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Formulation Development and Evaluation of Ciprofloxacin HCl Transfersomes

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Abstract
Ciprofloxacin hydrochloride (CIP HCL) is broad spectrum antibiotic belongs to fluoroquinolones which is active against both gram-positive and gram-negative bacteria. It has low permeability. The aim of the present study is to formulate CIP loaded Transferosomes to overcome the barrier function of the skin and enhance the permeability. CIP loaded Transferosomes was optimized using **2^3** full factorial design using sigma tech software, were independent variables are Span80 (X_1), Tween80 (X_2) and Phospholipid as (X_3) and the responses are entrapment efficiency (Y1), particles size determination (Y2) and *in vitro* drug release (Y3). In the Preformulation studies it was proven that the drug solubility in 7.4 pH was 1820.22±0.151 mcg/ml, and FTIR shows no interaction between drug and excipients used. Thin film hydration technique is use for preparation of Transfersomes. It was concluded that the factorial design (2^3) by composite method total of 18 formulations was carried out which had the ability to obtain an optimized CIP HCL Transfersomes with maximize EE% (88.97±0.31), minimize particle size (90.1±0.21), and maximize transdermal flux (5.341±4.21). SEM of optimized CIP Transfersomes appeared as spherical, well identified, unilamellar nanovesicles. After optimization of formulation variables, it was found that the optimized formulation was suggested to contain 525, 525 and 315 mg of X1, X2, and X3, respectively. Therefore, Ciprofloxacin Hcl loaded Transfersomes are successfully optimize for transdermal drug delivery.

Keywords
Transfersomes; Ciprofloxacin Hydrochloride; antibacterial activity; entrapment efficiency; Carbapol 934

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1.INTRODUCTION
Bacterial skin infections are common, even though the skin forms such an effective barrier that people may constantly come into contact with bacteria without skin problems occurring. Any break in the skin makes it more likely for bacterial disease to establish itself, so it is important to keep cuts and scrapes clean and covered. Infectious skin diseases are also more prevalent in those people with suppressed immune systems or with conditions such as diabetes, where circulation is impaired. [1]
Ciprofloxacin is a fluoroquinolone antibiotic that possesses strong activity against gram-negative bacteria. Ciprofloxacin proves to be effective in the treatment of infections caused by microorganisms like Enterobacteriaceae, Escherichia coli, Vibrio, Pseudomonas aeruginosa etc [2]. Hence, it is approved for the treatment of 14 types of infections,

especially urinary tract infections, bone-joint infections, typhoid fever, pneumonia, bacterial conjunctivitis, corneal ulcer, bronchitis, acute sinusitis, and infection of skin or subcutaneous tissue. The mechanism of antibacterial action of quinolone, including ciprofloxacin, involves interfering with replication and transcription of DNA via inhibiting bacterial DNA gyrase/ topoisomerase II and DNA topoisomerase IV, and further preventing DNA of bacteria from unwinding and duplicating. Thus, complexes of quinolone-enzyme-DNA are formed, leading to the production of cellular poisons and cell death. [3,4].

The topical application of CIP is problematic in the treatment of cutaneous diseases due to poor skin penetration. Conventional formulations are given in overcome this issue and compensate for low permeability. In recent years, the use of lipid vesicles as carriers for topical drugs has attracted great attention due to their ability to overcome the barrier properties of the skin [5].

Transfersomes are ultra-flexible vesicles with a bilayer structure. They can penetrate the skin easily and overcome the barrier function by squeezing through the intracellular lipid of the stratum corneum [6]. After application of Transfersomes on the skin, they move from the dry stratum corneum to a deep hydrated layer according to the osmotic gradient. The presence of surfactant in their structure helps in solubilizing the lipid in stratum corneum and permits high penetration of the vesicles [7].

2.MATERIALS AND METHODS

2.1 Materials

Ciprofloxacin Hydrochloride was purchased from BMR Chemicals, Hyderabad, India. soya lecithin was purchased from Himedia, Methanol and chloroform was purchased from Fischer Scientifics Mumbai India, span 80 and tween 80 was procured from S.D fine chemicals, Potassium dihydrogen phosphate and sodium hydroxide were obtained from Fischer Scientifics Mumbai India and Carbopol 934 was procured from S.D fine chemicals.

