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Formulation and development of oxiconazole based ethosomal gel system for dermal delivery

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ABSTRACT

Last few decades the act of regular drug for the wound healing improving in many parts of India depends on the sources such as herbal, semi-synthetic and synthetic medicines. Despite the fact that pharmaceutical innovation currently has focused on the improvement of nano drug delivery systems such as liposomes, niosomes, nanosomes, ethosomes, dendrimers, nanoparticle etc. This could be a novel approach towards skin mediated drug delivery system. An attempt was made to formulate highly effective oxiconazole gel. In this current work oxiconazole, loaded ethosomes were formulated in the form of the gel for the treatment for the skin infection. The preformulation study confirms low interparticle friction between the drug and polymers with better flow property. FTIR and DSC analysis confirm that there is no prominent chemical reaction between oxiconazole and polymers. Ethosomes has been prepared by using the injection method by introducing semi-synthetic and natural polymers. The prepared ethosomes are in the range of micrometer and found as spherical in shape confirmed by TEM and SEM analysis. The smaller size of the particle and polyethylene glycol plays an important role to enhance the skin penetration. Evaluation study (such as drug entrapment efficiency, percentage yield, drug content, pH, viscosity and spreadability) for ethosomes has been carried out and found optimum as per the IP. Formulation E16 has shown a better result in sustaining the oxiconazole as 93% in 12h. The kinetic release study follows non-Fickian behaviour.

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INTRODUCTION

Fungal infection mainly caused by a microscopic organism such as fungi readily available in the environment which can invade the epithelial tissue. Yeast, moulds, rusts and mushroom are belonging to the family of fungi can grow easily on animals and also obtain nutrients from the environment (Lopez-Martinez, R, 2010; Heitman, J, 2011). Fungal infection commonly affects the hair, nail and skin. In case of systemic infection, fungal pneumonia can occur to individuals depending on the favourable climates for the proliferation of fungi (Shapiro, RS *et al.,* 2011). The patient suffering from HIV, cancer, anaemia, blood disorder problem and diabetes are more prone to infected by a fungal infection.

Table 1: Formulation of oxiconazole containing ethosomal gel (%)

Formulation	Drug (%)	CH (%)	HPMC (%)	SA (%)	CP (%)	Soya Lcithin (%)	Ethanol (%)	Polyethylene glycol (%)
E1	1	1	-	-	-	3	45	2
E2	1	1.5	-	-	-	3	45	2
E3	1	2	-	-	-	3	45	2
E4	1	2.5	-	-	-	3	45	2
E5	1	-	1	-	-	3	45	2
E6	1	-	1.5	-	-	3	45	2
E7	1	-	2	-	-	3	45	2
E8	1	-	2.5	-	-	3	45	2
E9	1	-	-	1	-	3	45	2
E10	1	-	-	1.5	-	3	45	2
E11	1	-	-	2	-	3	45	2
E12	1	-	-	2.5	-	3	45	2
E13	1	-	-	-	1	3	45	2
E14	1	-	-	-	1.5	3	45	2
E15	1	-	-	-	2	3	45	2
E16	1	-	-	-	2.5	3	45	2

Oxiconazole is an expansive range imidazole antifungal specialist. Like other imidazole antifungals, it can enhance film penetrability to zinc, enlarging its cytotoxicity (Roychowdhury, S *et al.,* 2012). It is a biopharmaceutical grouping framework Class II sedate having low fluid solvency and poor foundational retention. The significant downside of this medication is low fluid dissolvability and its hydrophobic nature. Thus, extraordinary strategies are utilized to upgrade the dissolvability of this ineffective water solvent medication which incorporates the utilization of surfactants, co-surfactants, co-solvents, etc. (Touitou, E *et al.,* 2000). In spite of having numerous favourable circumstances of gels, noteworthy confinement is in the conveyance of hydrophobic drugs (Sinha, VR *et al.,* 2004). Ethosomes is a magnificent vehicle for hydrophobic medication due to its smaller size of the particle and the advancement in the formulation. ethosomal gel provides a better carrier and enhance the skin conveyance of different drugs (Pardeike, J *et al.,* 2009; Khullar, R *et al.,* 2012; Touitou, E *et al.,* 2000) or used as a superior vehicle to the conveyance of hydrophobic medications.

