PREPARATION AND EVALUATIONOF TRANSFERSOMAL GEL OF AZITHROMYCIN

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ABSTRACT

Acne vulgaris is a disease of the pilosebaceous follicle characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules and nodules). The aim of the present study was to statistically optimize the vesicular formulations (Transfersomes) for enhanced skin delivery of a model drug of Anti-acne category, which was potent candidate for the treatment of acne. The preformulation studies helped to determine the organoleptic properties of Azithromycin. Transfersome containing Azithromycin, Soya lecithin, Edge activator (Tween20), ethanol and water were formulated by stirring and the ultra-sonication method. Prepared Transfersome under various parameters like particle size and Encapsulation Efficiency were obtained. For all formulation Encapsulation Efficiency was 45.62 % to 83.63 %. The Zeta sizer gives Transfersome size between 144.23 to 317.36 nm, Zeta potential between -52.3 to 66.4 mV and PDI 0.243 to 0.354. All five Transfersome formulations were mix with gel base. Transfersomal gels were physically clear, yellowish, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, pH of all formulations were observed between 6.8 to 7.1 and Spreadability between 6.0 to 6.6 cm. percentage of drug content between 62.48 % to 91.75% and viscosity between 112 to 118 centi poice (cp) and % and extrudability was found between 153.8 to 184.4 g. But on the basis of drug release kinetics F-3 formulation was excellent because its % drug release was 97.91%. On the basis of % drug release and regression (R^2) values of all formulations showed that formulation F-3 possessed excellent release profile, so the F-3 Transfersome, drug release data was converted in different type of kinetic modeling and found best fitted model was zero order. The rate constants are

calculated from the slop of the respective plots the release mechanism of Transfersomal gel. **F-3** formulation can be further study for preclinical and clinical evaluations.

Keywords: Transfersome, Azithromycin, gel, FT-IR spectrogram, Edge activator, transdermal

INTRODUCTION

Acne vulgaris is a disease of the pilosebaceous follicle characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules and nodules)[1]. Its pathogenesis is multifactorial the interplay of hormonal, bacterial and immunological (inflammatory) factors results in the formation of acne lesions[2]. Although acne is not a life-threatening situation, it can have detrimental effects on the excellence of life of affected individuals[3]. Fortunately, acne is readily approachable to the wide-range of available medications, with the goals of therapy being to clear the lesions, prevent scarring, and limit any treatment-related side-effects and psychosocial sequelae[4]. Newer fixed-dose combination products target multiple acne pathogenic factors and offer simplified dosing regimens, which may potentially enhance both efficacy and patient adherence when evaluate with single agent therapy[5]. All forms of acne involve one or more of these pathophysiologic factors: Hyperkeratinization of the follicular epithelium with comed one configuration enlarged sebum production[6]. Bacterial proliferation of Propioni bacterium acnes (*P. acnes*) local immune hypersensitivity causing inflammation[7]. Experts believe the primary cause is a rise in androgen levels -androgen is a type of hormone[8]. Androgen levels rise when a human becomes an adolescent. Rising androgen levels make the oil glands under your skin grow; the enlarged gland produces more oil[9]. Unnecessary sebums can breakdown cellular walls in your pores, causing bacteria to grow. Some studies designate that susceptibility to acne could also be genetic[10]. Some medications which contain androgen and lithium may cause acne. In such conditions transdermal drug delivery remains the most preferential mode of administration. But, stratum corneum forms the most formidable barrier for penetration of drug through skin[11]. To overcome stratum corneum barrier, use of lipid vesicles like transfersomes in delivery systems has concerned increasing attention in recently ears.

The aim of the present study was to statistically optimize the vesicular formulations (Transferosomes) for enhanced skin delivery of a model drug of Anti-acne category, which was

effective candidate for the treatment of acne. Azithromycin is an antibiotic widely used for the treatment of acne. In oral dosage forms it produces pseudomonas colitis while in topical dosage forms it has side effects like irritation, skin rash, itching etc. It's topical bioavailability is also less[12]. So, to overcome these limitations an attempt has been made to prepare transfersomes of Azithromycin and optimize it for enhanced delivery through the skin.

MATERIALS AND METHODS

Azithromycin was gifted by Cipco Pharmaceuticals, Indore. Soya lecithin and Tween 20 were purchased from Finar Chemical (India) Pvt Ltd., Ahmedabad. All other used chemicals belong to AR grade.

