gurjar, B. N. & B. S. and S. K Development and Evaluation of Cinnamon Oil and Aloe Vera Containing Anti-Acne Gel. *Int. J. Pharm. Ind. Res*, **2** (4), 446–451 (2012).
17. Kalpesh, K. *et al.* ; 2012, Development and Evaluation of Herbal Anti-Acne Formulation. *Res. J. Pharm. Biol. Chem. Sci.*; ISSN: 09758585; *3* (3), 334–339.
18. Singh, V. K. et.al; 2013, Formulation and Evaluation of Topical Gel of Acelofenac Containing Piparine. *Indo Am. J. Pharm Res.; 3* (7).
19. Guleri, K. T. & Preet, K. L. Formulation and Evaluation of Topical Gel of Acelofenac. *J. Drug Deliv. Ther*, **3** (6), 51–53 (2013).
20. Felmingham, D., Robbins, M. J., Sangharajkam., L. A. & R. G. L In vitro Activity of Some 14-, 15-, and 16-Membered Macrolides against Staphylococcus Spp., Legionella Spp., Mycoplasma Spp. and Urea Plasma Urealyticum. *Drugs under Experimental and Clinical Research, PMID-*, **1650694** (2), 91–99 (1991).
21. Wu, P. S., Li, Y. S., Kuo, Y. C., Tsai, S. J. J. & Lin, C. C. ; 2019, Preparation and Evaluation of Novel Transfersomes Combined with the Natural Antioxidant Resveratrol. *Molecules; 24* (3), 600.

22. Surini, S. & Joshita Djajadisastra, S. Formulation and in Vitro Penetration Study of Transfersomes Gel Containing Gotu Kola Leaves Extract (Centella Asiatica L. Urban). *J. Young Pharm*, **10** (1), 27–31 (2018).