The current research aimed to formulate and evaluation of oxiconazole based ethosome gel using different polymers at different compositions to sustain the release rate of the active drug. The preformulation study has been performed between active drug and a different ration of individual polymers. Drug and polymer compatibility study was performed by FTIR and DSC analysis. Surface morphology and size of the particle was determined by SEM and TEM analysis. Various parameters have been evaluated for ethosomes such as drug entrapment efficiency, percentage yield, drug content, pH, viscosity and Spreadability.

MATERIALS AND METHODS

Reagents and Chemicals

Oxiconazole and hydroxypropyl methylcellulose (HPMC K4M) were obtained from Cipla research laboratories, India. Chitosan and sodium alginate analytical research grade was purchased from Sigma Aldrich and used as received. Similarly, carbopol and all other excipients were of analytical research grade and used as received from Divya Chemicals, India.

Detail of Instrumentation

Analytical weighing balance (ContechA224, India), pH meter (Elico LI120, India), UV visible spectrophotometer (1800, ShimadzuCorp., Japan), FTIR (Bruker Alpha, India), DSC (Q 1000 DSC System (TA Instruments, USA), Magnetic stirrer with Hotplate (Shimadzu Mumbai, India), Franz Diffusion cell Apparatus (EMFDC 06 Orchid scientific, Nasik), Scanning Electron Microscope (Hitachi, Tokyo, Japan), Transition Electron Microscope (Hitachi, Tokyo, Japan)

Formulation of Ethosomal Gel

The ethosome has been prepared by using the injection method with slight modification of the procedure (Haque, SE and Sheela, A, 2015) shown in Table 1. The required amount of oxiconazole as an antifungal drug is prepared separately with methanol and added with different polymeric solutions prepared (such as HPMC, Sodium alginate, chi-

tosan and carbopol at different concentrations) individually in a water bath at 30°C. The aqueous phase (water) is added in a fine stream to the ethanol with constant stirring at 700 rpm for 15 minutes in a well-sealed container. The obtained solution was left to cool down at room temperature till 1h.

Physicochemical evaluation

Entrapment efficiency

The entrapment efficiency of the antifungal drug oxiconazole is determined by UV Visible spectrophotometer. This technique is used to find out the drug content in the ethosome. The prepared gel has been diluted into 10 ml of methanol with proper stirring by using magnetic stirrer. A homogeneous solution has been obtained and kept for a centrifuge. The centrifuge was carried out at 1200 rpm for 30 min. The supernatant liquid has been analyzed under UV Visible spectrophotometer at 296 nm with suitable dilution. The EE was calculated by formula mention below.

% Entrapment efficiency = (Amount of drug entrapped / Amount of drug added) X 100

Percentage Yield

It is calculated to determine whether the drug entrapment facing startling polymer was efficient. The product results expect close to measuring final output which should compare with the raw materials weight.

Percentage yield = (Practical yield / theoretical yield) X 100

Drug Content

Prepared gel was weighed up to 1gm and diluted with buffer sample pH 6.8 and make the volume up to 50 ml. From the solution, 5ml was pipetted out in 25 ml volumetric flask, and the volume was made up utilizing phosphate buffer pH 6.8. The absorbance was estimated under UV Visible spectrophotometer at 296nm. Medication content was calculated by utilizing a standard curve of the oxiconazole.

pH

The pH of various formulations from ethosome was evaluated by using digital pH meter. It was calibrated before use. The measurement of pH of each formulation was done in triplicate, and the average was calculated.

Viscosity

Brookfield viscometer is used to measure the viscosity of the prepared gel. The gels were poured in a beaker and rotated at 50 rpm, and the corresponding reading shown on the viscometer was noted. The viscosity of the gel was obtained by Brookfield viscometer. The viscosity was measured in cps. Experiments were carried out in triplicates for all the formulations in case of ethosome.

Spreadability

Prepared gel present in the form of ethosome was taken for spreadability test. Approximately 350mg of the gel was weighed, and then, applied on the glass plate to determine the spreadability of the gel. Another glass plate was dropped at a height of 5 cm to the previously applied glass plate. After one minute the diameter of the circle was measured and the test as performed in triplicate, and average values were calculated.