Preformulation Studies

The preformulation studies helped to determine the organoleptic properties, solubility,melting point, partition coefficient, UV Spectroscopy of Azithromycin.

Determination of λ_{max}: 10 µg/ml solution of was scanned in UV-spectrophotometer range from 200-400nm using double beam visible spectrophotometer.

Calibration Curve: Azithromycin (10 mg) was dissolved in 1ml 6.8 pH phosphate buffer and volume was made up to 10 ml volumetric flask using 6.8 pH phosphate buffers (1000 µg/ml). 1 ml of stock solution (1 mg/ml) was further diluted with 6.8 pH phosphate buffer, up to 10 ml. This solution (100µg/ml) was further diluted to 6.8 pH phosphate buffer, to obtain solutions of 10 to 50µg/ml. Absorption of each solution was measured at 212 nm using Systronics UV-2203 UV/Vis double beam spectrophotometer and 6.8 pH phosphate buffer, as a reference standard.

Drug- excipient compatibility:

(a) Physical compatibility: solubility, color changes, dissolution, sedimentation rate, phase separation or immiscibility and liquefaction.

(b) Chemical compatibility: undesirable reaction between drug and excipients to monitor if compounds undergo oxidation, hydrolysis, reduction, decarboxylation, precipitation and racemization determined by FT-IR Spectroscopy.

Formulation of Azithromycin Loaded Transfersomes

First, different proportions of Soya lecithin (phosphatidylcholine) were dissolved in ethanol and heated (upto 30 ± 2^{O}C) on water bath in closed vessel. Edge activator (Tween20) and Drug azithromycin were uniformly dispersed in distilled water then heated (upto 30 ± 2^{O}C) on water bath. This drug dispersion in water was added slowly as a fine stream to the above ethanol solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25±1°C) for 45 minutes. Then probe sonicator was used to further size reduction by ultra sound cavitation to form small unilamellar vesicles. Finally, samples were stored in a dark room at 25 °C.

Characterization of Azithromycin Loaded Transfersomes

Microscopic observation of prepared transferosomes: An optical microscope with a camera attachment was used to observe the shape of the prepared transferosomes formulation.

Vesicle Size and Polydispersity Index (PDI): The vesicles size and size distribution and Polydispersity Index were determined by Dynamic Light Scattering method (DLS)

Zeta potential: The zeta potential was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

Entrapment efficiency: Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded transfersomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The transfersomes along with encapsulated drug were separated at the bottom of the tubes. Plain transfersomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 212 nm.

Preparation of Transfersome Enriched Gel

Viscosity of the transfersomal dispersions was low. Hence to achieve the desired rheological characteristics and texture for transdermal application, the optimized dispersion was converted into a gel. Various gelling agents like Carbopol Ultrez 10 and Carbopol 940 were evaluated for their gelling ability. Based on the compatibility with transfersomal dispersions, feel, aesthetic appeal and ease of spreadability Carbopol Ultrez 10 was selected as the gelling agent. Gel dosage forms were prepared using a serial mixture of deionized water and glycerin in the ratio of 4.5:0.5 w/w as the vehicle and Carbopol Ultrex 10. Different concentrations of Ultrex 10 ranging from 0.5-1% w/w were used for gelling and the concentration giving the optimum viscosity was chosen for further studies. For the final formulation 0.75% w/w Ultrez 10 was selected and dispersed into the vehicle to give the total drug concentration of 0.1 % w/w. Triethanolamine was added to adjust the pH to 7, and then remaining vehicle was added to give a total weight of 20 g. Gel was dispersed thoroughly using an overhead stirrer at the speed of 800 rpm for 3h.

Characterization of Transfersomes Containing Gel

Transfersomes containing gels were Characterized using standard methods of parameters e.g. physical appearance, pH, viscosity, % Drug content, Spreadability, Extrudability and *In-vitro* drug release.

Kinetic Modeling of Optimized Formulation

Drug release data was fitted into the different kinetic model e.g. Zero order, First order, Higuchi model and Peppas-Korsemeyer of kinetics, to know the pattern of drug release and it mechanism.