2.2Methods

2.2.1 Preparation of CIP-Loaded Transfersomes

CIP Transfersomes were prepared using the thin film hydration technique with three independent variables like Span 80 (X_1), Tween 80 (X_2) as surfactant and phospholipid (X_3) as vesicle forming agent as shown in Table 3. A factorial design (2^3) is applied to prepare different formulations of CIP Transfersomes by using Sigma tech software. Precise amount of drug, surfactant and phospholipid were dissolved in a mixture of organic solvents consisting of chloroform and methanol (2:1, v/v) in a dry, round-bottom flask. The organic solvent was allowed to evaporate using a rotary evaporator (Aditya scientific evaporator) adjusted to 60 rpm, at 45° C under low pressure to prepare a thin lipid film on the wall of the round-bottom flask. The dry thin lipid film formed was subjected to hydration with phosphate-buffered saline (pH 7.4) by rotation for 1 h at 60 rpm at room temperature. The formed lipid vesicles were allowed to swell for 2 h at room temperature (25°C). The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe Sonicator (Mangaldeep tech solutions) for 30 min to reduce the vesicle size and stored at 4°C for further investigation [8,9]

2.2.2PREFORMULATION STUDIES [10]

- **Melting point:** determination of melting point of drug was done by capillary method using melting point apparatus.
- **Solubility**: For determining the saturated solubility of the drug in distilled water, methanol, 6.8 and 7.4 phosphate buffer (5ml) were taken in a 100ml conical flask. After adding excess amount of drug in each flask it was closed with stopper. These flasks were kept in rotary shaker rotating at 50. Then test samples were collected at specified time and filtered, diluted with the solvent; the absorbance of the drug was taken with UV Visible Spectrophotometer.
- **Drug – Excipient Compatibility Study**: FTIR spectrum was taken for pure drug and physical mixture of excipients with drug by potassium bromide pellet method. The samples were analyzed between wave numbers 4000 and 400 cm^{-136}.

2.2.3CHARACTERIZATION OF TRANSFERSOMES

- **Determination of Entrapment Efficiency (EE %)**

5ml of CIP Transfersomes suspension was ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4^0C so as to separate the entrap drug from the un-entrap drug. After removal of the supernatant, the sediment was lysed using methanol and then analyzed at 272 nm using a UV spectrophotometer, (Elico, PG instruments). The EE% of CIP in the prepared Transfersomes was calculated applying the following equation [8]

EE% = [Amount of Entrapped CIP/Total Amount of CIP] /100

- **Surface morphology of Transfersomes**

Scanning electron microscopy (SEM) is used to determine the shape and size of formulated CIP loaded Transfersomes. [9,10]

- **Determination of Particle Size and Zeta Potential**

Particle size and zeta potential were measured for all prepared CIP Transfersomes using the dynamic light scattering (DLS) technique at 25°C using the Particle Size System [8,9].

- **Determination of *In Vitro* diffusion of CIP Transfersomes**

A diffusion study of Transfersomes formulations was carried out using Franz diffusion cell through dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. Franz diffusion cell contain two compartments upper donor and lower receptor compartment. The receptor compartment was filled with 7.4 pH and donor compartment contain 5 ml of transferosome suspension on dialysis membrane with exposure area of 2cm^2 to receptor medium and whole assembly was kept on magnetic stirrer at 600rpm for a period of 10 hours and samples were withdrawn at specified time interval of 1 hr and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 272nm. Steady state Flux (**Jss**) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area (µg/cm^2) against a time (h) plot. Permeability coefficient (**Kp) =Jss/Co,**(Co = initial CIP concentration)[8,11].

- **Release kinetic profile**

The drug release kinetics studies were estimated to determine the type of release mechanism followed. Release kinetic study of optimized formulation was studied for different kinetic equations (zero order, first order, and Higuchi) [12].

