CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3376716

Available online at: http://www.iajps.com Research Article

FORMULATION DEVELOPMENT AND EVALUATION STUDIES IN *IN-VITRO* OF ECONAZOLE NITRATE TRANSFEROSOMAL GEL

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Abstract:

Econazole nitrate is mainly used in the treatment of fungal infections. The basic idea behind the development of such a system is to maintain a sustain release of drug from the dosage form and for target delivery. Econazole nitrate has short half life (4hrs) when Econazole nitrate was formulated as transferosomes, half life can be increased and the desired effect can be obtained. In the research work an attempt was made to formulate and evaluated the transferosomal gel for sustained effect. Estimation of Econazole nitrate was carried out by U.V spectrophotometer at λ max 271.5 nm using water as solvent, which had a good reproducibility and this method was used entire study. Formulations were prepared by using soya lecithin as a lipid polymer and solvent such has ethanol the size of transferosomes, morphology, entrapment efficiency, solubility studies and drug release were evaluated. Entrapment efficiency ranging from 65.45 to 80.11% was obtained. Particle size of transferosomes was found to be in the range of 368 to 931 nm. In 24 hrs the drug release was observed ranging from 79.08% to 88.72%. Drug release from the gel was observed that 79.90%. In order to reduce the probable mechanism of drug from the dosage form, the result of in vitro dissolution studies were fitted to various kinetics equations. When the data subjected to zero order and first order kinetics model, a linear relationship was observed with high R^2 values for zero order model as compared to first order model and suggested that the formulations followed zero order sustained release.

Key words: *Transferosomes, Econazole nitrate, Soya lecithin, Sustain release, Zero order.*

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Please cite this article in press V. Viswanath et al., ***Formulation Development and Evaluation Studies in In-Vitro of Econazole Nitrate Transferosomal Gel.,*** *Indo Am. J. P. Sci, 2019; 06(08).*

INTRODUCTION:

For many decades treatment of acute illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, suppositories, pills, ointments, creams, aerosols, injectables as drug carriers. These types of conventional drug delivery systems are known to provide a prompt release of drug.

Therefore, to achieve as well as to maintain drug concentration within the therapeutically effective range needed for treatment, it is often needed to take these types of drug delivery systems several times a day. Thus the salient aspect of conventional method of drug delivery is the fluctuation in concentration between dosages. With some drugs this fluctuation causes no problem, but with drugs that cause toxic reactions when administered above a narrow therapeutic concentration range, such variations can cause undesirable side effects.[1]

The novel drug delivery system thus aims at releasing one or more drugs continuously at a pre determined pattern for a fixed period of time, either systemically or to a specified target organ. When the aim is to deliver the drugs through the skin in predetermined and controlled fashion, it is known as trans dermal drug delivery[2,3].

Formulations on skin can be classified into two categories according to target site of action of the containing drugs. One has systemic action after drug uptake from the cutaneous micro vascular network and the other exhibits local effects in the skin[4].

MATERIALS & METHODS:

Econazole nitrate (Yarrow chemicals Ltd, Mumbai
Span 80 (S.D. Fine chemicals Ltd, India)
Tween 80 (S.D. Fine chemicals Ltd)
Soya phosphatidyl choline (S.D. Fine chemicals)
Methanol (S.D. Fine chemicals Ltd)

PRE-FORMULATION STUDIES

Preformulation testing is the primary step in the rationale development of dosage forms of a drug substance. It can be defined as a study of physical and chemical properties of a drug substance alone and when combined with excipients. The overall aim of preformulation testing is to produce information useful to the formulator in increasing stable, effective and safe dosage form.

Hence preformulation studies were carried out on the obtained samples of drug for identification and compatibility studies.

Identification of Drug

The obtained sample was examined by Infrared absorption spectral analysis and was compared with the reference standard IR spectrum of Econazole Nitrate.

Method: IR Spectra of drug and blends were recorded on an IR spectrophotometer (Shimadzu Corporation, Japan) in the range of 4000-400 Cm^{-1} using potassium bromide discs.

Determination of Melting point

Melting point of Econazole Nitrate was determined by open capillary method. Melting-point apparatus is most frequently used for the determination of the melting point of a solid. A few crystals of the compound are placed in a thin walled capillary tube 10-15 cm long, about 1 mm in inside diameter, and closed at one end.

Determination of solubility

The known excess amount of drug was added to methanol, dichloro methane and DMSO, Chloroform and these samples were rotated at 20 rpm in a water bath (37 ± 0.5°C) for 2 hours. The samples were then filtered through 0.45µm membrane filter, suitably diluted, and analyzed visually.

Determination of pH

Dissolve 100mg of Econazole Nitrate pure drug in the solvent in which it was miscible and stir with a clean glass rod. Let the sample stand for 1h to allow the temperature to stabilize. Immerse the electrode(s) of the pH meter into the water sample and turn the beaker slightly to obtain good contact between the solvent and the electrode.

The electrode(s) require immersion 30 seconds or longer in the sample before reading to allow the meter to stabilize. Read and record the pH value to the nearest tenth of a whole number.