Figures

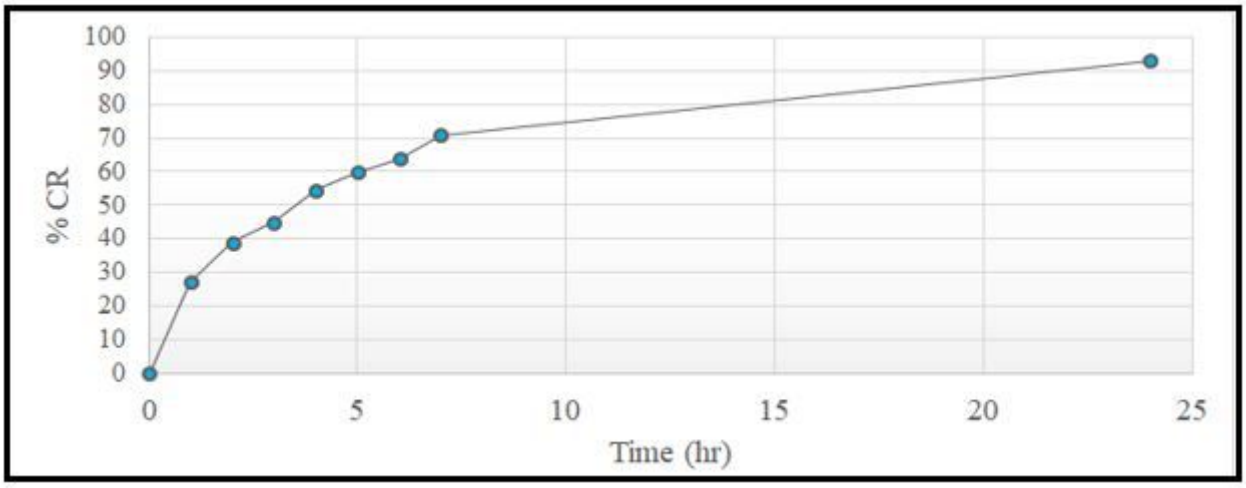

Figure 1

In-vitro diffusion study of Transferosomes

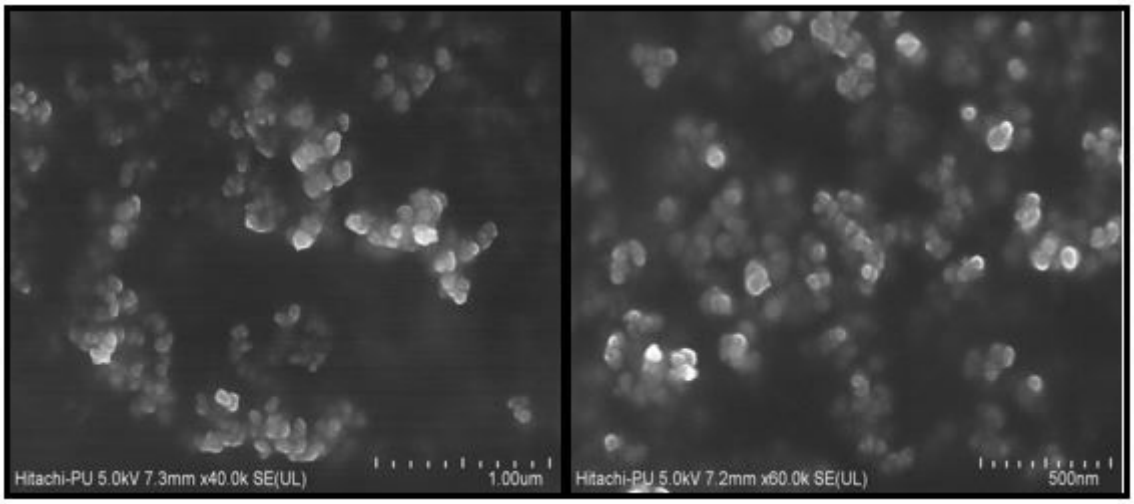

Figure 2

SEM analysis of Transferosomes