- **Stability studies**

The stability studies of CIP Transfersomes of optimize formulation were conducted at refrigerated temperature (4°C) and room temperature as per Guidelines of International Conference on Harmonization (ICH). Samples were analyzed for physical appearance drug content, and *in vitro* diffusion studies after 15, 30 and 45 days [13].

3. RESULT AND DISCUSSION

- **Melting point:**

The reference melting point of CIP HCL was 252^0 C and practically observed melting point was 256^0 C (shown in Table 1).

Table 1: Melting Point of CIP HCL

Reference Value	Observed Value
252°C	**256 °C**

- **Solubility:**

The solubility of CIP HCL was checked, as CIP HCL belongs to BSC class III solubility was not the issue in pre-formulation studies (Table 2).

Table 2: Solubility of Ciprofloxacin HCL

Solvents	**Concentration(mcg/ml) of drug**
Water	**812.23±0.11**
Methanol	**661.11±0.120**
6.8 pH	**1230.7±0.96**
7.4 pH	**1820.22±0.151**

All values are expressed as mean ± SD, (n=3)

- **FT-IR Spectroscopic Analysis:**

As shown in Figure 1 (a) and (b), The FTIR spectra, observed that the characteristic absorption peaks of pure CIP HCL were obtained at 3529, 2455, 1308, 1143 cm-1 corresponding to C-OH, C=O, C-F, C-N. The spectral data suggests that the major peaks for drugs are obtained as nearer value and there were no considerable changes in IR peaks in all physical mixtures of drug and excipients. This indicates that the drugs were molecularly dispersed in the excipients thereby indicating the absence of any interactions.

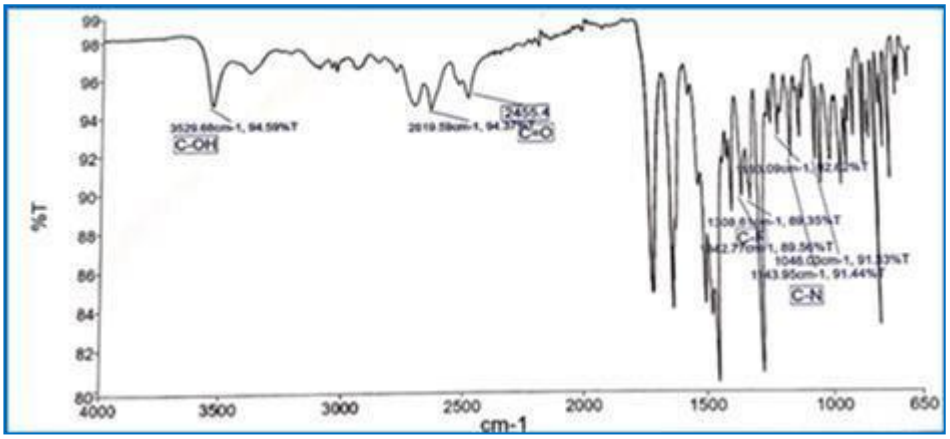

Figure 1(a): FTIR of pure Ciprofloxacin HCL

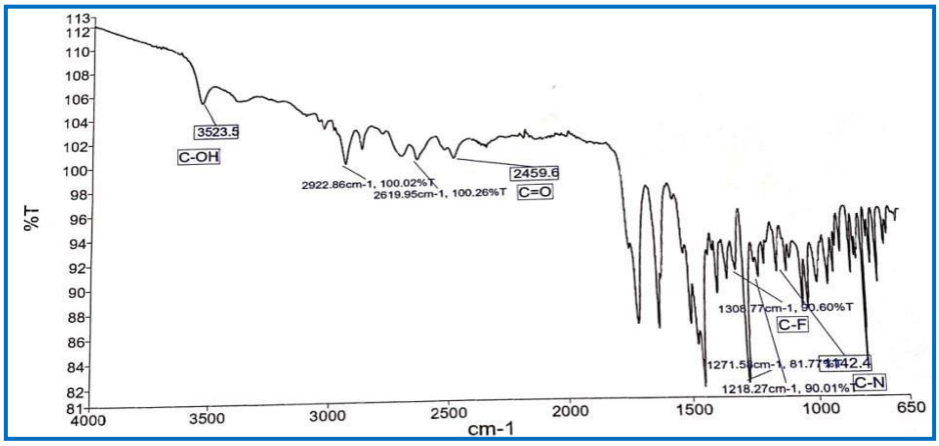

Figure 1(b): IR spectra for pure CIP HCL along with SPAN 80, TWEEN 80, SOYA LECITHIN