Stability Study

Stability study is performed for optimized formulation shows greatest drug release and hence can be termed as ‘best formulation’ from within those that are developed. Stability study was carried out for 1 month; the formulation was kept in stability chamber at $40^{o}C$ and at 75% relative humidity and 4 ^{o}C. After one month the formulation was checked for parameters like phase separation pH and drug content.

RESULT AND DISCUSSION

Transfersomes of Azithromycin was been successfully formulated. The preformulation studies helped to determine the organoleptic properties of Azithromycin which was white crystalline Tasteless, odorless power. After that solubility determined in various solvents Azithromycin was freely soluble in ethanol and methanol, soluble in buffer pH 6.8, sparingly soluble in 0.1N NaOH, 0.1N HCl and water. Melting point was obtained as 122-125 ^{O}C and Partition Coefficient was found 3.033. λ_{max} at 212 nm, also calibration curve was obtained with following linear equation y=0.014x + 0.002 and R^2 = 0.999. Drug:Excipient Compatibility Studies also confirmed by FT-IR spectrogram using Clarck's Analysis.

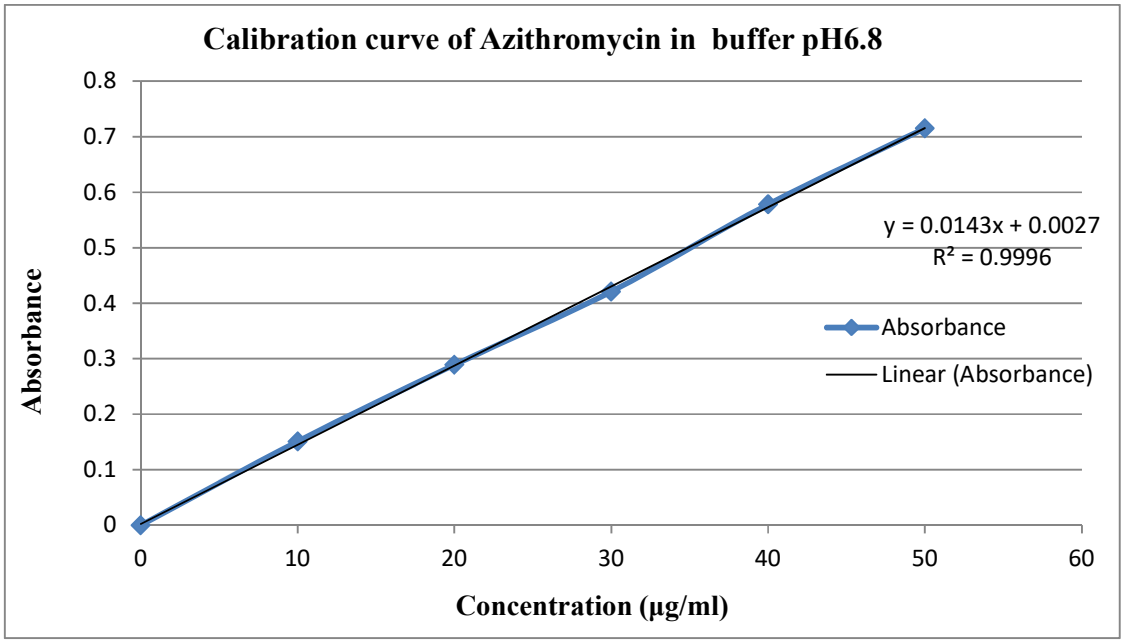

Figure 5: Calibration curve of Azithromycin in Phosphate buffer pH6.8

Table No. 9: Important band frequencies in IR spectrum of Azithromycin

S. No.	Frequency (cm^{-1})	Peak Assigned	Azithromycin FT-IR (cm^{-1})	Drug + Polymer
1.	3200-3000	OH str.	3363	3393
2.	3000-2840	CH_3 str	2928	2917, 3002
3.	1382-1266	C-N	1404	1410
4.	1275-1200	C-O str. ether	1319	1314

Formulation of Different Batches of Azithromycin Loaded Transfersomes

Transfersome containing Azithromycin, Soya lecithin, Edge activator (Tween20), ethanol and water were formulated by stirring and the ultra-sonication method. Prepared Transfersome under various parameters like particle size and Encapsulation Efficiency were obtained. For all formulation Encapsulation Efficiency was 45.62 % to 83.63 %. The Zeta sizer gives Transfersome size between 144.23 to 317.36 nm, Zeta potential between -52.3 to 66.4 mV and PDI 0.243 to 0.354.