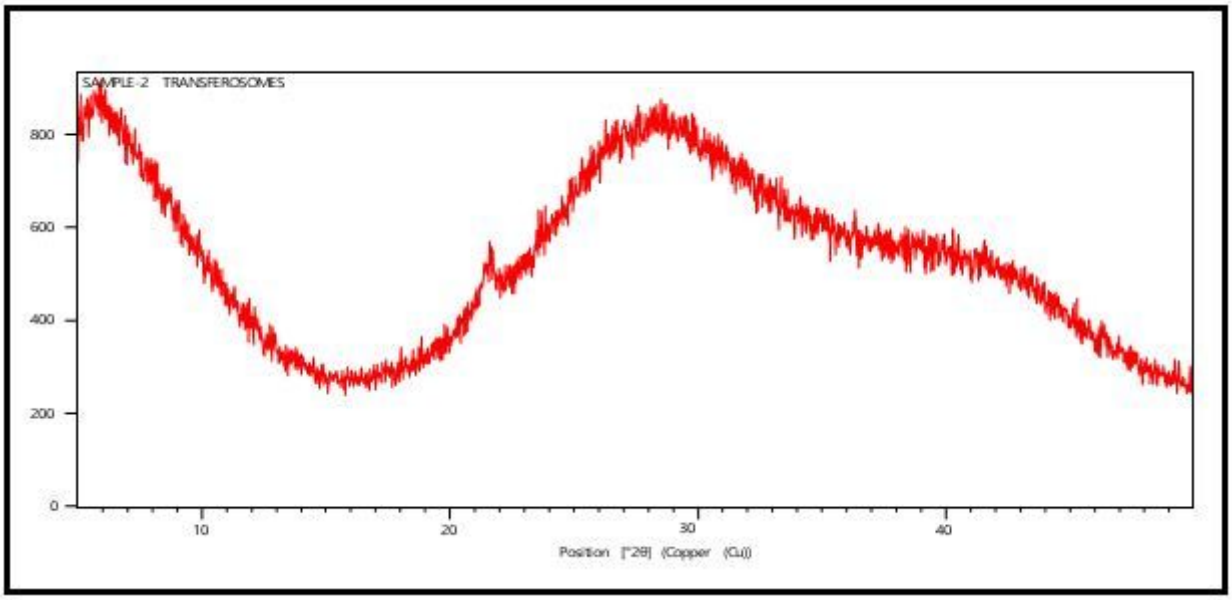

Figure 3

XRD of Cephalexin Transferosomes

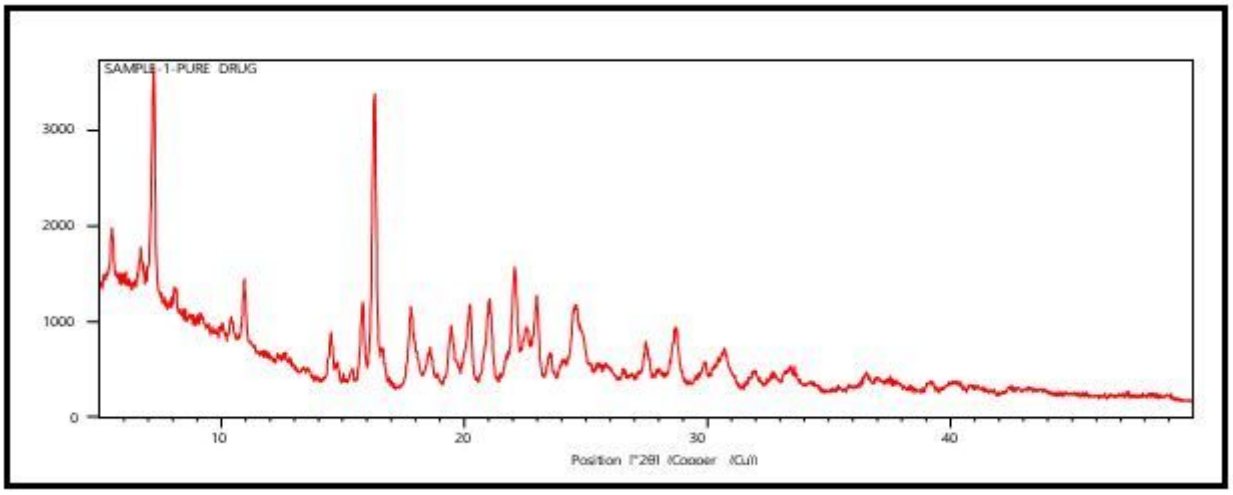

Figure 4

XRD analysis of Cephalexin

Figure 5

Optimized Transferosomal Gel

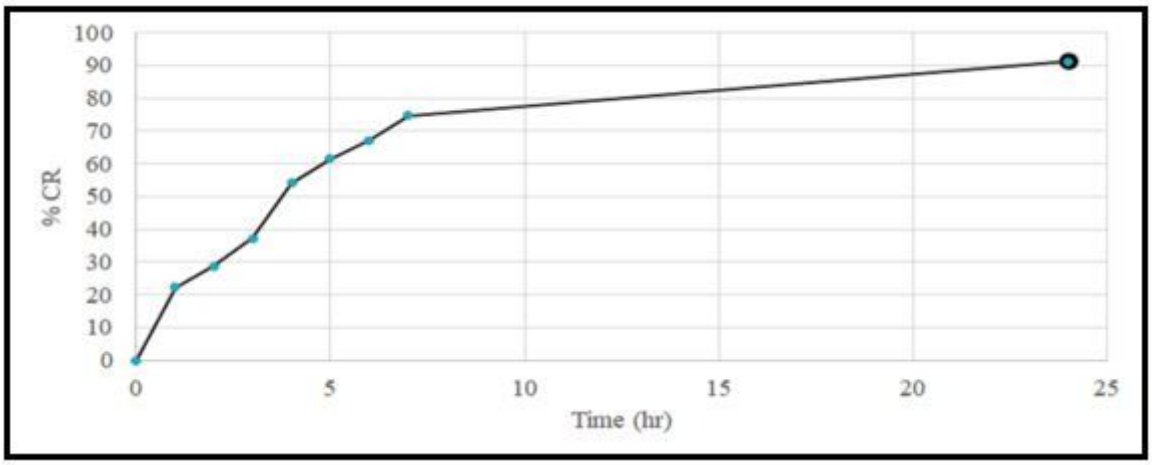