- **Entrapment Efficiency:**

As shown in Table 4, it was found that the prepared CIP Transfersomes exhibited a good EE%, with values ranging from (59±3.21%) for X1-At-2L to (88.97±0.31%) for X1X2.From the results of EE%, it was found that Transfersomes prepared with high level of Span 80 have a higher EE%. These results may be attributed to the hydrophilic lipophilic balance (HLB) values of these surfactants. The HLB values of Span 80 and Tween 80 were 4.3 and 15, respectively. Hence, according to HLB values, the affinity of surfactant to phospholipid was expected to be higher in case of Span 80 than in case of Tween 80 due to the higher lipophilicity of Span 80. The EE% was increased in the case of 525 mg of Span80 and phospholipid 315 mg, as compared to 375 mg of Span80 and phospholipid 465, respectively given in Figure 2. These results are in good agreement with those of Abdallah et al. who reported that the EE% of nystatin in the prepared Transfersomes was higher in case of Span 80 than in case of Tween 80.

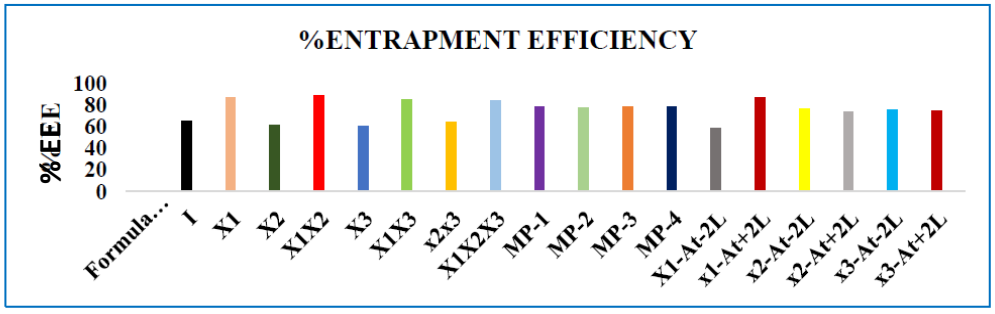

Figure 2: Entrapment Efficiency of all formulations

- **Particle Size (Y2) And Zeta Potential of Prepared CIP Transfersomes**

The prepared CIP Transfersomes were tested for particle size and zeta potential. The vesicle size ranged from (108.4±0.21nm) for X1X2 (201±0.22 nm) for X1-At-2L. From the results of particle size, it was found that all prepared CIP Transfersomes have a particle size less than 200 nm, and as such are effective for transdermal applications. Plots shows that particle size of the prepared CIP Transfersomes increased with increasing X1 and decreased as X2 and X3 increased.

From the previous results it was found that CIP Transfersomes particle size increases with the increase in concentration of span80 and decreases with the decrease in tween80 and phospholipids. This may be attributed to the general concept of the use of surfactant with a lower HLB which resulted in the preparation of vesicles with smaller sizes. Hence, the Transfersomes prepared with Span 80 (lipophilic surfactant) have the smaller size than those prepared with Tween 80 (a hydrophilic surfactant). The direct proportionality between vesicle size and surfactant HLB can be explained by the reduction in surface free energy resulting from high hydrophobicity. The same explanation applies for the decrease in vesicle size as the lipids increased.

- ***In vitro* diffusion studies**

Diffusion studies of all formulations were carried out using dialysis membrane for 10 hours and samples were analysed using double beam Uv spectrophotometer (Figure 3).