Compatibility Studies

The compatibility of drug and polymers under experimental condition is important prerequisite before formulation. Incompatibility between drugs and excipients can alter stability and bioavailability of drugs, affecting its safety and efficacy.

Study of drug–excipient compatibility is an important process in the development of a stable solid dosage form. Drug–excipient compatibility testing at an early stage helps in the selection of excipients that increases the probability of developing a stable dosage form.

The compatibility between pure drug, surfactants, carriers and cholesterol were detected by FTIR spectra obtained on Brucker-Germany. The potassium bromide pellets were using pellet press. For the prepared pellets, the solid powder samples were ground together in a motor with 100 times quantity of KBr. The finally grounded powder was introduced into a stainless steel die. The powder was

pressed in the die between polished steel anvils at a p Preparation of Econazole nitrate Loaded Transfersomes

Soya-phosphatidylcholine was taken in a round bottom flask. Span80(sp) or Tween 80 (Tw)was put in the same round bottom flask. Methanol was then added to the same flask. The Econazole nitrate was also loaded in the same RBF. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotatory vaccum evaporator at 600C. This thin film was then hydrated by phosphate buffer saline to get the Transferosome.

Preparation of Econazole nitrate Loaded Transfersomes

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Incorporation of vesicular dispersions in gel

The carbopol gel (2%w/w) was prepared. Carbopol934 (2g) was dispersed in distilled water in which glycerol was previously added. The mixture was stirred until thickening occurred and then neutralized by the drop wise addition of 50% (w/w) triethanolamine to achieve a transparent gel of pH 6.6. Then the vesicular dispersions (transfersomes) were incorporated into carbopol gel. vesicular dispersions were mixed into vehicles by using a mechanical stirrer for 5min.

EVALUATION PARAMETERS

Particle size

The prepared transferosomes were undergone morphological studies by using optical microscopic metod. Small quantity of sample spreaded over clean slide. The slide was focused under optical light and images were snapped by using optical microscopy attached with Dewinter Microscopic camera software.

According to morphological evaluation analysis, all vesicles types seemed to have a spherical or oval shaped. These oval-shaped vesicles may have resulted from the transferosomes' deformation, which might occur during the sample preparation.

Entrapment efficiency

The probable reason for high entrapment efficiency was the presence of ethanol in the transferosomal formulations. Methanol increases the fluidity and the intra lamellar distance of vesicular membranes, which in probably responsible for better entrapment efficiency. The Entrapment efficiency of transferosomes depends on the surfactant concentration in the bilayer.

Initially with increasing surfactant concentration, there was an increased Entrapment efficiency. However after threshold level i.e above 15% a further increases in the surfactant concentration led to a decrease in Entrapment efficiency. This may due to the fact that at a certain concentration surfactant molecule get associated with the phospholipids bilayer resulting in better partitioning of drug.

So above 15% concentration surfactant molecules may start forming micelles in bi layer which results in pore formations in vesicles membrane and complete conversion of vesicle membrane into mixed micelles. These mixed micelles were reported to have a lower drug carrying capacity and poor skin permeation due to their structural features.

Drug content

Drug content uniformity was determined as triplicate by dissolving in methanol and dissolved transferosomes were undergone centrifugation at 3000rpm for 2hrs and filtered with Whatmann filter paper (0.45,) Whatman, Maidstone (UK). The solution was diluted to Beer's range and observed in UV-Spectrophotometer).

***In vitro* Diffusion Studies**

Franz diffusion studies of all the formulation of transferosomes of Econazole nitrate were carried out in pH 7.4 phosphate buffer. The study was performed for 24hrs and cumulative percentage drug release was calculated at different time intervals. The in vitro drug release profile for the formulation (F1 to F6), (F7 to F12) were tabulated in. The plot of time Vs cumulative % drug release formulations (F1 to F6) to (F7 to F12) were plotted and depicted in figures. Effects of various surfactants and their concentration on drug release were studied.

In vitro drug release studies from gel through a cellophane membrane: Transferosomal gel formulation was subjected to in vitro drug release studies using a cellophane membrane. The cumulative amount of drug release was calculated.

RESULTS AND DISCUSSION:

Table 1: Calibration Curve data for Econazole Nitrate at 271.5nm

S. No	Concentration in µg/ml	Absorbance at 271.5nm
1.	0	0
2.	20	0.214
3.	40	0.418
4.	60	0.596
5.	80	0.760
6.	100	0.931

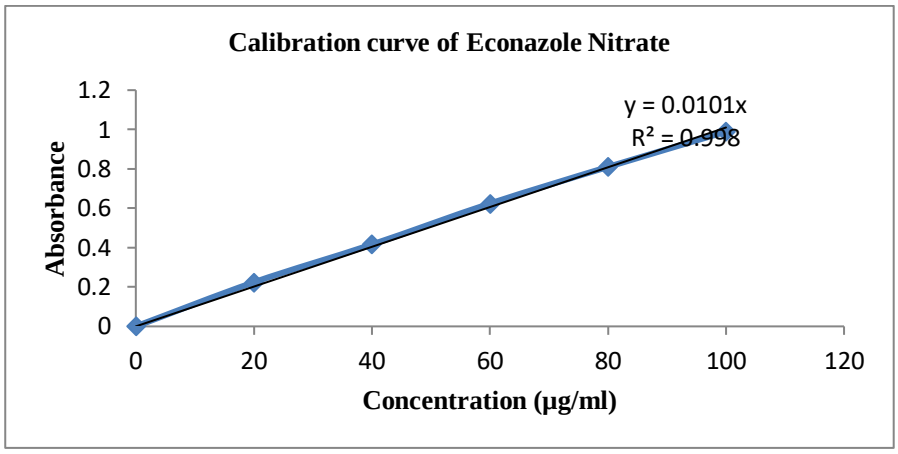

Figure 1: Calibration curve of Econazole Nitrate

Identification of Econazole nitrate

The IR spectrum of pure drug was found to be similar to that of standard spectrum of Econazole Nitrate. The spectrum of Econazole Nitrate shows the following groups at their frequencies shown in 1037, 1330, 1412, 1586, 2923, 3108 cm-1.

Determination of melting point

The melting point of Econazole Nitrate was found to be 164-1660C which complied with the BP standards.

Drug-Polymer compatibility

Compatibility studies of pure drug Econazole nitrate with polymers were carried out prior formulation of transferosomes. IR spectra of pure drug and polymer were taken; All the characteristic peaks of Econazole nitrate were present in spectra at respective wavelength. Thus, indicating compatibility between drug and polymers. It shows that there was no significant change in the chemical integrity of the drug.

