FORMULATION AND EVALUATION OF TRANSFEROSOMAL GEL LOADED WITH CEFADROXIL FOR TRANSDERMAL DELIVERY

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Abstract:

In the present research work transferosomal gel was developed for an effective treatment of skin infection using cefadroxil as model drug. The Transferosomes were prepared by thin film hydration method using Phospholipon 90H as a Lipid, Sodium deoxycholate as a surfactant. The transfersomal gels were prepared by dispersion method using Carbopol 934 as a gelling agent. The vesicles were characterized on the basis of entrapment efficiency, vesicle size, Poly dispersity Index, Zeta potential, morphological Characterization of Transferosomes, SEM. The transferosomal gels were evaluated on the basis of Physical appearance, Drug Content, pH, Rhelogical studies, spreadability, Extrudability, In vitro drug release studies, and stability Studies.

Keywords: Cefadroxil, transferosomes, vesicles, gel, antimicrobial.

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, Cefadroxil.
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, Cefadroxil, 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 systems, 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. [13] 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.

Materials and Method

List of materials used

Table.1: List of Chemicals

S. No.	Materials	Manufacturer
1.	Cefadroxil	Zeiss Pharma Ltd
2.	Lipoid-90-H	Shree Pharma International
3.	Sodium Deoxycholate	Alpha chemika
4.	Methanol	Alpha chemika
5.	Phosphate Buffer	Wagle Industrial Estate, Thane, Maharashtra
6.	Carbopol 934	Alpha chemika
7.	Methyl Paraben	Hexon Laboratories Private Ltd., Nashik
8.	Propylene glycol	Nice Chemicals Pvt Ltd., Cochin
9.	Triethanolamine	Thermo Fisher Scientific India Pvt.Ltd., Mumbai

Table.2: List of Equipments

S.No	Instruments	Manufacturer
1.	Digital Weighing Balance	Shimadzu, Japan
2.	UV/VIS Spectrophotometer	Shimadzu, Japan
3.	Magnetic Stirrer	Remi Equipments, Mumbai
4.	Melting Point Apparatus	Remi Equipments, Mumbai
5.	pH Meter	Ohaus, USA
6.	Infrared Spectrophotometer (FTIR)	Perkin Elmer, Germany
7.	Franz Diffusion cell assembly	Orchid Scientific, Mumbai
8.	Water bath	Sunshine Scientific Equipments
9.	Brookfield Digital Viscometer	Dolphin Pharmacy Instruments Pvt.Ltd., Mumbai

FORMULATION

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 (Cefadroxil) 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[14].

Formulation of Transferosomal Gel:

Transferosomal gel were prepared by using sedimentation method i.e. Transferosomes, 5g of Carbopol 934, 1g of propylene glycol and 0.2g of methyl paraben. Optimization of the prepared gel was done based on concentration of Carbopol 934 (5g, 10g, 15g, 20, 25g, 30g). 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[15].

EVALUATION

Evaluation parameters of Transferosome formulations:

Drug entrapment efficiency:

The drug entrapment efficiency of formulated transfersomes was estimated by separating the transferosomes by ultracentrifugation at 10000 rpm for 30 min. the sum of free Cefadroxil in the supernatant was calculated by UV spectrophotometer at 232nm. The drug loading efficiency in the prepared transfersomes was calculated by the following formula:

Entrapment efficiency (%) = Tp-Tf/Tp x100

Where, Tp = Total amount drug, Tf =free drug

Vesicle size:

The transferosomes samples were suspended in Milli-Q water and screened for vesicle size at 25^{o}C by Zetasizer (Nano-ZS90, Malvern Instruments, UK). The disposable cuvettes were used for sample analysis. The results were reported as the mean ± standard deviation for tree replicates

Polydispersity Index

The transferosomes samples were suspended in Milli-Q water and screened for PDI at 25^{o}C by Zetasizer (Nano-ZS90, Malvern Instruments, UK). The disposable cuvettes were used for sample analysis. The results were reported as the mean ± standard deviation for tree replicates

Zeta Potential:

The transferosomes samples were suspended in Milli-Q water and screened for zeta potential at 25^{o}C by Zetasizer (Nano-ZS90, Malvern Instruments, UK). The disposable cuvettes were used for sample analysis. The results were reported as the mean ± standard deviation for tree replicates[16].

Scanning Electron Microscope(SEM) :

Scanning electron microscope is used to attain scanning electron micrographs of Cefadroxil containing Transferosomes. The instrument used for this purpose is Hitachi S-4800scanning electron microscope. The microsphere were assembled directly on the SEM sample stub, using double sided sticking tape, and coated with gold film (thickness 200nm) under reduced pressure (0.001 torr)[17].

Evaluation parameters of Transferosomal Gel formulations:

Physical appearance

The prepared transferosomal gel formulations were inspected visually for their color, homogeneity, consistency, grittiness and phase separation.

Drug content

Drug content of the transferosomal gel was determined by dissolving an accurately weighed quantity of 1 g gel in about 100ml of methanol. 2ml of this solution was diluted to 10ml with methanol solutions were then filtered and spectrophotometrically analyzed for drug content at 285nm. Drug content was determined from the standard curve of cefadroxil[18, 19].

pH Determination

1g of gel was accurately weighed and dispersed in 100ml of distilled water. The pH of dispersion was measured by using digital pH meter.

Rheological studies

Brookfield digital viscometer was used to measure the viscosity (in cps) of the prepared transferosomal gel formulation. The spindle number 62 was rotated at 50rpm for the viscosity measurement. The viscosity of the formulated batches was determined using a cone and plate viscometer with spindle 7(Brookfield engineering Laboratories). The assembly was connected to a thermostatically controlled circulating water bath maintained at 25^{o} C. The formulation whose viscosity was to be determined was added to a beaker covered with thermometer jacket. Spindle was allowed to move freely into the transferosomal gel. And reading was noted.

Spreadability

Spreadabilty of the formulation was determined by using an apparatus designed and developed in the laboratory especially for the project and diagram of the apparatus. Two rectangular glass plates of standard dimension were selected. 500mg of the sample was placed on one of the glass plate. Second plate was placed over the other one to sandwich sample between plates. A 20gm weight was placed on the top of upper plate to provide a uniform thin film of the sample between the plates. Weight was removed excess of the of the gel sample was scrapped off from the edges. The top plate was then subjected to pull by using string to which 50gm weight was applied. The time required by the upper plate to travel a distance of 6cm and separate from the lower plate was noted.

A shorter interval indicated better spreadability. Experiment was repeated and averages of three attempts were calculated for each formulation using formula

Spreadability= (M×L) /T

M= weight tied to upper side

L = length of the glass slide

T= time in second

Extrudability

The development formulations were filled in collapsible metal tubes and crimped at one end. After removing the cap tube is pressed to extrude the product from the tube[20].

In Vitro drug release of transferosomal gel formulations loaded with Cefadroxil

The in Vitro drug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on dialysis membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer pH 7.4 was used as a dissolution media. The temperature of the cell was maintained at 37^{o} C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar blank set was run simultaneously as a control. Sample (5ml) was withdrawn at suitable time intervals and replaced with equal amount of fresh dissolution media. Samples were analyzed spectrophotometrically at 285nm and the cumulative % drug release was calculated. The difference between the reading of drug release and control was used as the actual reading in each case[21].

In- vitro release kinetics of Transferosomal gel formulations:

Zero- order kinetics:

Following this profile, prescription dosage formulation emits the same volume of medication per unit of time, rendering it the perfect type of drug release for achieving pharmacologically extended operation. This model can be represented in a simple way using the following relation:

$$Qt = Qo + Kot$$

Where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution (most time, $Q_o = 0$) and K_o is the zero order release constant.

First- order kinetics

The following relation expresses this model:

$$\log Qt = \log Qo + \frac{k1t}{2.303}$$

Where Q_o is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution and K1 is the zero order release constant.

A graph of the decimal logarithm of the drug's published number Vs time would be linear as a result. Pharmaceutical dosage formulations that adopt this dissolution profile, such as those

containing water- soluble drugs in porous matrices, release medication proportionally to the amount of drug remaining in their interior, resulting in a reduction in the amount of drug released per unit of time.

Higuchi model

Higuchi devised a number of experimental models to investigate the release of water- soluble and low- soluble drugs in semi-solid and solid matrixes. For drug particles scattered in a uniform matrix acting as diffusion media, mathematical expressions were obtained.