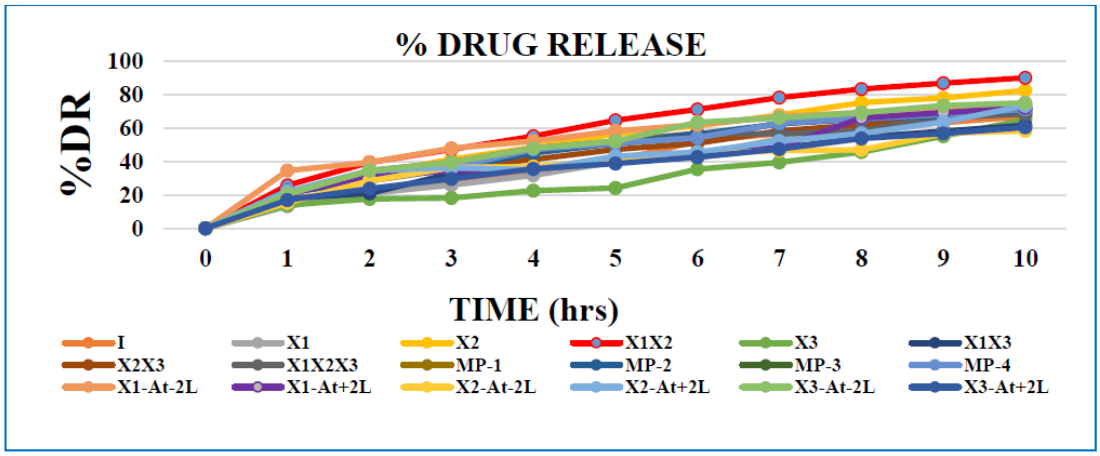

Figure 3: *In vitro* diffusion studies all Transfersomal formulations

Table-3 Formulation factors for the multilevel factorial design.

Independent Factors	Low level	High level
X1 - Span 80 (mg)	375	525
X2 - Tween 80 (mg)	375	525
X3 – Phospholipids (mg)	315	565

Dependent variables	Goals
Y1 – entrapment efficiency	Maximize
Y2 – drug release	Maximize
Y3- particles size	Minimize

Table 4: Formulation design with responses

SNO.	Combinations	Span80 X1	Tween80 X2	Phospholipids X3	Y1 (%EE)	Y2 (% drug release)	Y3 (particle size) nm
1	I	375	375	315	65.39±1.03	67.04±0.21	162.1±1.03
2	X1	525	375	315	87.45±0.31	60.4±0.64	122.2±3.13
3	X2	375	525	315	61.59±3.21	82.5±0.4	183.9±0.23
4	X1X2	525	525	315	88.97±0.31	90.1±0.21	108.4±0.21
5	X3	375	375	465	60.83±0.11	65.0±1.21	163.1±0.64
6	X1X3	525	375	465	84.91±1.7	61.7±0.64	132.2±0.61
7	X2X3	375	525	465	64.38±2.02	69.2±0.34	120.8±0.4
8	X1X2X3	525	525	465	83.9±0.33	68.4±0.7	158.3±1.6
9	MP-1	450	450	390	78.25±0.56	71.22±0.11	120.8±0.21
10	MP-2	450	450	390	77.98±1.02	70.88±0.23	120±0.21
11	MP-3	450	450	390	78.01±0.56	71.22±0.11	120±0.21
12	MP-4	450	450	390	78.11±0.56	71.22±0.11	120±0.21
13	X1-At-2L	300	450	390	59±3.21	74.2±3.1	172±0.44
14	X1-At+2L	600	450	390	87±1.22	72.3±0.42	201±0.22
15	X2-At-2L	450	300	390	77±0.53	58.4±2.42	166±0.34
16	X2-AT+2L	450	600	390	74±0.43	73.4±1.04	145±0.33
17	X3-At-2L	450	450	240	76±0.62	75.1±0.35	188±1.87
18	X3-At+2L	450	450	540	75±1.43	60.4±0.42	112±0.33

The values are expressed as mean, ±SD (n=3)