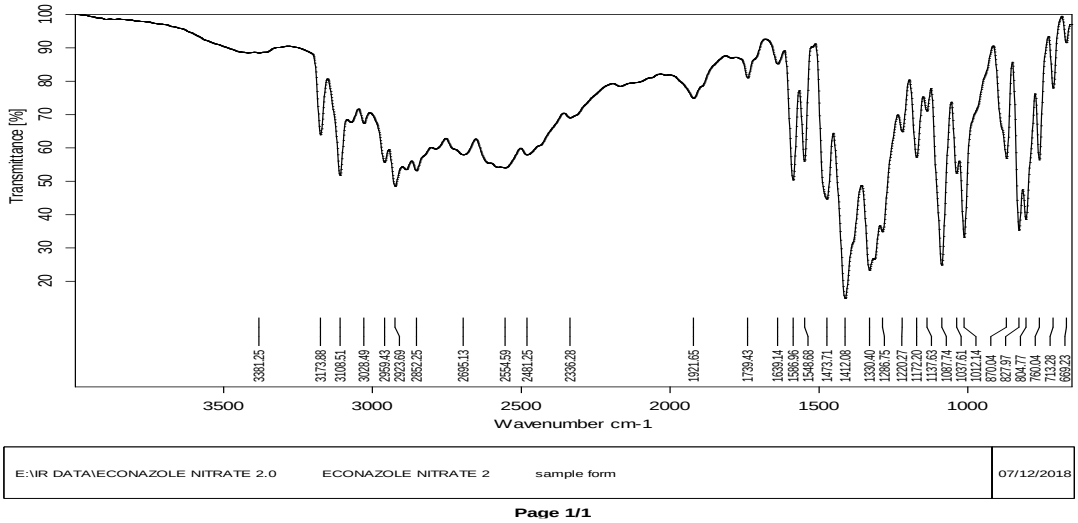

Figure 2: Econazole nitrate drug

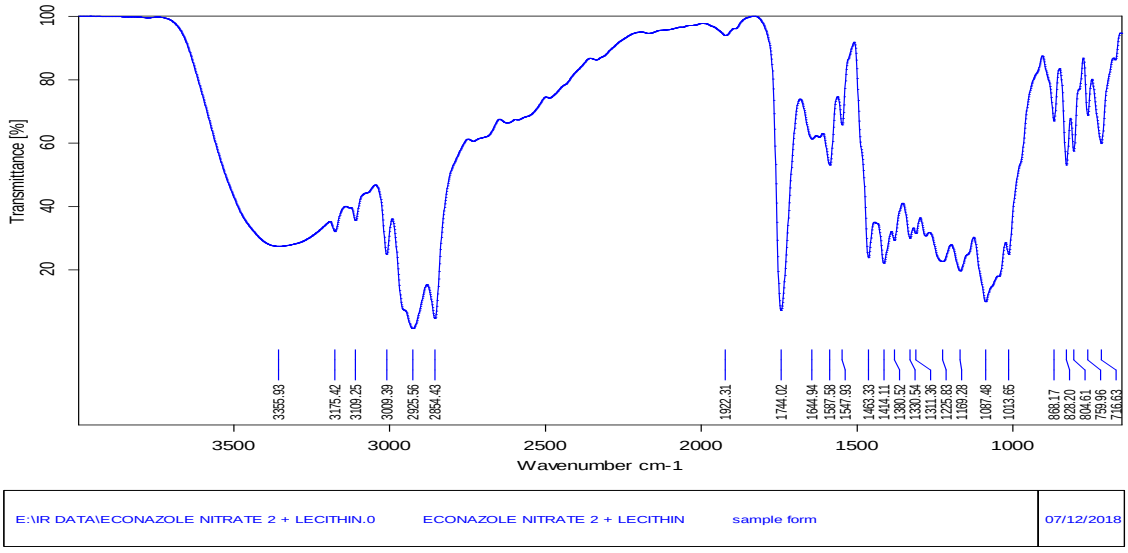

Figure 3: FTIR of Econazole nitrate + Soya Lecithin

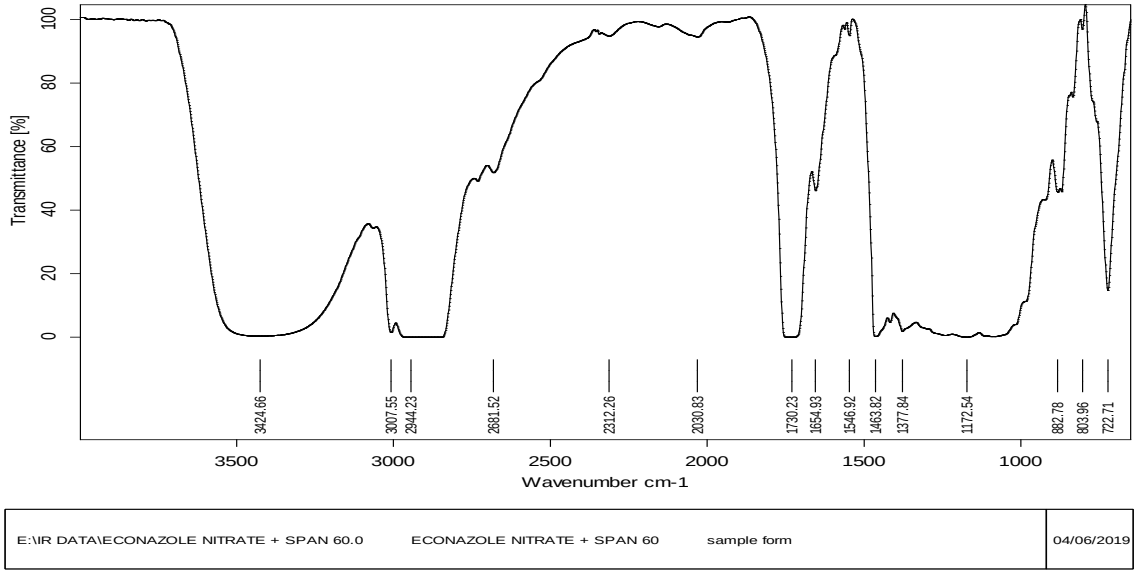

Figure 4: FTIR spectra of Pure Econazole Nitrate + Span 60

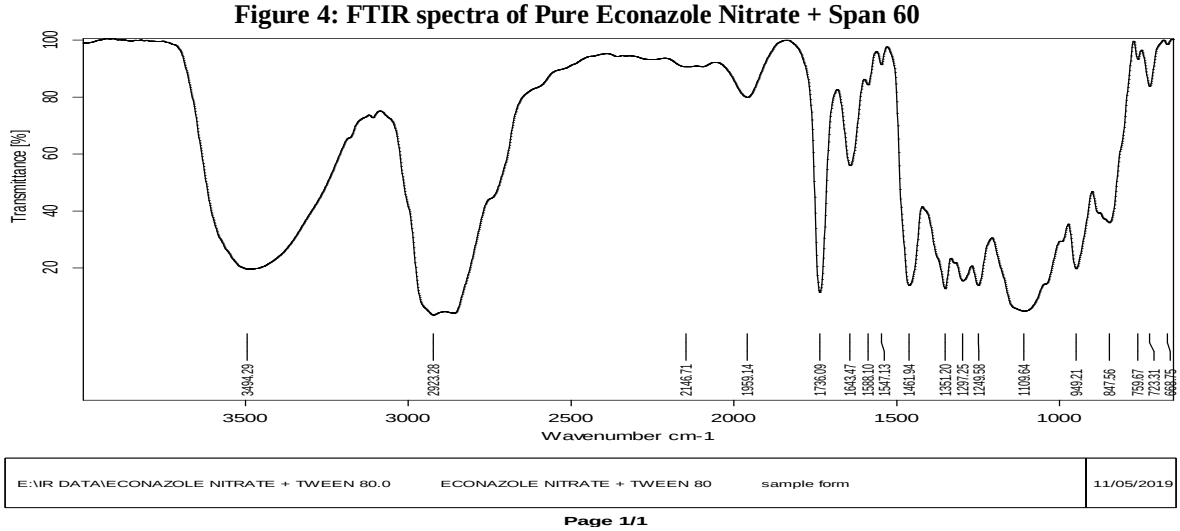

Figure 5: FTIR spectra of Pure Econazole Nitrate + Tween 80

Table 2: Particle size, Drug content, Entrapment Efficiency of F1 to F12 Formulations