The simplified Higuchi model is expressed as:

$$\boxed{Q = KH.t1/2}$$

The amount of drug release in time t is Q, and Higuchi dissolution constant is KH. The Higuchi model depicts drug release as a square root time dependent diffusion mechanism based on Fick's law. This association can be used to explain the degradation of water- soluble medications from a number a modified released prescription dosage formulation, such as transdermal systems and matrix tablets.

Korsmeyer- Peppas model:

Korsmeyer et al. used a simple empirical equation to describe general solute release behavior from controlled release polymer matrixes:

$$\boxed{\frac{Mt}{M\infty} = at^n}$$

Where, $M_t/M\infty$ is fraction of drug released is a kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log $M_t/M\infty$ versus log time curve. Regardless of the release process, Peppas stated that the above equation could accurately explain the release of solutes from slabs, spheres cylinders and disks. Peppas used this n value in order to characterize different release mechanism, concluding for values for slab, of n= 0.5 for Fickian diffusion and higher values of n, between 0.5 and 0.1, or n= 1.0, for mass transfer following a non- Fickian model. In case of a cylinder n= 0.45 instead of 0.5 and 0.89 instead of 0.1. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent n the portion of the release curve where $M_t/M\infty < 0.6$ should only be used. To use this equation, the release must be one- dimensional and the device width – thickness or length- thickness relationship must be at least ten. To account for the lag time (1) at the start of drug release from the pharmaceutical dosage type, a modified version of this equation was developed:

$$\frac{Mt}{M\infty} = a(t - l)n$$

When there is the possibility of a burst effect, b, this equation become:

$$\boxed{\frac{Mt}{M\infty} = at^n + b}$$

The 1 and b values would be zero if there was no lag time or burst effect, and only atn would be used. This statistical model, also known as power Law has been used to explain the release of a number of prescription adjusted release dosage types on a daily basis[22].

Stability studies

The main objective of stability testing is to give evidence on the changes of quality of drug product with respect to time under the influence of various environmental factors such as temperature, humidity, and light and enables recommended storage conditions; re-test periods and shelf lives to be accomplished. According to the ICH guidelines the optimized formulation was kept for accelerated stability for six months. Microspheres were kept in stability chamber maintained at temperature of 40ºC±2ºC/75% RH±5% RH. During the study period, the formulation was monitored at prearranged time intervals of 0, 15, 30, 45, 60, 75, 90, 180 days for change in physical appearance, drug content and in- vitro release characteristics[23].

RESULT AND DISCUSSION

FORMULATION

Formulation of Transferosomes formulations:

Transferosomes were prepared by using thin-film hydration method. Carriers that are used for the preparation of transferosomes were mentioned in **table 3.**

Table 3: Composition of Transferosomes

Sr. No	Formulation Code	Drug (g)	Lipoid 90H(g)	Sodium deoxycholate (g)	Methanol (ml)	Phosphate buffer(ml)
1.	T1	100	665	35	10	10
2.	T2	100	630	70	10	10
3.	T3	100	595	105	10	10
4.	T4	100	560	140	10	10
5.	T5	100	525	175	10	10
6.	T6	100	490	210	10	10

Formulation of Transferosomal Gel:

Transferosomal gel was prepared by using sedimentation method. Carriers that are used for the preparation of transferosomes were mentioned in **table 4.**

Table 4: Formulation of Transferosomal Gel loaded with Cefadroxil

Sr. No	Formulation Code	Carbopol 934(g)	Propylene glycol(g)	Methyl paraben(ml)	Triethanolamine(ml)	Water(ml)
1.	TG1	5	1	0.2	1	Q.S
2.	TG2	10	1	0.2	1	Q.S
3.	TG3	15	1	0.2	1	Q.S
4.	TG4	20	1	0.2	1	Q.S
5.	TG5	25	1	0.2	1	Q.S
6.	TG6	30	1	0.2	1	Q.S

EVALUATION

Evaluation of Transferosomes loaded with Cefadroxil

Entrapment Efficiency

Percentage Drug Entrapment and loading of all formulation was given in table 5.

Table 5: Percentage Entrapment efficiency of Transferosomes

S.No.	Formulation Code	(%)Entrapment efficiency
1	T1	66.96±1.89
2	T2	79.87±2.35
3	T3	55.69±2.84
4	T4	61.54±2.02
5	T5	65.98±2.11
6	T6	65.89±2.99

Mean±SD,n=3

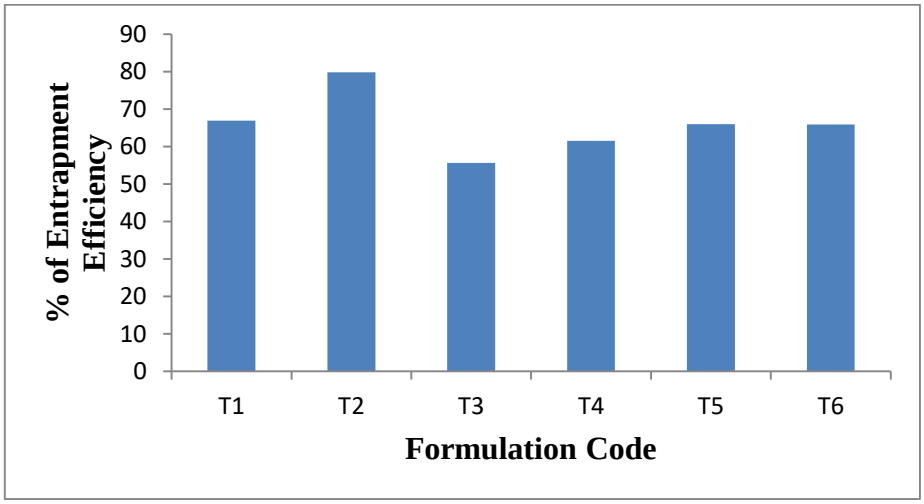

Figure 1: Entrapment Efficiency

Discussion: The entrapment efficiency data for Transferosomes ranges from 55.69±2.84 to 79.87±2.35 (as shown in table 5 and figure 1) reflecting variability influenced by several critical factors. The higher efficiencies observed in formulation like T2 could be attributed to optimal lipid composition and preparation method that promote effective drug encapsulation. Due to low stirring speed, high drug polymer interaction, low solubility of drug in continuous phase, low concentration of emulsifier leads to high entrapment efficiencies. Conversely, formulation T1, T3, T4, T5 and T6 shows lower efficiencies because of high stirring speed, low drug polymer interaction, high solubility of drug in continuous phase, high concentration of emulsifier leads to low entrapment efficiencies.

The best entrapment efficiency of T2 shows best result in entrapment efficiency with range of (79.87±2.35). Lower size of transferosomes is needed since they are to be entrapped into gel and lower size would also be beneficial for localization of the active constituents into deeper layer of the skin particular to target the causative agent for skin infections.

Vesicle Size

Table 6: Vesicle Size of Transferosomes formulations

S.No.	FormulationCode	Vesicle Size(nm)
1	T1	272±5
2	T2	248±3
3	T3	256±2
4	T4	273±4
5	T5	251±6
6	T6	261±4