Table 5: Jss and Kp of Transfersomal formulations

S NO.	FORMULATIONS	FLUX (Jss)	PERMEABILITY COEFFICIENT (KP)
1	I	3.77±2.07	0.856±0.12
2	X1	3.45±1.02	0.785±0.11
3	X2	4.75±4.21	1.072±0.62
4	X1X2	5.34±4.21	1.213±2.40
5	X3	3.005±0.11	0.06±0.01
6	X1X3	2.759±2.34	0.627±0.20
7	X2X3	4.006±0.45	0.910±3.42
8	X1X2X3	4.088±3.22	0.929±0.12
9	MP-1	4.22±0.42	0.95±1.42
10	MP-2	4.19±2.11	0.95±0.51
11	MP-3	4.22±0.41	0.95±0.11
12	MP-4	4.22±0.41	0.95±0.11
13	X1-At-2L	4.58±2.32	1.04±1.01
14	x1-At+2L	3.918±1.02	0.89±0.52
15	x2-At-2L	3.37±4.42	0.76±0.45
16	x2-At+2L	3.83±1.12	0.87±1.34
17	x3-At-2L	4.50±0.35	1.02±1.21
18	x3-At+2L	3.40±0.53	0.77±2.05

The values are expressed as mean, ±SD (n=3)

As shown in Table 5, The permeation increases as Tween 80 increases rather than Span 80, which could be attributed to an increase in the hydrophilicity of Tween 80, which causes it to act as a solubilizing agent for the drug, facilitating drug release. The permeation rate also decreased as the lipids increases, which may be due to the increased bilayer hydrophobicity and stability. Thus, the permeability decreased. From the results of in vitro permeation and EE%, it was found that there was an inverse relationship between them. The higher the EE% of the CIP in prepared Transfersomes, the slower the in vitro permeation.

- **Contour plots:** Figure 4 shows contour plots for %EE, Particle size and zeta potential.

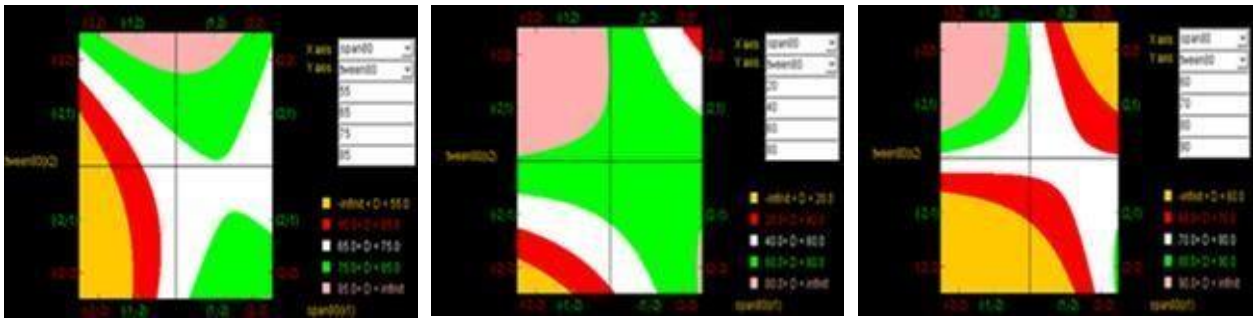

Figure 4: contour plots for %EE, particle size and *in vitro* diffusion

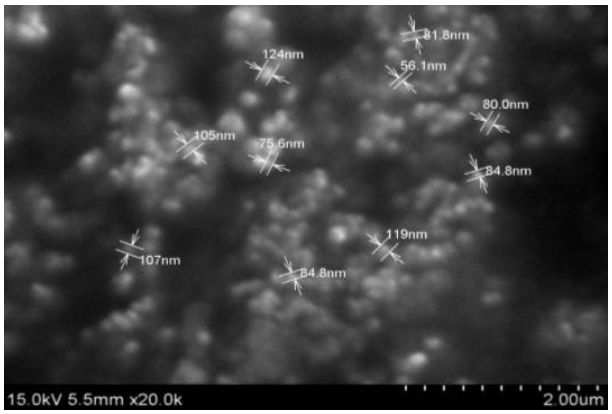

Figure 5: SEM of optimize formulation

3.1 OPTIMIZE FORMULATION:

Table 6 shows the composition of the optimized formula (X1X2). it was found that the optimized formulation was contain 525, 525 and 315 mg of X1, X2, and X3, respectively.