Table No. 10: Formulation Different batches of transfersome

Batch code	Azithromycin (mg)	Soya lecithin (PC)	Tween20 (%w/v)	Ethanol (ml)	Water
F-1	100	0.5	2	10	10
F-2	100	1.0	2	10	10
F-3	100	1.5	2	10	15
F-4	100	2.0	2	10	15
F-5	100	2.5	2	10	15

Characterization of Azithromycin Loaded Transfersomes Batches

Table No. 11: Characterization of Azithromycin Loaded Transfersomes

Batch code	Vesicle Size(nm)	Zeta Potential (mV)	PDI	% Entrapment Efficiency
F-1	144.23	-52.3	0.243 ±0.03	65.43
F-2	132.41	-63.5	0.294 ±0.04	71.84
F-3	210.12	-64.6	0.265 ±0.03	83.63
F-4	279.34	-66.2	0.328 ±0.01	52.71
F-5	317.36	-66.4	0.354 ±0.02	45.62

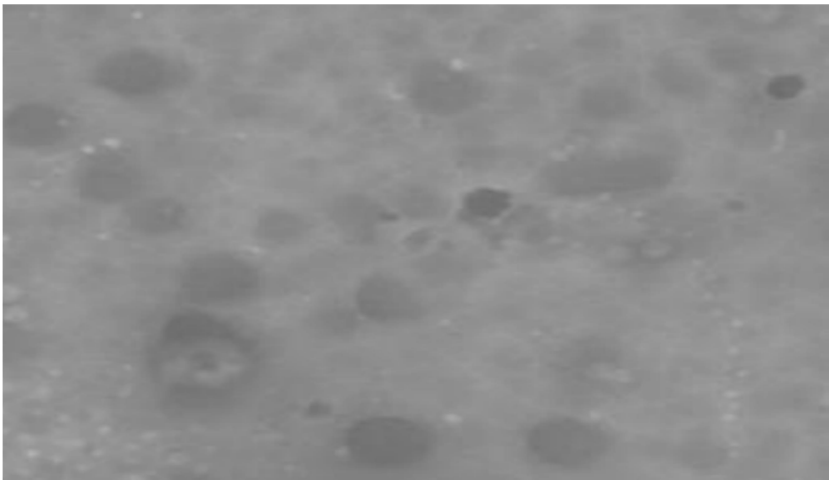

Figure 8: Microscopic Observation of optimized batch (F-3) of transfersomes

Preparation of Transferosomal Gel Base

Using the different transfersome batches (F-1 to F-5), Different transferosomal gels were prepared according to formulation described in table below:

Table No. 12: Formulation of transferosomal gel base

S. No.	Ingredients	Quantity
1	Transfersomal dispersions equivalent to Azithromycin	0.1 %
2	Carbopol Ultrez 10	0.75 %
3	Glycerin	10 %
4	Deionized Water	90 ml
5	Triethanolamine	q.s.

Evaluation of Prepared Transferosomal Gels

All five Transfersome formulations were mix with gel base. Transfersomal gels were physically clear, yellowish, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, pH of all formulations were observed between 6.8 to 7.1 and Spreadability between 6.0 to 6.6 cm. percentage of drug content between 62.48 % to 91.75% and viscosity between 112 to 118 centi poice (cp) and % and extrudability was found between 153.8 to 184.4 g.

Table No. 13: Evaluation of transferosomal gels of Azithromycin

Batch code	Physical Appearance	pH	Viscosity (cp)	% Drug Content	Spread ability (g.cm/sec)	Extrudability (g)
F-1	Yellowish, Clear	6.9	112 ± 1.8	78.42 ± 1.3	6.3 ± 0.5	156.5
F-2	Yellowish, Clear	6.8	115 ± 2.0	79.53 ±1.2	6.6 ± 0.0	153.8
F-3	Yellowish, Clear	**7.0**	**117 ± 0.8**	**91.75 ± 0.9**	**6.0 ± 0.4**	**173.9**
F-4	Yellowish, Clear	7.1	112 ± 1.2	65.77 ± 0.8	6.6 ± 0.1	183.3
F-5	Yellowish, Clear	7.1	118 ± 2.6	62.48 ± 0.3	6.1 ± 0.3	184.4