Mean±SD,n=3

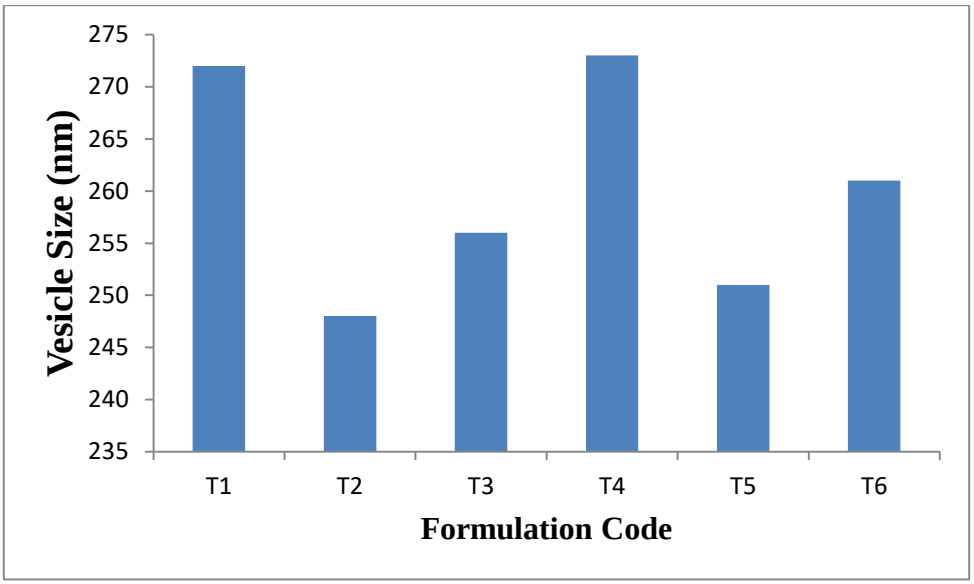

Figure 2: Vesicle Size of Transferosomes formulations

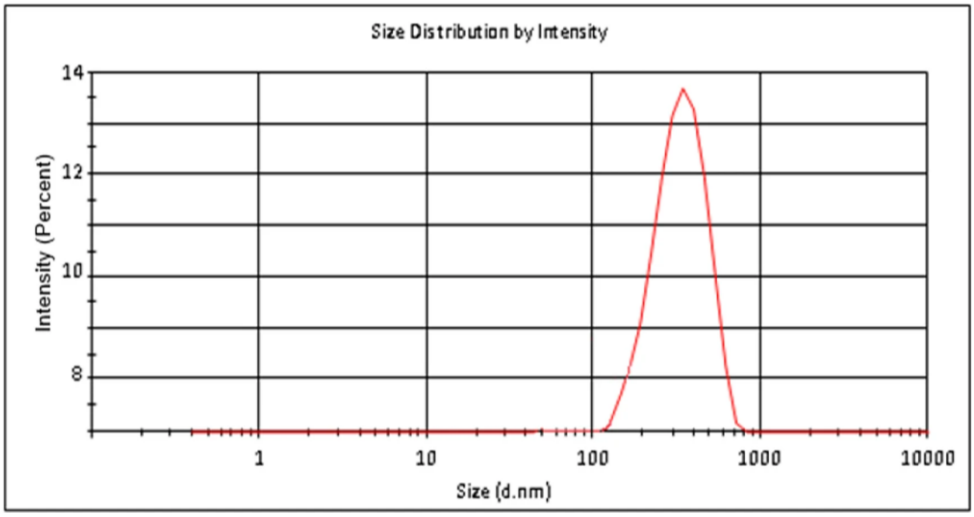

Figure 3: Vesicle Size of T2 formulation

Discussion: The Vesicle size data for Transferosomes ranges from 251±3 to 272±5 (as shown in table 6 and figure 2) reflecting variability influenced by several critical factors. The smaller vesicle size in T2 (248±3) could be due to optimal preparation condition, such as prolonged or adequate sonication time ensures the breakdown of larger vesicles smaller ones. The use of unsaturated fatty acids or a lower concentration of lipids can helps form smaller vesicles. Optimal hydration conditions can produce more uniform and smaller vesicles. In contrast, the larger vesicles size in T1 >T4 > T6 > T3> T5 could of be due to suboptimal preparation condition, such as unappropriate sonication time, higher concentration of lipids can result in the formation of larger vesicles. Suboptimal hydration conditions can affect vesicle size, leading to larger vesicles.

The best Vesicle size was found in T2 (248±3) smaller vesicle sizes can enhances drug delivery efficiency, cellular uptake, and stability. Smaller vesicles tend to be more stable, reducing the likelihood of aggregation.

Polydispersity Index:

Table 7: Polydispersity Index of Transferosomes formulations

S.No	Formulation code	Polydispersity Index
1.	T1	0.489±0.40
2.	T2	0.389±0.32
3.	T3	0.468±0.45
4.	T4	0.415±0.32
5.	T5	0.429±0.23
6.	T6	0.526±0.45

Mean ± SD; n = 3

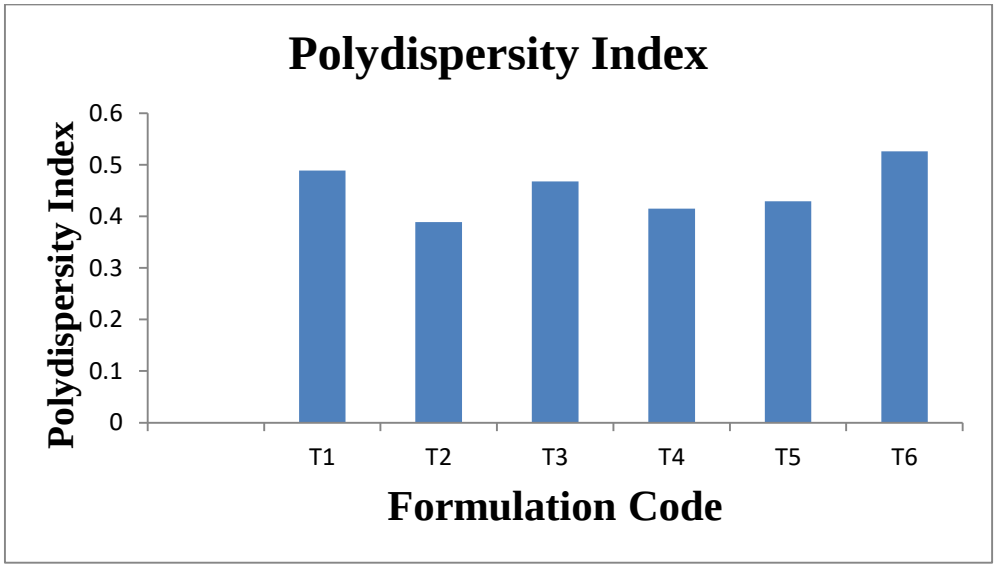

Figure 4: Polydispersity Index of Transferosome formulations

Discussion:

The PDI data for Transferosomes ranges from 0.389±0.32 to 0.526±0.45 (as shown in table 7 and figure 4) reflecting variability influenced by several critical factors. The PDI of T6 > T1> T3 > T5 >T4 indicates wider size disrtibution, suggesting more variability in particles size. Potentially less stable and reproducible**.**

The best PDI was found in T2 (0.389±0.32) due to its more uniform particle size distribution, which contributes to better stability and reproducibility**.**

Zeta Potential

Table 8: Zeta Potential of Transferosomes formulations

S.No.	Formulation Code	Zeta Potential(mV)
1	T1	-19.53±0.91
2	T2	-35.9±0.52
3	T3	-22.30±0.42
4	T4	-21.49±0.43
5	T5	-20.30±0.33
6	T6	-23.93±0.31