SEM Analysis

Scanning electron microscopy (SEM) was conducted to analyze the surface morphology of ethosomes. SEM analysis can also use to determine the shape of the formulated ethosomes. A drop of the formulated gel was mounted on clear glass stub, air dried and visualized under SEM.

***In vitro* Drug Release**

Franz diffusion cell of vertical form is used to determine in vitro drug release study. From each formulation of ethosomes 3 mg of the freshly prepared gel was spread on the donor side of the cellulose nitrate membrane grade 110 (each sample done in a triplicate manner). The cellulose membrane soaked with isopropyl alcohol to make it more hydrophobic. In receptor vessel, 1litre of saline phosphate buffer (pH-7.4) with methanol was used. The study was carried out at 37 ±0.5°C temperature and the speed of agitator maintained as 400rpm for 12h. After a regular interval of time, a 5ml sample was collected and replaced with the same buffer solution. Collected samples were marked and kept it for analysis under UV Visible spectrophotometer. A suitable dilution has been made for each sample and concentration was measured at 296nm. The obtained result reveals that % of drug release at regular interval of time from the prepared gel.

Release Kinetic Studies

The release kinetic study of oxiconazole based ethosome has been conducted by using the dissolution profile. The kinetic study was evaluated by the following equation mentioned below.

- Zero-order: $M_t = M_o + K_o t$
- First order: $\ln M_t = \ln M_o + K_1 t$
- Higuchi model: $M_t = K_H \sqrt{t}$
- Korsmeyer–Peppas model: $M_t/M_o = K_k t^n$

Where M_t is the amount of drug dissolved at time t, M_o the initial amount of drug, $K_1$is the first order release constant, K_0 the zero order release constant, K_H the Higuchi rate constant, K_k the Korsmeyer–Peppas model release constant and n is the diffusional release exponent indicative of the operating release mechanism. The correlation coefficient (R^2) value was used as an indicator of the best fitting, for each of the models considered.

RESULTS AND DISCUSSION

Pre-formulation study for drug and polymers

Bulk density and tapped density

Bulk density and tapped density mainly depend on the nature of the compound and its size. These properties of a compound may vary due to the crystallization, milling or in the formulation. It also provides true knowledge of the size of the final dosage form. Obtained results of bulk and tapped density has been reported in Table 2 for drug-polymer ratios. The bulk density of the drug-polymer ratios was found to be 0.299 to 0.542 gm/ml and the tapped density shows 0.211 to 0.41 gm/ml. Obtained result shows low interparticle friction between the drug and polymers with better flow property.

Table 2: Pre-formulation study for drug and polymers

Formulation	Bulk density	Tapped density
F1	0.299	0.271
F2	0.321	0.253
F3	0.343	0.232
F4	0.371	0.211
F5	0.402	0.357
F6	0.441	0.41
F7	0.455	0.394
F8	0.468	0.32
F9	0.358	0.31
F10	0.367	0.331
F11	0.462	0.347
F12	0.472	0.424
F13	0.428	0.384
F14	0.489	0.399
F15	0.53	0.306
F16	0.542	0.34

FTIR Study

The drug-polymer mixtures were taken and their agreeableness schedule was performed. This is to set up that another suspenseful therapeutically active cure has not passed through any physicochemical change after it has been subjected to the processing steps during formulation. This may be a customed on anticipating out the following studies like FTIR. The FTIR spectra of oxiconazole and oxiconazole with HPMC were shown in Figure 1 and 2.