Figure 6

In-vitro diffusion study of Transferosomes

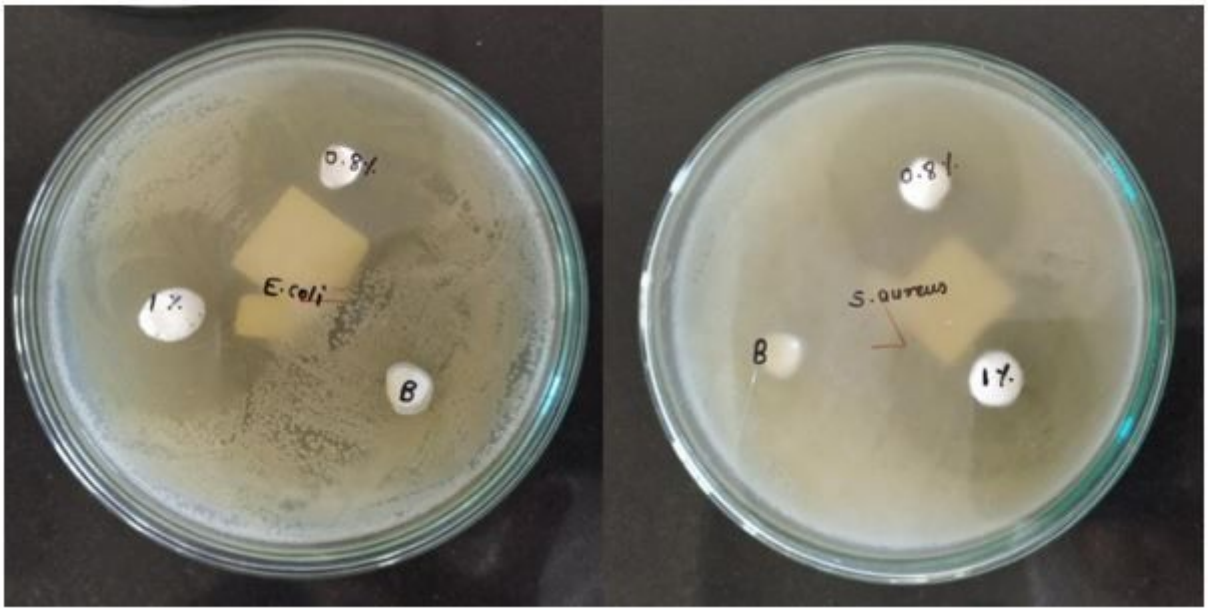

Figure 7

E. coli and S. aureus

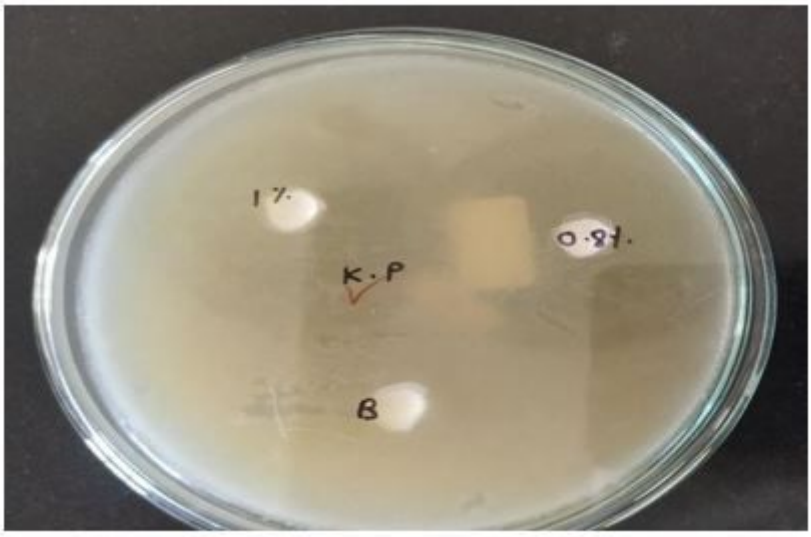

Figure 8

K. pneumonia