Mean ± SD; n = 3

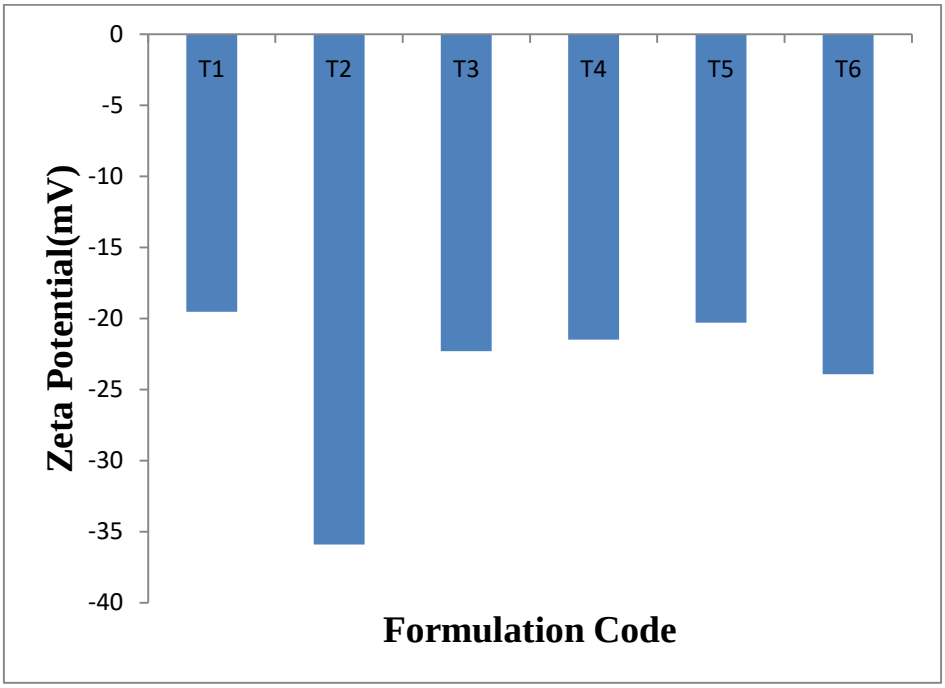

Figure 5: Zeta Potential of Transferosome formulations

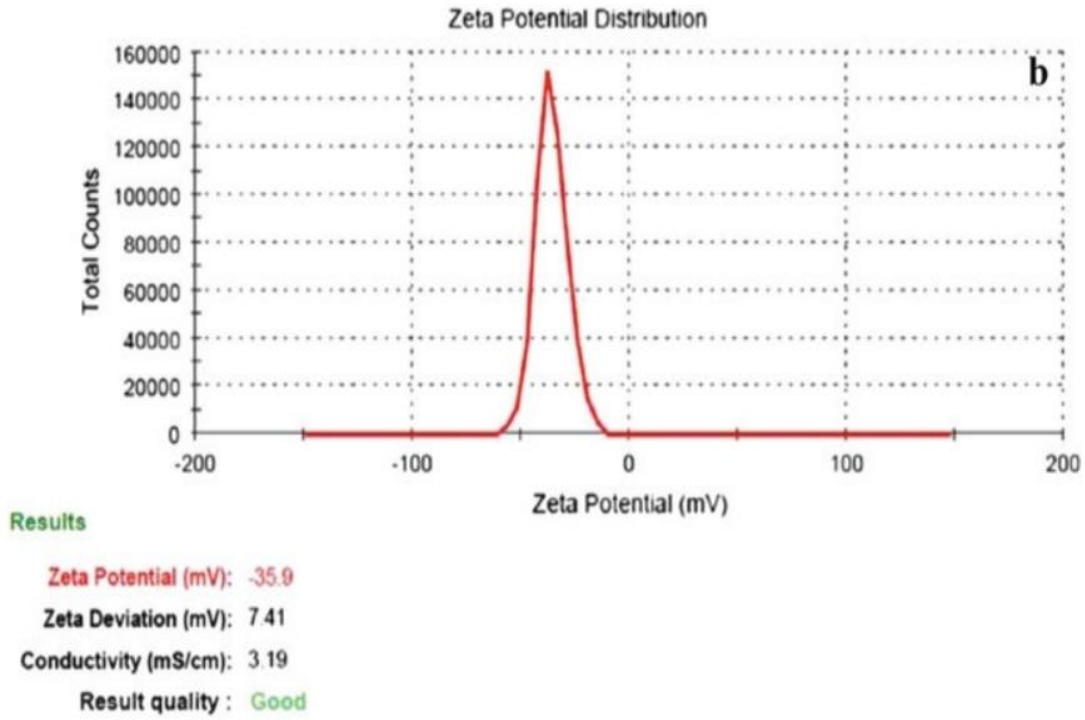

Figure 6: Zeta Potential of T2 formulation

Discussion: The Zeta Potential data of transferosomes formulations ranges are -19.53±0.91mV, -35.9±0.52mV**,** -22.30±0.42mV, -21.49±0.043mV, -20.30±0.33mV, -23.93±0.31mV were T1, T2,T3, T4, T5, T6 respectively. The high Zeta potential of T2 (-35.9±0.52mV) is likely due to a optimal values such as lipid composition, inclusion of charged molecules, the pH and iconic strength of the dispersion medium, presence of stabilizing agent, and the preparation methods used. The type and concentration of lipids used in T2 might leads to a higher surface charge density. In contrast, while other formulations shows low zeta potential such as in T1 < T5 < T4< T3 <T6is likely due to suboptimal values such as lipid composition, use of weakly charged lipids, presence of impurities, particle aggregations and suboptimal preparation methods.

The best zeta potential was in the T2 (-35.9±0.52mV) ensures better colloidal stability, reduces risk of aggregation, and contributes to improved performance and longer shelf life of the formulation.

Morphological Characterization of Transferososmes

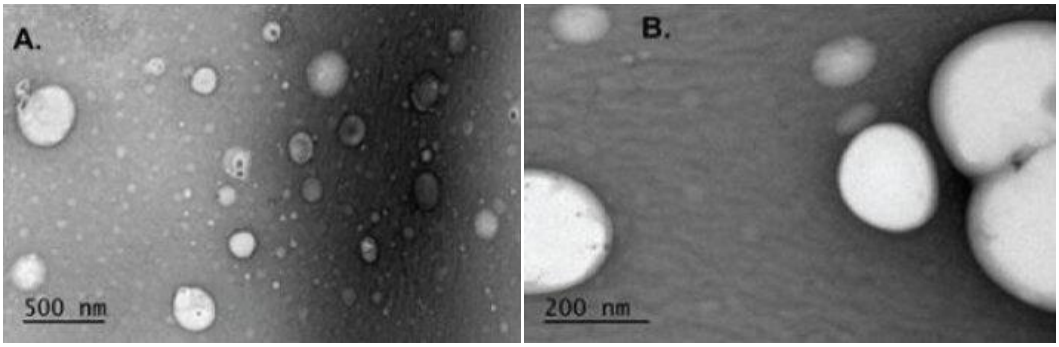

Figure.7: Scanning electron microscopy photograph of T2 A) X10,000 Magnifaction, B) x 20,000 Magnification

Discussion: The T2 formulation likely contain an optimal mix of lipids that favors the formation of spherical vesicles. The preparation methods for T2 formulation is probably designed to produce and maintain spherical shape. The high zeta potential of T2 formulation results in strong repulsive force, reducing aggregation and helping maintain a spherical shape. While, the other formulations like T1, T3,T4, T5, T6 likely contain an suboptimal mix of lipids that favors rod like structures. Variation in preparation conditions such as temperature, hydration rate, or lack of sufficient shear forces could lead to rod like structures. Lower zeta potential results in weak repulsive forces , leading to more aggregation and less control over shapes.

Hence, The Best Representation of scanning electron microcopy was found in T2 formulation which shows proper spherical shape and less aggregation of particles. The peripheral appearance of formulated transferosomes based on saturation of polymer solution, produced smooth and high yield of transferosomes. The un-dissolved polymer solution generates irregular and rod shape particles. In this, preparation the polymer was fully saturated and leading to the smooth and spherical configuration, independently distribution homogeneous particles and no evidence of collapsed particles.

Evaluation of Transferosomal Gel loaded with Cefadroxil

Physical evaluation

Table 9: Physical evaluation of Transferosomal Gel formulations

Formulation Code	Color	Phase separation	Homogeneity
TG1	Off White	No	Good
TG2	Off White	No	Good
TG3	Off White	No	Good
TG4	Off White	No	Good
TG5	Off White	No	Good
TG6	Off White	No	Good

Discussion: All the prepared transferosomal gel formulations were off white preparations with a smooth and homogeneous appearance. Results have been discussed in Table 9.

Drug Content:

Table 10: Drug Content

Sr. No	Formulation Code	Drug Content (%)
1.	TG1	76.21±1.35
2.	TG2	73.45±0.87
3.	TG3	72.29±0.98
4.	TG4	85.21±1.16
5.	TG5	75.88±1.31
6.	TG6	74.88±1.10

Mean ± SD; n = 3

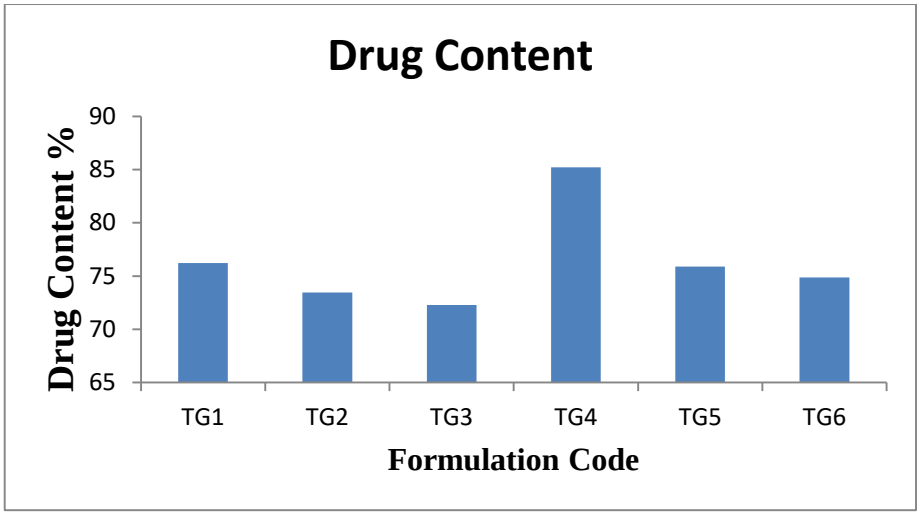

Figure 8: Drug Content of formulations

Discussion:

The Drug content data of Transferosomal gel ranges from 72.29±0.98 to 85.21±1.16 (as shown in table 10 and figure 8). The TG1, TG2, TG3,TG5 and TG6 shows low drug content there is many factors leading to low drug contents, like suboptimal formulation process (inefficient mixing, encapsulation or stabilization) can lead to lower drug content. Larger particles or unevenly distributed particles may have smaller surface area for drug encapsulation, resulting in lower drug content. In contrast, the TG4 shows high drug content due to optimized formulation process include efficient formulation techniques, such as proper mixing , encapsulation can enhances the drug loading capacity of the transferosomes leads to high drug content. Smaller and uniformly distributed particles can provide a larger surface area for drug absorption, increasing drug content.