S. No	Formulation Code	Particle size (nm)	Drug content (%)	Entrapment efficiency (%)
1	F1	620	87.65	74.14
2	F2	741	81.43	69.42
3	F3	368	88.91	80.11
4	F4	721	90.31	71.78
5	F5	866	87.41	70.65
6	F6	871	94.15	65.41
7	F7	644	83.46	69.12
8	F8	702	80.45	66.12
9	F9	794	89.14	72.44
10	F10	462	85.16	74.25
11	F11	866	84.25	68.36
12	F12	931	88.16	72.11

Table 3: Cumulative drug release % of F1 to F6

Time (hr)	**Formulation (F)**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
1	12.54	13.50	16.87	13.27	11.44	11.68
2	20.66	16.09	20.41	23.36	27.57	23.48
3	29.99	20.82	23.99	26.91	30.13	27.96
4	32.57	29.01	27.60	31.60	37.29	34.61
5	39.73	32.72	33.49	35.22	42.23	39.20
6	41.26	38.74	38.31	39.99	46.08	42.77
7	48.51	44.82	45.43	42.59	48.81	45.31
8	51.26	50.96	51.50	50.74	52.71	48.93
12	64.30	60.58	60.99	61.18	62.37	57.89
24	82.03	81.69	79.57	87.21	85.84	81.81

Table 4: Cumulative drug release % of F7 to F12

Time (hr)	**Formulation (F)**					
	F7	**F8**	**F9**	**F10**	**F11**	**F12**
0	0	0	0	0	0	0
1	16.77	19.88	15.70	21.13	13.05	19.28
2	31.32	28.78	20.35	24.87	17.93	24.01
3	32.83	34.04	27.28	28.64	25.23	28.78
4	37.94	39.35	32.03	33.61	30.23	35.87
5	41.91	42.22	40.20	42.16	32.90	44.16
6	48.31	50.09	45.08	46.10	39.15	49.13
7	51.17	53.06	47.76	47.72	47.84	53.01
8	60.05	63.51	51.59	54.05	51.86	56.93
12	71.41	74.07	62.18	65.13	63.05	65.41
24	87.67	88.45	82.97	83.37	79.08	88.72