All values are mean of triplicate value (n=3) ± S.D

***In-Vitro* Drug Release Studies**

Table No. 14: *In-vitro* cumulative % drug release from transferosomal gels

Time (hrs)	Cumulative %drug release from transferosomal gels				
	F-1	**F-2**	**F-3**	**F-4**	**F-5**
0	0	0	0	0	0
1	18.25	19.62	21.61	22.68	24.31
2	24.34	39.68	44.64	41.88	37.40
3	50.93	59.22	60.41	49.93	50.98
4	78.54	82.18	73.72	61.49	60.92
5	84.27	88.56	87.01	72.81	69.17
6	92.77	95.56	97.91	81.53	80.02

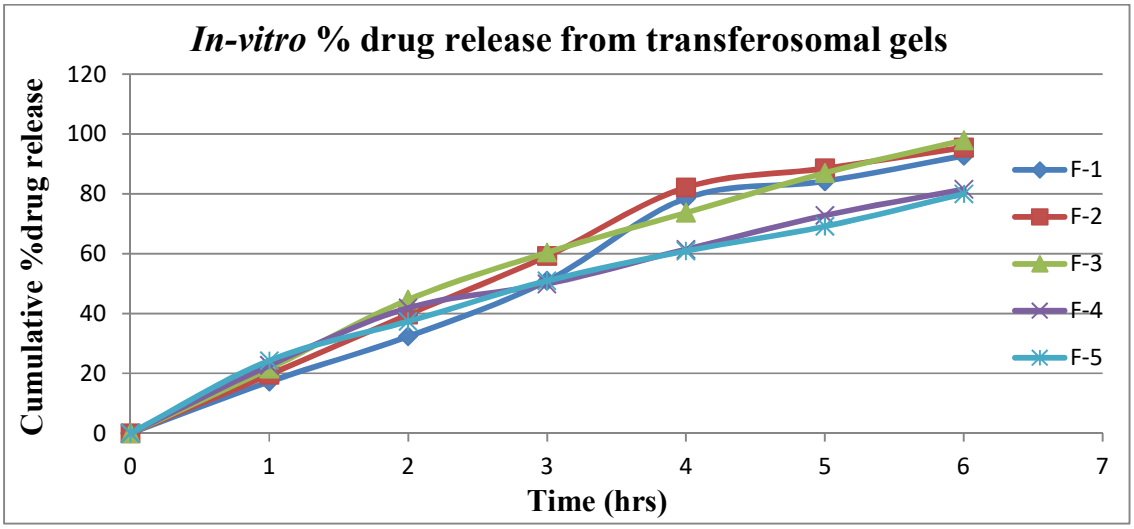

Figure 9: *In-vitro* % drug release from transferosomal gels

Table No. 15: Regression Coefficient (R^2) values of all of transferosomal gel formulations

Model	F1	F2	**F3**	F4	F5
R^2	0.975	0.969	**0.980**	0.967	0.968

Results: On the basis of % drug release and regression (R^2) values of all formulations showed that formulation F-3 possessed excellent release profile.

Kinetic Modeling of optimized formulation (F-3)