Thus, the best result shown in TG4 with its higher drug content 85% is more suitable for gel formulations. Higher drug content ensures greater efficacy, consistent dosing, and cost effectiveness. The formulation process, particle size, and drug – excipients compatibility play critical role in achieving high drug content in gel formulations. And also shows that good capacity of formulation to hold the drug.

pH determination

Table 11: pH determination Transferosomal Gel formulations

Formulation Code	pH
TG1	6.2±0.47
TG2	6.4±0.25
TG3	6.5±1.98
TG4	6.0±1.14
TG5	6.7±0.37
TG6	6.5±0.42

Mean ± SD; n = 3

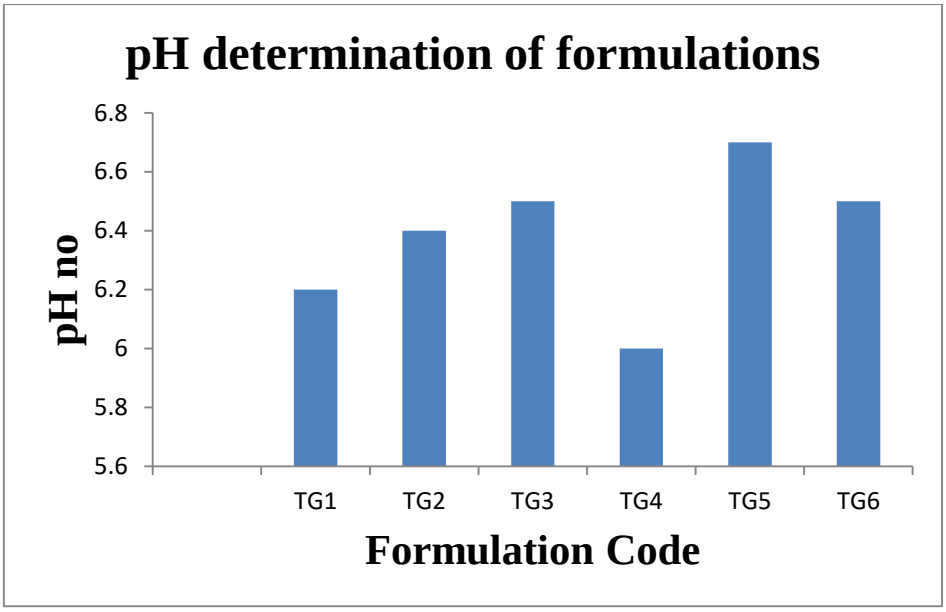

Figure 9: pH determination formulations

Discussion: The pH data for Transferosomal gel ranges from 6.0±1.14 to 6.7±0.37 (as shown in table 11 and figure 9). The TG4 shows low pH range 6.0±1.14 because active ingredients in gel formulation are more stable and effective at lower pH levels. Acidic pH can enhance the penetration of active ingredient through the stratum corneum, making formulation more effective. Lower pH can help in reducing skin irritation and sensitivity, making the formulation suitable for sensitive skin types. Lower pH environments can inhibit the growth of harmful bacteria and fungi, contributing to the preservation and safety of the product. Formulations (TG5 >TG6 >TG3 >TG2 >TG1) with a high pH can disrupt the skin's acid mantle, leading to irritation, dryness and increased susceptibility to infections.

The best formulation of TG4 with low pH (6.0±1.14) is generally more favorable for gel formulations, which lies normal pH range of the skin and was considered acceptance to avoid any irritation upon application to the skin.

Rheological Studies:

Table 12: Viscosity of the Transferosomal Gel formulations

Formulation Code	Spindle no.	Revolutions per minute(rpm)	Torque (%)	Viscosity (cps)
TG1	S63	50	88.5	1474±33.4
TG2	S63	50	81.5	1425±31.1
TG3	S63	50	82.8	1534±26.51
TG4	S63	50	88.2	1379±22.50
TG5	S63	50	86.3	1581±31.39
TG6	S63	50	88.1	1612±33.4

Mean ± SD; n = 3

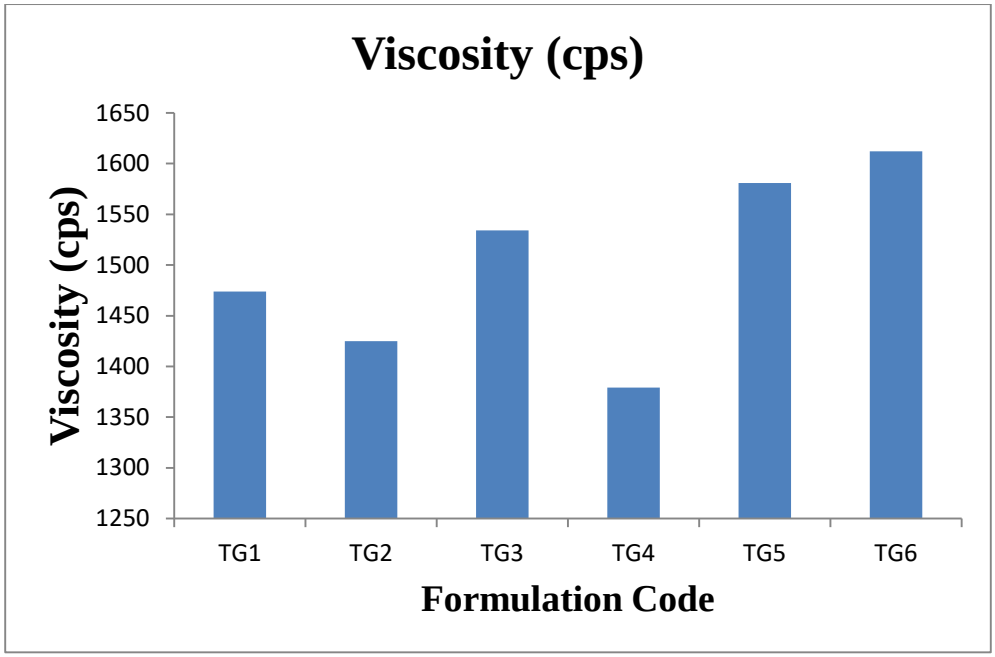

Figure 10: Viscosity of the Transferosomal gel formulations

Discussion:

The rheological behavior of all formulated transferosomal gel was studies using Brookfield viscometer at a speed of 50rpm and spindle no.63 was used. The viscosity of TG1, TG2, TG3, TG4, TG5, TG6 were 1474±33.4, 1425±31.1, 1534±26.51, 1379±22.50, 1581±31.39, and 1612±33.4 respectively.

The viscosity values indicate that the formulations are efficient to hold the transferosomal formulation. Moreover the TG4 (1379±22.50) formulation has low viscosity which can be spread evenly over the skin and will maintain contact for longer period of time with the skin and thereby leading to maximum therapeutic effect.