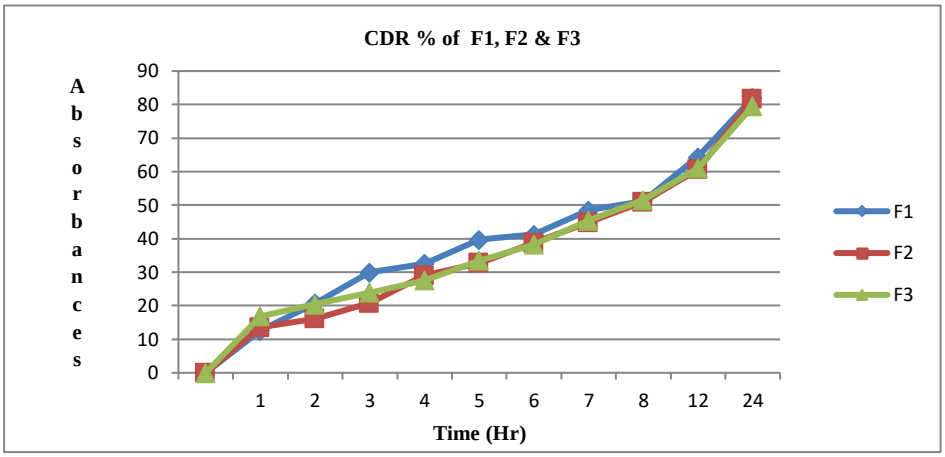

Figure 6: Cumulative drug release % of F1, F2 & F3

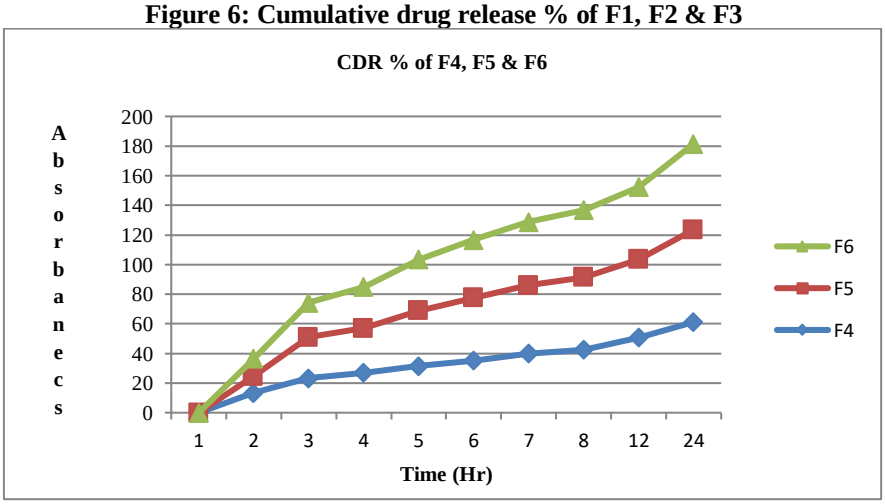

Figure 7: Cumulative drug release % of F4, F5 & F6

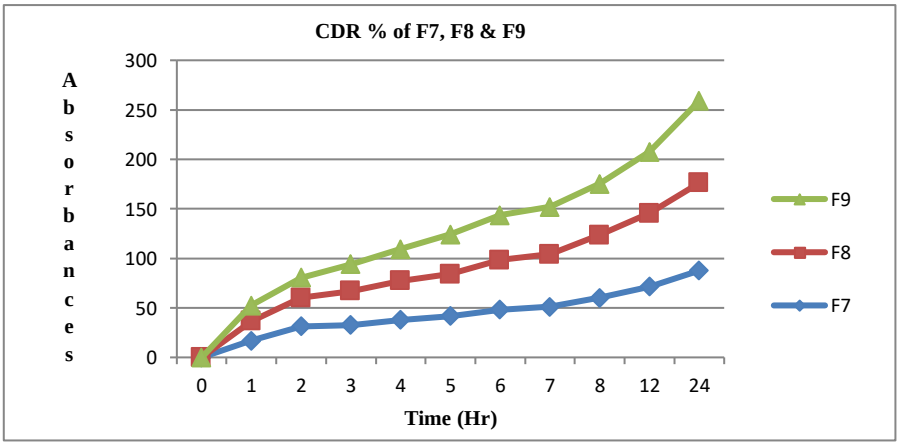

Figure 8: Cumulative drug release % of F7, F8 & F9

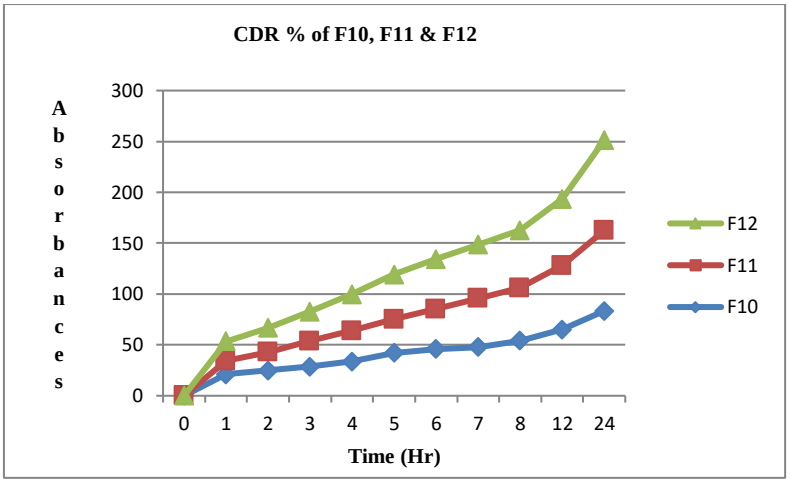

Figure 9: Cumulative drug release % of F10, F11 & F12

Table 5: Release Kinetics Data of all the Formulations (F1 to F12)

Formulation code	Zero order R^2	First order R^2	Higuchi's R^2	Korsemeyer Peppa's	
				N	R^2
F1	0.848	0.984	0.986	0.6	0.978
F2	0.886	0.991	0.977	0.633	0.969
F3	0.872	0.982	0.979	0.541	0.965
F4	0.91	0.993	0.992	0.594	0.988
F5	0.843	0.988	0.987	0.59	0.942
F6	0.862	0.987	0.993	0.585	0.969
F7	0.819	0.984	0.98	0.513	0.97
F8	0.806	0.978	0.974	0.495	0.982
F9	0.857	0.989	0.989	0.56	0.984
F10	0.842	0.978	0.988	0.473	0.973
F11	0.851	0.971	0.974	0.614	0.978
F12	0.844	0.992	0.987	0.518	0.978