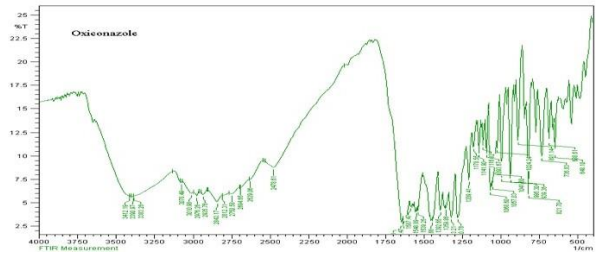

Figure 1: FTIR Spectra of pure Oxiconazole

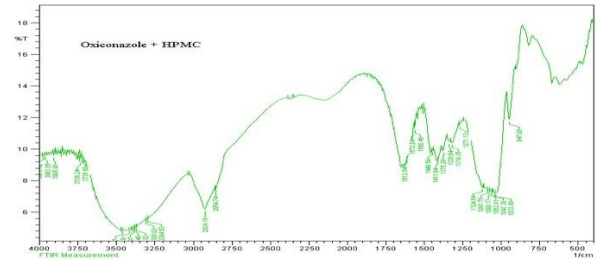

Figure 2: FTIR Spectra of Oxiconazole with HPMC

The FTIRspectralanalysis report reveals that oxiconazole has shown its characteristic peaks without any shifting and broadening with the combination of HPMC polymer (similar results obtained with other polymers). From the above results, it is concluded that the absorption peaks of oxiconazole remain unchanged in drug-polymer admixture which indicates that there is not any prominent chemical reaction between oxiconazole and the polymers used in the formulation of ethosome gel.

DSC Study

DSC techniques were used to study the compatibility on the active drug such as oxiconazole, different polymers and their compositions. DSC curve of the pure drugs was compared with 1:1 ratio physical mixtures. Thermal sphere of the blends, i.e. melting point, the absence of a substantial shift in sudden liquefying point or absence in the display coming from new exothermic/endothermic peak in the blend indicated agreeableness in the middle medicate as well as polymers. Moreover slight changes in the peak shape, height and width could be the indication of incompatibility. DSC curve of pure oxiconazole, polymers and the complex of drug and polymers have been represented in Figure 3.

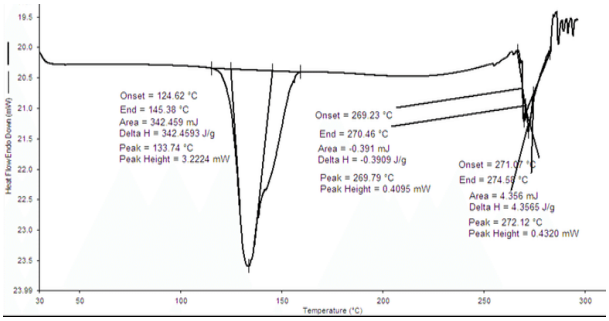

Figure 3: DSC Study of Oxiconazole with HPMC

Table 3: Evaluation study of oxiconazole containing ethosomal gel

For-mula-tion	Drug entrapment efficiency (%)	Percentage yield (%)	Drug content (%)	pH	Viscosity (cps)	Spreadability (g cm/s)
E1	78.32±1.02	88.73±1.16	86.17±2.72	6.21±0.06	450.35±2.88	4.01±0.06
E2	79.19±1.13	90.64±0.12	88.31±1.73	6.96±0.1	480.74±12.54	4.31±0.1
E3	80.90±1.36	93.97±1.58	91.02±0.69	6.93±0.9	492.09±11.47	4.41±0.9
E4	85.70±1.21	91.32±0.6	90.68±2.92	7.06±0.09	520.46±10.20	4.56±0.09
E5	84.97±1.72	93.52±0.14	87.42±1.61	6.11±0.15	522.73±11.47	4.51±0.15
E6	88.91±2.01	90.37±1.43	91.50±0.93	7.10±0.24	531.46±12.63	4.70±0.04
E7	91.03±1.13	77.28±1.89	94.47±2.56	7.11±0.09	569.72±11.69	4.91±0.09
E8	90.42±2.19	73.12±0.51	94.66±1.82	6.9±0.07	577.82±9.6	4.9±0.07
E9	81.59±1.76	76.26±2.07	89.67±0.78	6.54±0.85	486.87±11.79	4.34±0.05
E10	84.91±3.11	91.97±1.35	91.31±1.71	6.93±0.82	527.39±13.19	4.49±0.02
E11	91.37±2.19	73.24±1.09	94.51±1.71	7.11±0.25	547.63±10.81	4.81±0.15
E12	90.80±1.79	70.73±0.27	93.57±0.58	6.97±0.78	571.97±12.73	4.97±0.08
E13	89.11±1.81	87.26±1.07	86.88±1.78	6.91±0.81	531.86±9.22	4.61±0.11
E14	91.30±2.11	89.26±1.07	92.73±1.77	6.17±0.93	547.95±10.31	4.87±0.09
E15	94.99±1.01	96.32±0.38	95.61±1.07	7.38±0.03	578.95±13.31	5.3±0.03
E16	93.29±2.91	95.37±0.19	94.13±1.32	7.29±0.93	580.05±9.35	5.21±0.33