Spreadability

Table 13: Spreadability coefficient of Transferosomal Gel formulations

Sr. No	Formulation Code	M(gm)	L(cm)	T(sec)	Spreadability
1.	TG1	50	6	10	25.4±0.54
2.	TG2	50	6	10	23.07±0.47
3.	TG3	50	6	15	20± 0.34
4.	TG4	50	6	11	30± 0.46
5.	TG5	50	6	11	26.27 ± 0.65
6.	TG6	50	6	16	21.42± 0.24

Mean ± SD; n = 3

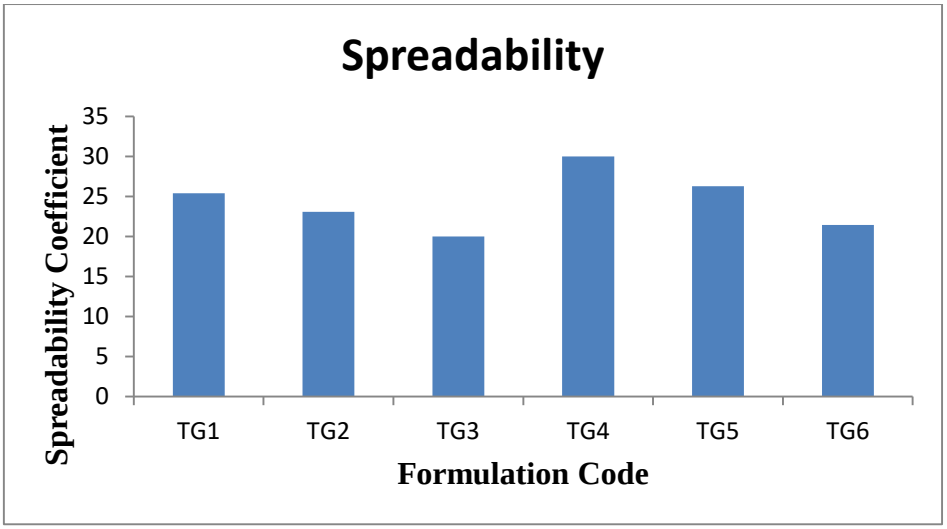

Figure 11: Spreadability coefficient of formulations

Discussion: The spreadability data of Transferosomal gel ranges from 20± 0.34 to 30± 0.46 (as shown in table 13 and figure 11). Formulations (TG3<TG6 < TG2< TG1<TG5) which shows low spread ability because larger particle size or unevenly distribution particles can increases resistance to spreading. Higher concentration of gelling agents can lead to lower spread ability. Higher viscosity of TG3 which can make the gel thicker and less easy to spread. While, the TG4 shows high Spread ability (30±0.46) because smaller or uniformly distributed particles in gel can lead to better spread ability. Gel with lower viscosity can easily spread over the skin.

Thus, the best result shown in TG4 (30±0.46) with higher spread ability is more suitable for gel formulation. Its lower viscosity and potentially better rheological properties contribute to easier and more effective application, enhancing the overall performance of the gel formulation. And also spread ability values indicate that the formulations are efficient to hold the transferosomal formulation. Moreover the TG4 formulation has lower viscosity which can be spread evenly over the skin and will maintain contact for longer period of time with the skin and thereby leading to maximum therapeutic effect.

Extrudability

Table 14: Extrudability of Transferosomal .Gel formulations

Sr. No.	Formulation code	Weight extruded from the tube(gm)
1.	TG1	0.69±0.128
2.	TG2	0.73± 0.121
3.	TG3	0.77±0.20
4.	TG4	1.1±0.36
5.	TG5	0.81±0.15
6.	TG6	0.89±0.151

Mean ± SD; n = 3

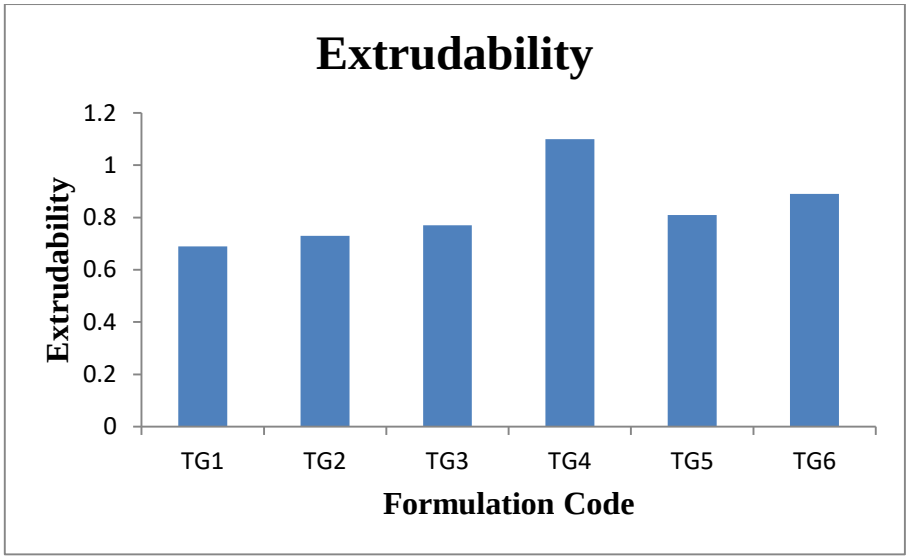

Figure 12: Extrudability of formulations

Discussion: The extrudability data of Transferosomal gel ranges from 0.69±.128 to 1.1±0.096 (as shown in table 14 and figure 12). The TG1< TG2 <TG3 <TG5 <TG6 shows low extrudability, because higher viscosity making it thicker and more resistant to flow. This requires more force to extrude the gel. Larger or unevenly distributed particles can increase internal friction, making it harder to extrude the gel. While, the formulation TG4 shows high extrudability because TG4 has lower viscosity making it easier to push through the nozzle of the container. Optimum viscosity gels require less force to extrude. Smaller and more uniformly distributed particles can reduce internal friction, allowing the gel to flow more easily.

Thus, the best result shown in TG4 with higher extrudability value (1.1± 0.36) is more suitable for gel formulations. Its low viscosity and potentially better flow properties contribute to easier and ensuring effective application of the gel**.**

IN –vitro Drug Release Studies of Transferosomal gel formulations

IN-vitro drug release studies revealed that the release of cefadroxil from different formulations varies with characteristics and composition of excipients. All the six formulations showed a chronomodulated pattern of drug release. The comparison of the drug release profile of all formulations showed that formulation TG4 shows maximum drug release of 95.56%. And all the others release studies of formulations showed in Table no. 15

Table 15: In Vitro drug release study

Time (hr)	**Cumulative Percentage of drug release**					
	TG1	**TG2**	**TG3**	**TG4**	**TG5**	**TG6**
0	0	0	0	0	0	0
0.5	19.62 ±0.23	19.78 ±0.28	19.78 ±0.45	20.25 ±0.39	18.67 ±0.63	19.48 ±0.34
1	35.45 ±0.28	35.67 ±0.24	38.78 ±0.34	40.56 ±0.28	34.44 ±0.46	28.45 ±0.56

2	45.44 ±0.45	45.67 ±0.34	51.89 ±0.54	48.98 ±0.21	43.56 ±0.45	41.67 ±0.49
4	65.06 ±0.34	62.78 ±0.091	62.65 ±0.48	69.98 ±0.32	59.25 ±0.35	62.78 ±0.45
6	69.89 ±0.41	68.89 ±0.34	72.67 ±0.39	75.65 ±0.23	75.71 ±0.48	64.67 ±0.38
8	82.76 ±0.56	83.67 ±0.34	80.78 ±0.38	89.98 ±0.51	84.78 ±0.58	79.89 ±0.24
10	88.98 ±0.34	88.67 ±0.41	85.78 ±0.48	95.56 ±0.49	87.65 ±0.26	82.56 ±0.11