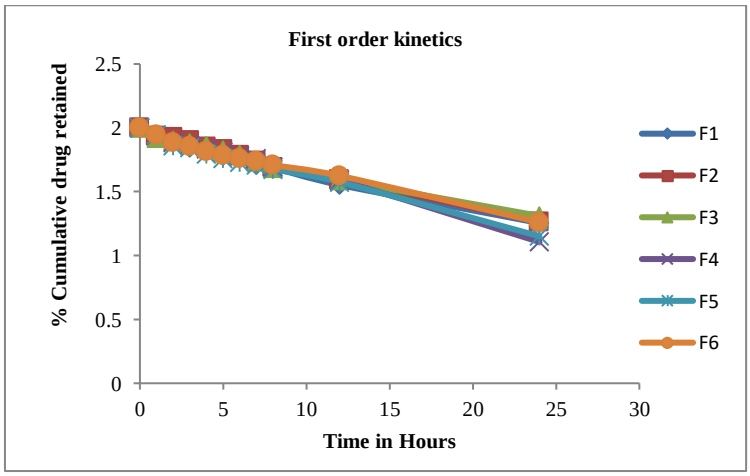

Figure 10: Time Vs Drug retained (First order kinetics) of formulations F1 to F6

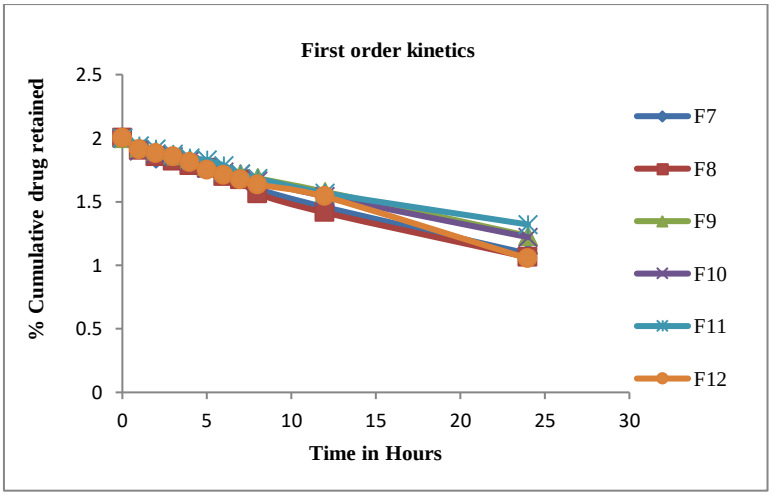

Figure 11: Time Vs Drug retained (First order kinetics) of formulations F7 to F12

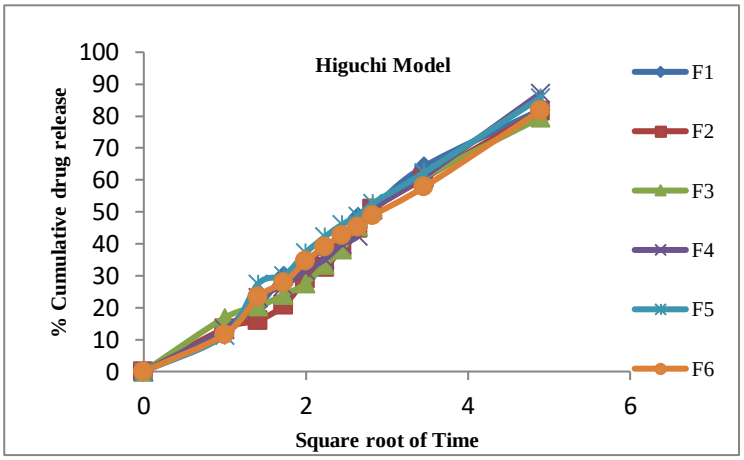

Figure 12: Square root of Time Vs % CDR (Higuchi Release mechanism) of Formulation F1 to F6

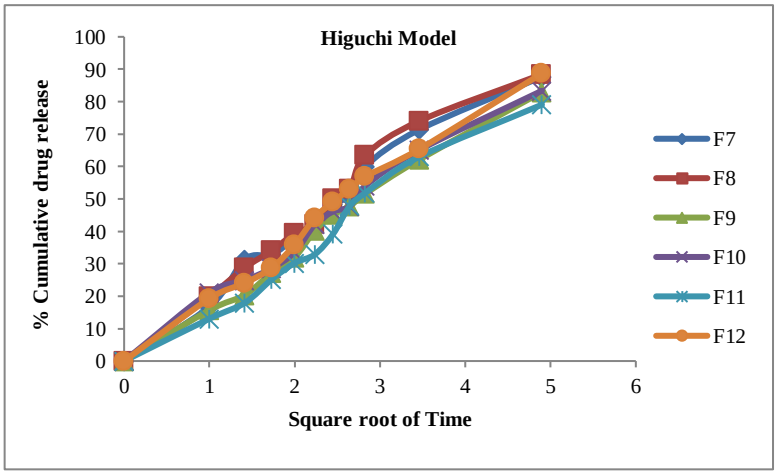

Figure 13: Square root of Time Vs % CDR (Higuchi Release mechanism) of Formulation F7 to F12

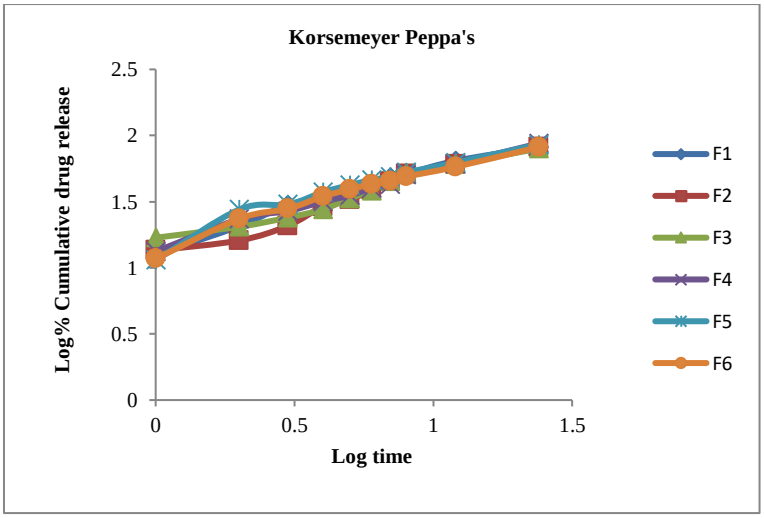

Figure 14: Log Time Vs %CDR (Korsmeyer-Peppas Release Mechanism) of Formulations F1 to F6