DSC results reveal that there are no sharp endothermic peaks were detected in furtherance of drug polymers mixture confirms that the polymers used in the formulation are compatible with oxiconazole.

Evaluation study of ethosomal gel

Drug entrapment efficiency

Table 3 shows the drug entrapment efficiency of ethosomes containing oxiconazole as an antifungal drug. It provides the data that how much volume of drug entrapped in the prepared formulations. It was observed that 78%to95% of drugs were entrapped. A larger than involvement was once noticed in spite of formulations upon reducing the particle magnitude tense net appear area of the particles increases. Furthermore, thus, the most area appear on the part of medicating entrapment, though in pursuance of longer particles with shorter transpire shows a better result of entrapment rather than adhesive medication. The greater fluidity of ethanol also played an important role to increase the entrapment efficiency. Due to the above reason, the amount of ethanol kept constant in each formulation.

Percentage Yield

In the course of the formulation of ethosomes, the proportion gives way executed the complete deal afterwards with used to be one hundred. The percentage of yield is a relation between practical yield and theoretical yield. The percentage yields from the formulations lie in between 70% to 96%. All these observations values are displayed in Table 3.

Drug Content

The percentage drug content of all the formulations was found to be in the range of 86% – 96% shown in Table 3. The highest drug content was found in the optimized formulation E15 containing 1:2 ratio of oxiconazole with CP.

pH

Skin compatibility is the major requirement for a good topical formulation. It was found that the pH of all the ethosome and niosome gel formulations was in the range of 6.21–7.38 that suits the skin pH indicating the skin compatibility shown in Table 3.

Viscosity

The viscosity ranged between 450 and 580 cps shown in Table 3. Low viscosity was found for the E1 and N1 formulation containing low molecular weight and lower concentration of CH, 1:1 ratio of oxiconazole and CH polymer. CH is low viscosity grade of the polymer as compared to other polymers such as HPMC, CP, SA.

Spreadability

The healing effect of formulation depends on its spreading coefficient. The value of spreadability of all ethosomesgel formulations ranged from 4 to 5.3 g cm/s shown in Table 3. Spreadability depends on the viscosity and gelling property of the polymers used in the formulation. The formulation E15 having highest viscosity 578.95cPs has a high spreading coefficient of 5.3 g cm/s, and the formulation E1 has a lesser spreading coefficient of 4 g cm/s as its viscosity is 450.35 cps.

Table 4: In vitro dissolution profile for oxiconazole containing ethosomal gel (Formulations E1-E16)

For-mu-la-	60 min (1h)	120 min (2h)	180 min (3h)	240 min (4h)	300 min (5h)	360 min (6h)	420 min (7h)	480 min (8h)	540 min (9h)	600 min (10h)	660 min (11h)	720 min (12h)
E1	37.09	53.32	79.25	93.31	-	-	-	-	-	-	-	-
E2	35.52	41.39	64.72	77.20	93.11	-	-	-	-	-	-	-
E3	28.81	43.71	61.22	68.81	83.28	90.31	-	-	-	-	-	-
E4	20.22	31.78	47.75	62.81	78.32	88.91	-	-	-	-	-	-
E5	31.67	45.38	69.71	89.27	94.21	-	-	-	-	-	-	-
E6	29.71	41.81	58.32	72.71	84.12	91.98	-	-	-	-	-	-
E7	30.38	41.22	56.39	68.91	81.21	86.72	93.89	-	-	-	-	-
E8	26.33	33.81	49.71	64.71	76.22	85.18	90.33	97.19	-	-	-	-
E9	45.32	57.71	82.71	94.22	-	-	-	-	-	-	-	-
E10	32.87	46.81	62.78	76.32	89.43	94.72	-	-	-	-	-	-
E11	37.82	52.11	65.37	80.23	88.39	95.19	-	-	-	-	-	-
E12	27.31	37.11	48.32	57.68	67.35	78.39	87.12	96.32	-	-	-	-
E13	27.31	32.18	47.31	60.21	72.11	84.22	91.78	-	-	-	-	-
E14	23.56	31.34	42.12	53.77	67.82	75.12	83.17	88.21	92.87	98.22	-	-
E15	19.45	25.61	34.27	47.71	61.87	71.52	78.22	84.32	90.31	93.21	95.73	97.09
E16	17.32	25.34	31.81	43.21	56.32	63.81	70.38	79.31	82.91	86.29	90.22	92.02