Mean ± SD; n = 3

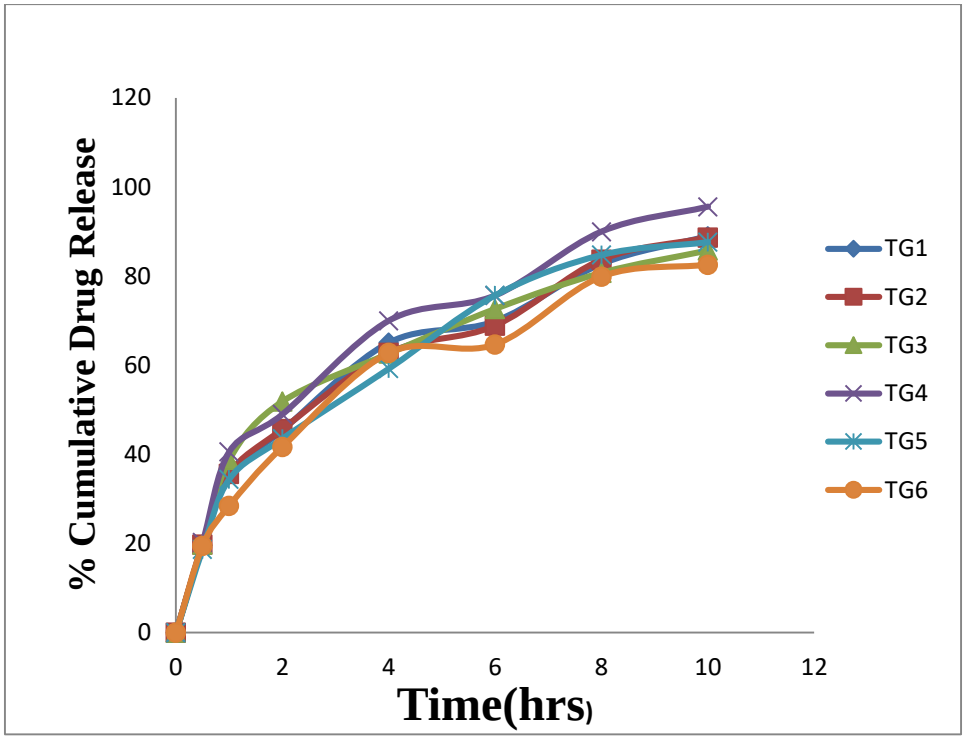

Figure 13: Dissolution profile of Formulation TG1 to TG6 of Transferosomal Gel

Discussion:

From the table 15 and figure 13. The in vitro drug release pattern of initial burst release of surface adsorbed drug was observed followed by slow and sustained release of entrapped drug from the transferosomes. The initial burst effect on the surface release of cefadroxil may be due to the loosely associated cefadroxil on the surface of transferosomes. The burst release is clinically significant to achieve initial high drug concentration in the target tissue. The slow release of the drug is controlled by the speed of the degradation of polymer. During the drug release study the reservoir condition was maintained by regularly replacing the dialysis medium. The two phase drug release drugs behavior might be responsible that the initial rapid drug release is due to the release drugs weakly networked with hydrophobic moiety and the following steady release is due to the release of drug robustly interacted with hydrophobic core of transferosomes.

In vitro kinetics release:

Table 16: In- vitro drug release data for formulation TG4

Time(hrs)	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	20.25	1.306	79.75	1.902
1	1	0.000	40.56	1.608	59.44	1.774
2	1.41421	0.301	48.98	1.690	51.02	1.708
4	2	0.602	69.98	1.845	30.02	1.477
6	2.44949	0.778	75.65	1.879	24.35	1.386
8	2.82843	0.903	89.98	1.954	10.02	1.001
10	3.16228	1	95.56	1.980	4.44	0.647

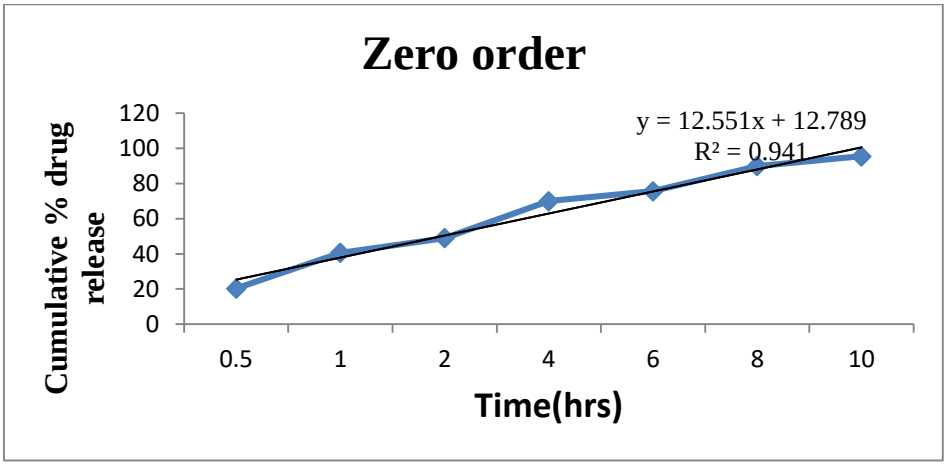

Figure 14: Representative of Zero order Release Kinetics of Formulation TG4 (Cumulative % drug released Vs Time)

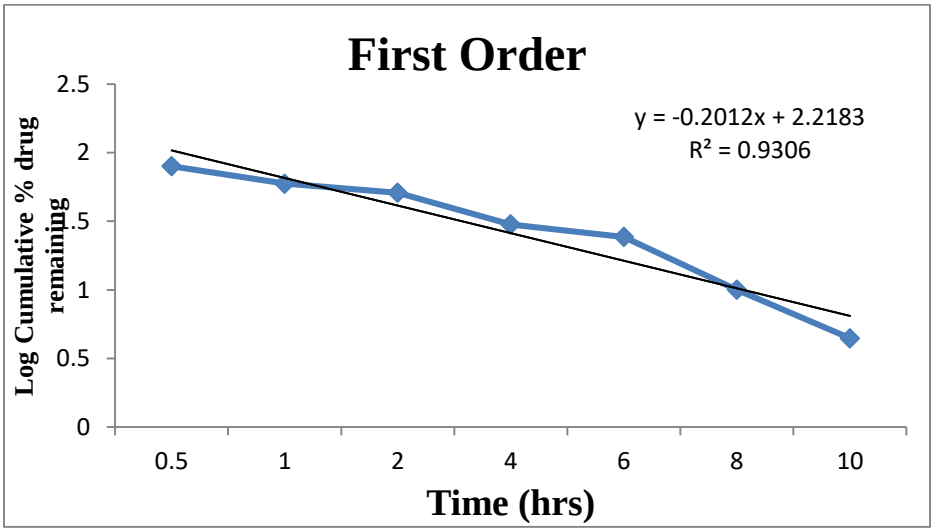

Fig.15: Representative of First order Release Kinetics of Formulation TG4 (Log Cumulative % drug remaining Vs Time)

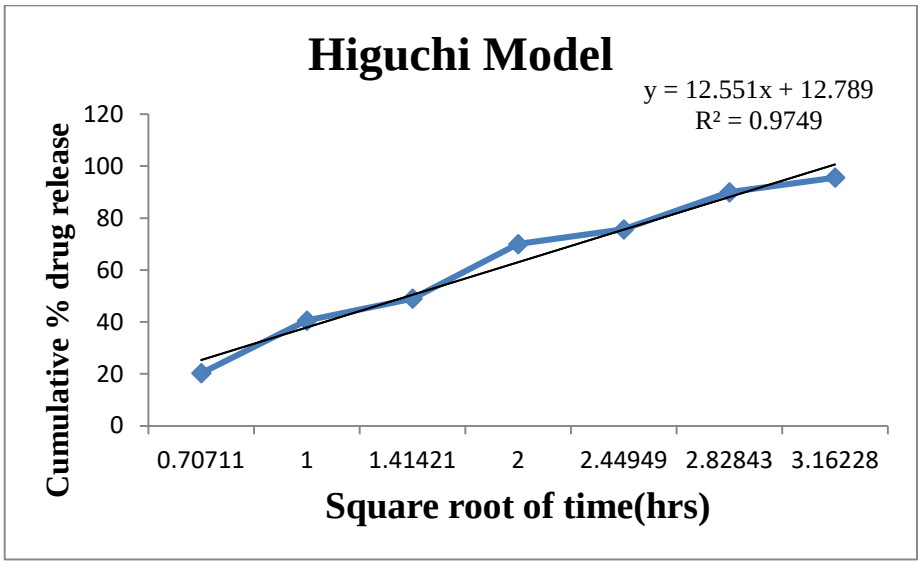

Fig.16: Representative of Higuchi Release Kinetics of Formulation TG4 (Cumulative % drug release Vs. Square Root Time)