Table 6. Composition of factors and response for the optimized CIP Transfersomes.

Independent variables		Responses
X1	525	Y1 88.97±0.31
X2	525	Y2 90.1±0.21
X3	315	Y3 120.8±0.21

- As shown in Figure 5, SEM photographs of optimized CIP Transfersomes appeared as spherical, well identified, unilamellar nanovesicles. Figure 6 and 7 shows zeta potential and particle size distribution of optimize CIP Transfersomes.
- **Release kinetic profile optimize Transfersomes**

The drug release kinetics studies were estimated to determine the type of release mechanism followed. Release kinetic study of CIP Transfersomes of optimized formulation was studied for different kinetic equations [zero order, first order and Higuchi equation shown in Figure 8(a), (b) and (c)].

R^2 values for the optimized formulation were found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion-controlled release mechanism. 'n' value was estimated from linear regression of log (Mt/M) vs log t and it was found that drug release follows Quasi fickian mechanism (see Table 7).

Table 7- Release kinetics for CIP Transfersomes

S.no.	Parameters	ZERO ORDER %CDR Vs T	FIRST ORDER Log % Remain Vs T	HIGUCHI % CDR Vs √T
1.	**Slope**	8.226	-0.097	0.0337
2.	**Intercept**	17.295	2.003	0.0762
3.	**Correlation**	0.9621	-0.9941	0.9995
4.	**R2**	0.9289	0.9926	0.9967

- **Stability studies**

Stability studies showed that Transfersomes is more stable at 4°C when compare to room temperatures (see Table 8). There was a change in color for the samples kept at room temperature, leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage.

Table 8: stability of CIP Transfersomes at 4±2^{0}C and R.T

DURATION	4±20C		Room Temperature	
	%DC	%CDR	%DC	%CDR
INITIAL	95.2±1.41	89.4±0.42	95.2±1.41	89.4±0.42
15 days	94.89±1.01	86.0±1.52	93.5±0.54	83.72±0.19
30 days	92.82±0.21	82.7±0.21	90.1±0.84	77.57±0.64
45 days	91.03±1.22	79.1±1.41	87.9±0.16	70.21±1.51

The values are expressed as mean, ±SD (n=3)