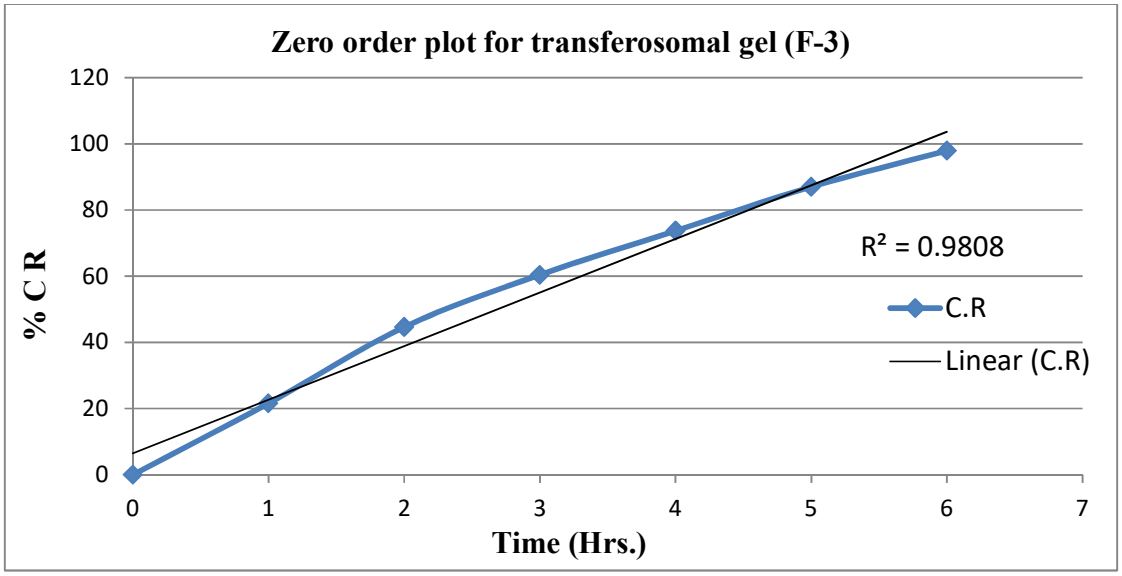

Figure 10: Zero order kinetic model for transferosomal gel (F-3)

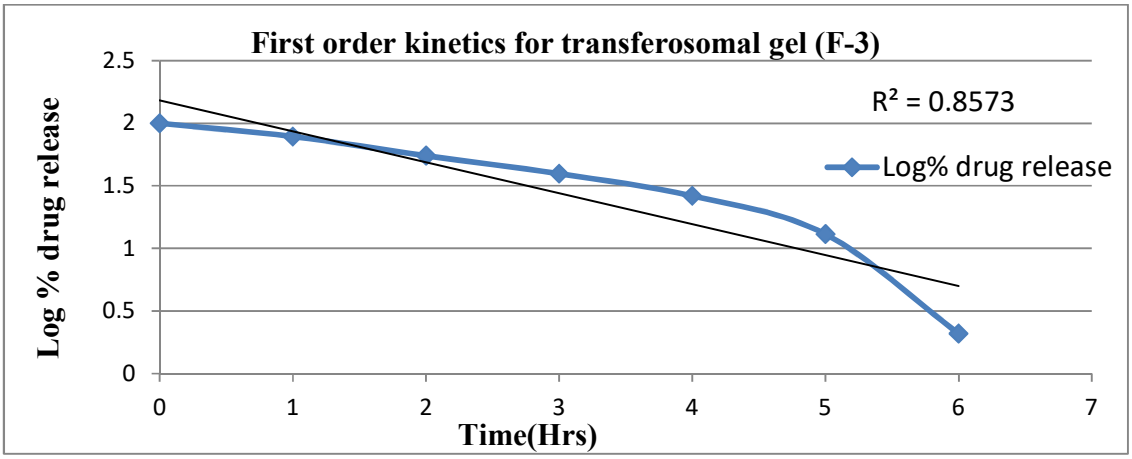

Figure 11: First order kinetic model transferosomal gel (F-3)

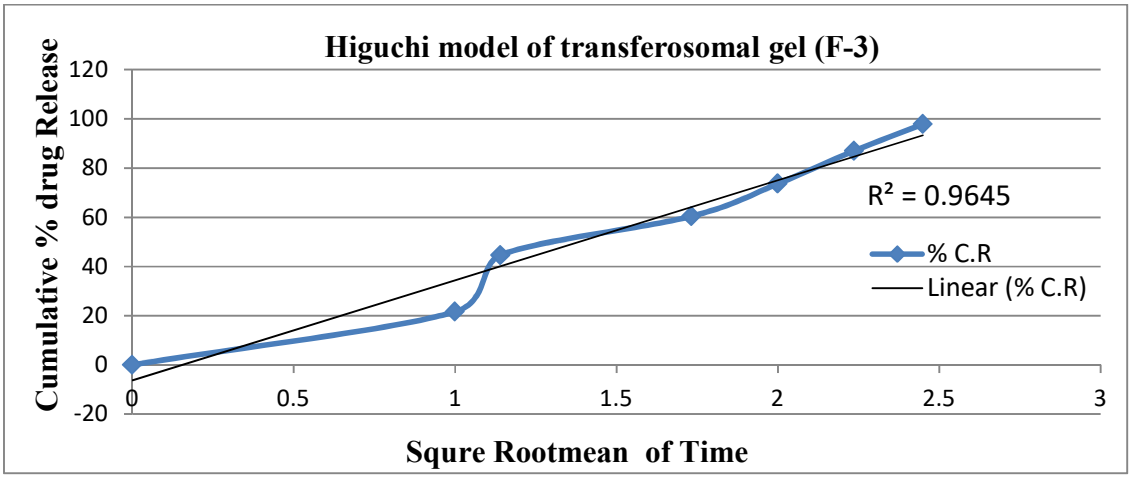

Figure 12: Higuchi model of kinetic (F-3)