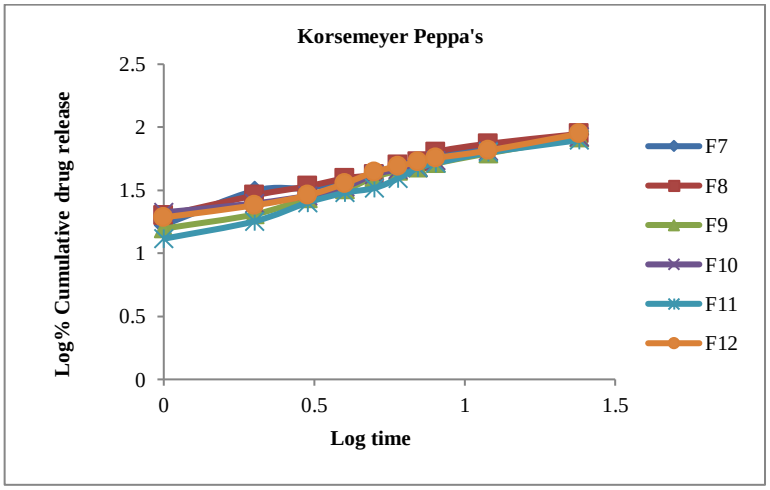

Figure 15: Log Time Vs %CDR (Korsmeyer-Peppas Release Mechanism) of Formulations F7 to F12

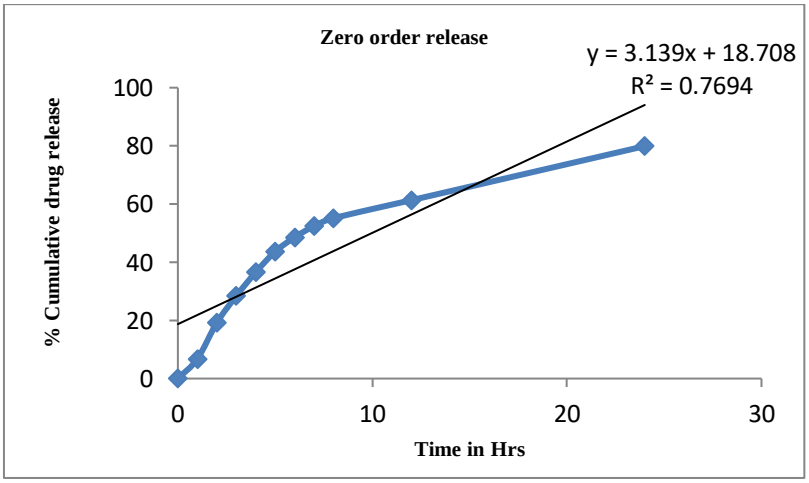

Figure 16: Time Vs % CDR (Zero order kinetics) of F3 Formulation Gel

The in vitro release data was subjected to zero order, first order, Higuchi's and Korsemeyers-Peppas model inorder to establish the drug release mechanism and kinetics of the drug release from the transferosomes and Transferosomal gel. When the data was subjected to zero order and first order

kinetics model, a linear relationship was observed with high R^2 value for zero order model as compared to first order model as compared to first order model

and its suggested that the formulations followed zero order sustained release.

Higuchi's model was applied to the in vitro release data, linearity was obtained with high R^2 values suggested that the drug release from the Transferosomes followed by different mechanism. In order to define perfect model which will represent a better fit for in vitro release data , Korsemeyer-Peppas model was applied which will define the exact mechanism. Good linearity with high R^2 values was observed with this model. The value of n obtained for all the formulations was >0.5 and <1.0, suggesting that the drug released followed non – fickianan diffusion.

CONCLUSION:

Transferosomal drug delivery system offers a simple and practical approach to achieved increase bioavailability, avoids first pass metabolism and modify drug release profiles essential for sustained, site specific and localized drug action. IR spectra of pure drug and with polymers are identical and do not show and incompatibility, thus the polymers are compatible with the drug.

All the prepared transferosomes were found to be a spherical shape. The drug content of formulation should satisfy and uniform distribution of drug within the transferosomal drug delivery system. The gel was found to have smooth appearance and texture in *in-vitro* release obeyed zero order kinetics with mechanism of release zero order followed by non fickian diffusion due to more lipophilic nature of polymers used.

The drug permeation was slow determined by Franz diffusion cell study and 79.90% of Econazole nitrate gel among all the formulations, F3 posses satisfactory swelling index, and in vitro drug release studies were extended period of time so F3 was considered to be the best formulations.

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