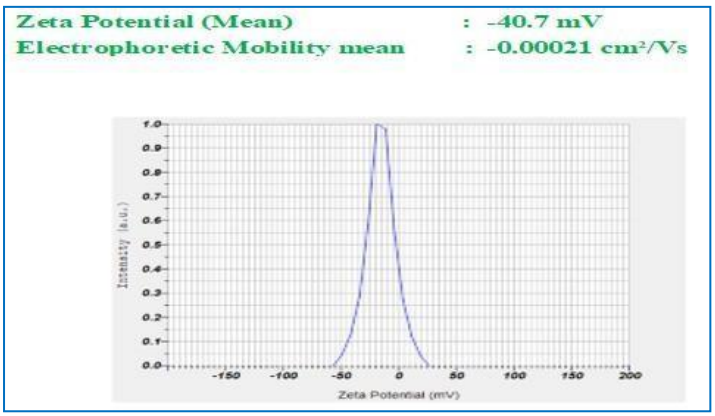

Figure 6: Zeta potential of optimize formulation

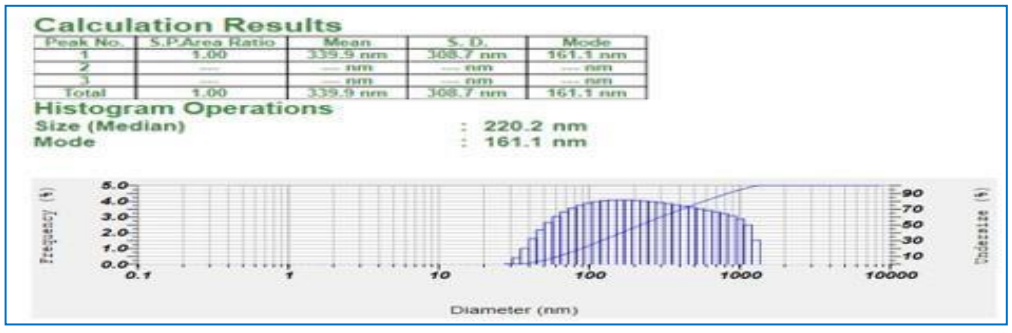

Figure 7: particle sizes of optimize formulation

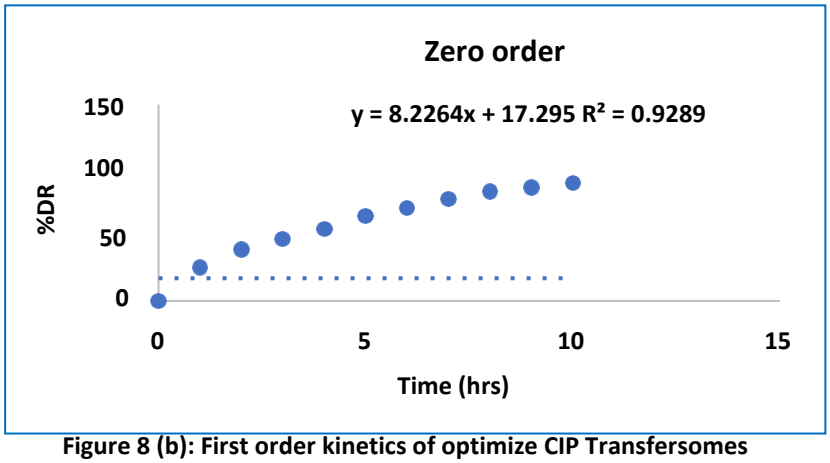

Figure 8 (b): First order kinetics of optimize CIP Transfersomes

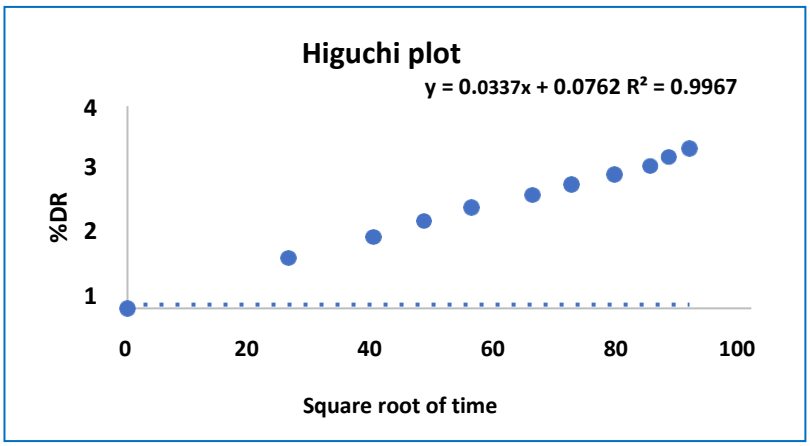

Figure 8(c): Higuchi plot of optimize CIP Transfersomes

4. CONCLUSION

The aim of the study was to enhance the permeability of CIP HCL by formulating the CIP loaded Transfersomes. CIP has broad spectrum antibiotic activity use to treat bacterial infections. In the Preformulation studies it was proven that the drug solubility in 7.4 pH was 1820.22±0.151 mcg/ml, and FTIR shows no interaction between drug and excipients used Transfersomes were developed by design of experiment using sigma tech software. It was concluded that the factorial design (2^3) by composite method total of 18 formulations was carried out which had the ability to obtain an optimized CIP HCL Transfersomes with maximize EE% (88.97±0.31), minimize particle size (90.1±0.21), and maximize transdermal flux (5.341±4.21). After optimization of formulation variables, it was found that the optimized formulation was suggested to contain 525, 525 and 315 mg of X1, X2, and X3, respectively. SEM of optimized CIP Transfersomes appeared as spherical, well identified, unilamellar nanovesicles. When release kinetics models were applied it follows Higuchi model, and the drug release follows Quasi fickian mechanism. Finally, Stability studies showed that Transfersomes is more stable at 4°C when compare to room temperature.

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