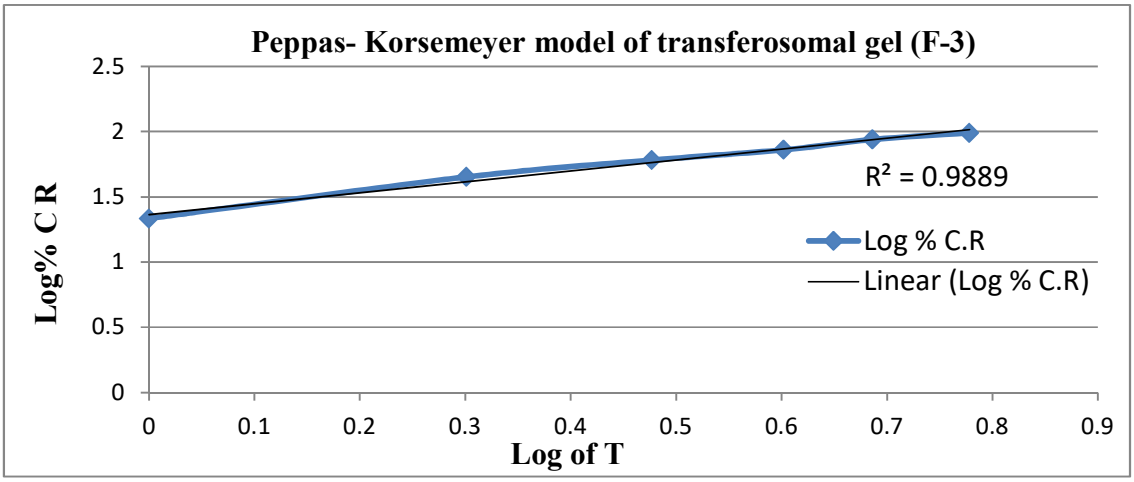

Figure 13: Higuchi model of kinetic (F-3)

Stability Study

Table No. 17: Stability of azithromycin loaded transferosomal gel (F-3) at different conditions

Formulation code	Phase separation		pH		Drug content (%)	
	4°C	40 °C	4°C	40 °C	4°C	40 °C
F-1	No	No	6.9	7.0	79.73 ± 1.1	75.83 ± 2.0
F-2	No	No	7.4	7.3	78.87 ± 1.8	74.48 ± 1.9
F-3	No	No	7.4	7.2	91.93 ± 1.9	88.73 ± 1.8
F-4	No	No	7.2	7.1	62.68 ± 0.6	63.73 ± 1.0
F-5	Yes	Yes	7.1	7.4	61.47 ± 1.9	59.23 ± 2.1

All values are mean of triplicate value (n=3) ± S.D

But on the basis of drug release kinetics F-3 formulation was excellent because its % drug release was 97.91%. With the 92.77 and 95.56% drug release of F-1 and F-2 formulation respectively was also good. Other formulations drug releases were F-4 (81.53) and F-5 (80.02). On the basis of % drug release and regression (R^2) values of all formulations showed that formulation F-3 possessed excellent release profile, so the F-3 Transfersome, drug release data was converted in different type of kinetic modeling and found best fitted model was zero order. Four gel formulations were F-1 to F-4 are stable but F-5was not stable at 4°C and 40 °C and phase separation occurs.

CONCLUSION

The prepared Transfersome containing Azithromycin was shown excellent promising results for all the evaluated parameters. On the basis of *in-vitro* drug release and drug content results, **F-3** formulation was excellent drug release as compare to other prepared Transfersome gel formulations which shows higher percentage of drug release. *In- vitro* drug release profile was applied on various kinetic models like Zero order, First order, Higuchi and Peppas-Korsemeyer model. The best fit with highest regression coefficient was found with Zero order. The rate constants are calculated from the slop of the respective plots the release mechanism of Transfersomal gel. **F-3** formulation can be further study for preclinical and clinical evaluations.

CONFLICTS OF INTERESTS

There are no conflicts of interests

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