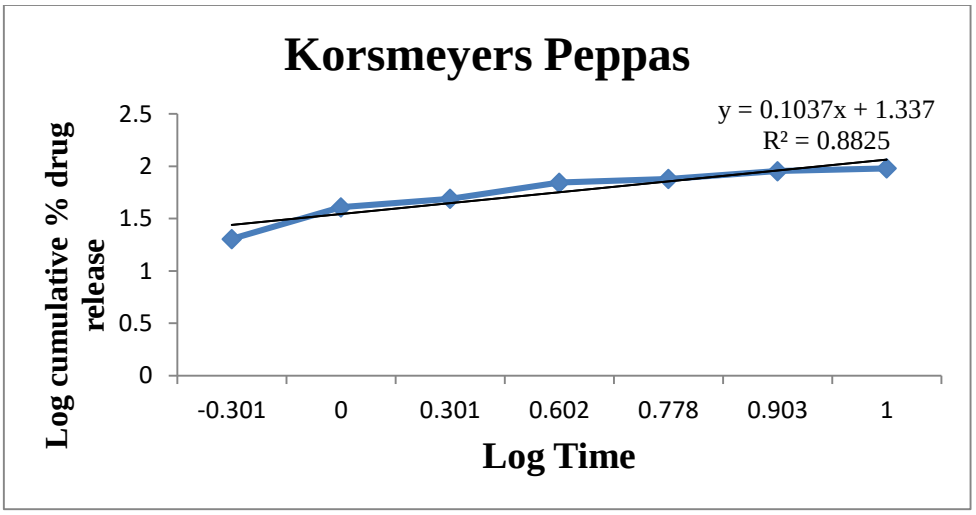

Fig.17: Representative of Korsmeyer- Peppas of Formulation TG4 (Log Cumulative % drug release Vs. Log Time)

Table 17: Release Kinetics Regression values of formulation TG4

Formulation Code	Zero Order Kinetics	First order Kinetics	Higuchi Model	Korsmeyer -Peppas
TG2	0.941	0.9306	0.9749	0.8825

Discussion: In case, R^2 value was calculated from the graph and reported in table and figure. Considering the determination coefficients, Higuchi model was found (R^2 =0.9749) to best fit the release data. This demonstrates that Cefadroxil were loaded in the Transferosomal gel and the drug was released from the transferosomes.

Stability studies:

Table 18: Stability study appearance Transferosomal Gel formulation TG4

Duration (days)	Appearance at 4±2ºC	Appearance at 25±2ºC/65%±5%RH	Appearance at 40±2ºC/75%±5%RH
0	White in Color	White in Color	White in Color
30	No change	No change	No change
60	No change	No change	No change
90	No change	No change	No change
120	No change	No change	No change
150	No change	No change	No change
180	No change	No change	No change

Table 19: Stability study of drug content of Transferosomal Gel formulation TG4

Duration(days)	Percentage of drug content at 4±2ºC	Percentage of drug content at 25±2ºC/65%±5%RH	Percentage of drug content at 40±2ºC/75%±5% RH
0	85.21±1.16	85.21±1.16	85.21±1.16
30	84.20±2.98	82.14±2.46	81.1±1.65
60	84.19±2.67	82.17±3.64	80.9±2.99
90	83.18±3.01	79.31±3.98	75.6±03.61
120	82.17±2.36	79.01±3.98	74.14±0.498
150	81.15±2.67	76.17±1.91	72.1±2.46
180	80.12±1.39	74.14±2.88	71.8±3.08

Table 20: Stability studies of in vitro drug release of Transferosomal Gel formulation TG2at 4±2ºC

Time (hrs)	Cumulative % drug release at 4±2ºC						
	0days	30days	60days	90days	120days	150days	180days
0	0	0	0	0	0	0	0
0.5	20.25 ±0.39	20.22 ±0.10	20.20 ±0.28	20.08 ±0.44	19.87 ±0.39	19.69 ±0.49	19.54 ±0.59
1	40.56± 0.28	40.1± 0.29	39.9± 0.55	39.01± 0.69	38.9± 0.38	38.05± 0.46	38.00± 0.28
2	48.89± 0.21	45.08± 0.25	45.00± 0.34	44.87± 0.56	44.78± 0.67	44.59± 0.45	43.32± 0.34
4	69.98± 0.32	53.30± 0.35	52.88± 0.22	52.65± 0.65	52.43± 0.30	52.18± 0.45	52.01± 0.65
6	75.65± 0.23	73.9± 0.61	73.40± 0.41	73.19± 0.35	73.01± 0.56	72.90± 0.45	72.69± 0.34

8	89.98± 0.51	87.12± 0.25	87.00± 0.21	86.98± 0.32	86.68± 0.45	85.41± 0.34	85.24± 0.28
10	95.56±0 .49	94.13±0 .20	93.98±0 .38	93.45±0 .26	92.15±0 .43	91.66±0 .36	91.12± 0.31

Mean ± SD; n = 3

Table 21: Stability studies of in vitro drug release of Transferosomal Gel formulation TG2 at 25ºC/65%±5%RH

Time (hrs)	**Cumulative% drug release at 25±2ºC/65%±5%RH**						
	0days	**30days**	**60days**	**90days**	**120days**	**150days**	**180days**
0	0	0	0	0	0	0	0
0.5	20.25 ±0.39	20.24 ±0.10	20.22 ±0.28	20.15 ±0.24	19.56 ±0.39	19.29 ±0.33	19.10 ±0.56
1	40.56± 0.28	30.0± 0.39	29.50± 0.25	29.00± 0.19	28.60± 0.38	28.15± 0.56	28.00± 0.48
2	48.98± 0.21	45.34± 0.38	45.07± 0.57	43.87± 0.22	43.78± 0.45	42.59± 0.34	42.32± 0.28
4	69.98± 0.32	53.10± 0.45	52.98± 0.23	52.55± 0.56	52.27± 0.16	52.08± 0.25	51.91± 0.32
6	75.65± 0.23	72.90± 0.28	72.40± 0.44	72.19± 0.51	72.01± 0.55	71.90± 0.41	71.69± 0.34
8	89.98± 0.51	88.12± 0.28	88.09± 0.66	87.98± 0.34	87.68± 0.56	87.41± 0.29	87.24± 0.23
10	95.56±0 .49	94.78±0 .38	94.12±0 .65	93.78±0 .23	93.56±0 .56	92.18±0 .89	91.45± 0.79

Mean ± SD; n = 3

Table 22: Stability studies of in vitro drug release of Transferosomal Gel formulation TG2 40±º2C/75%±5%RH

Time (hrs)	**Cumulative % drug release at 40±2ºC/75%±5%RH**						
	0days	**30days**	**60days**	**90days**	**120days**	**150days**	**180days**
0	0	0	0	0	0	0	0
0.5	20.25 ±0.39	19.54 ±0.46	19.35 ±0.37	19.15 ±0.56	19.01 ±0.35	18.89 ±0.45	18.10 ±0.49
1	40.56± 0.28	29.00± 0.52	28.50± 0.58	28.00± 0.43	27.60± 0.41	27.15± 0.35	26.00± 0.32
2	48.98± 0.21	43.34± 0.34	43.07± 0.45	42.87± 0.67	42.78± 0.62	41.59± 0.54	41.32± 0.48
4	69.98± 0.32	53.10± 0.42	52.98± 0.34	52.55± 0.31	51.27± 0.37	51.08± 0.44	50.91± 0.38

6	75.65± 0.23	72.90± 0.41	72.40± 0.52	72.19± 0.44	72.01± 0.48	71.90± 0.39	71.69± 0.42
8	89.98± 0.51	86.12± 0.58	86.09± 0.48	85.98± 0.43	84.68± 0.34	83.41± 0.29	82.24± 0.28
10	95.56 ±0.49	94.78±0 .054	94.56±0 .39	93.89±0 .27	93.45±0 .22	92.67±0. 44	91.34± 0.64

Mean ± SD; n = 3

Discussion: Stability of transferosomal gel containing drug was carried out for 180 days at **4±2ºC, 25±2ºC/65%±5%RH, 40±2ºC/75%±5%RH.** Responses obtained for different parameters for transferosomal gel during stability period. Transferosomes were found to be reasonably stable in terms of aggregation, fusion and/or vesicle disruption tendencies, over the studied storage period.

Stability studies for TG2 formulation were carried out at **4±2ºC, 25±2ºC/65%±5%RH, 40±2ºC/75%±5%RH** for a period of 180 days. Stability studies performed for transferosomal gel indicates that the prepared formulation shows that there was no significant variation found in physical appearance, but slightly decreases % drug content and cumulative % drug release of the transferosomal gel TG4 as the temperature increases. The results conclude that the TG4 formulation is stable after stability study for 180 days.

CONCULSION:

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. 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 Cefadroxil 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 Transferosomal gel can be effectively utilized for transdermal drug delivery in various skin conditions.

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