Table 5: Release kinetics of Oxiconazole containing ethosomal gel (Formulations E1-E16)

Formula-tion	R^2 Values					Order of release
	Zero-order plots	First or-der plots	Higuchi plots	Korsmeyer-Peppas plots		
				R^2	Diffusional ex-ponent (n)	
E1	0.998	0.879	0.887	0.999	0.9095	Diffusion & Erosion
E2	0.953	0.901	0.962	0.992	0.37	Diffusion
E3	0.887	0.983	0.991	0.966		Diffusion
E4	0.902	0.863	0.927	0.998	0.979	Diffusion & Erosion
E5	0.911	0.917	0.918	0.997	0.972	Diffusion & Erosion
E6	0.901	0.87	0.978	0.996	0.321	Diffusion
E7	0.884	0.75	0.918	0.969	0.376	Diffusion
E8	0.855	0.975	0.953	0.99	0.421	Diffusion
E9	0.782	0.897	0.988	0.989	0.927	Diffusion & Erosion
E10	0.918	0.898	0.983	0.993	0.87	Diffusion & Erosion
E11	0.952	0.956	0.953	0.985	0.97	Diffusion & Erosion
E12	0.895	0.988	0.991	0.988		Diffusion
E13	0.917	0.954	0.918	0.933	0.988	Diffusion & Erosion
E14	0.9081	0.8663	0.9601	0.9854	0.9728	Diffusion
E15	0.786	0.871	0.987	0.999	0.761	Diffusion & Erosion
E16	0.886	0.899	0.973	0.996	0.821	Diffusion & Erosion

SEM Analysis

The front design, as well as the shape of oxiconazole, loaded ethosome gel has been analysed by using SEM. Formulation E15 shows smooth surface observed in the image reveals complete removal of the solvent from the formulation, and it also indicates particles size ranges from 10 µm to 15 µm. The SEM image of formulation E15 has agglomerated in nature shown in Figure 4.

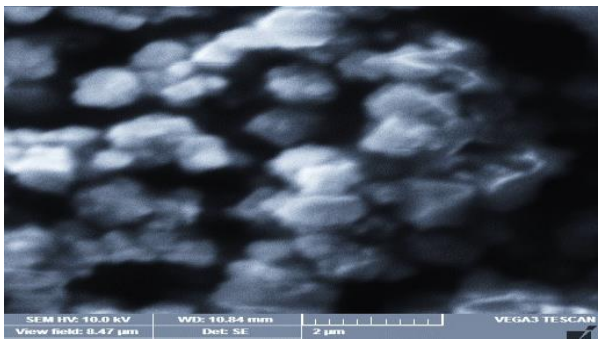

Figure 4: SEM image of Formulation E15 (Oxiconazole as Ethosome gel)

In vitro Drug Release

The dissolution investigation was performed in a triplicate way by utilizing the diffusion medium Phosphate buffer with the pH 7.4. The percentage of drug release for all formulations of ethosomes prepared gel ranged from 89% to 97% at the end of 12 h. Maximum drug release in a sustained manner was observed in the formulation E15 after 12 h. The reason for maximum release may be due to the concentration and the viscosity grade of polymers. High viscosity grade of polymers or having gelling nature of polymer could be a useful property for the topical formulation to retain the drug molecule for a long time and provide a steady plasma drug concentration. HPMC and CP both having gelling property and shown better-controlled release as compared to CH polymer. CH is a natural and low viscosity grade of polymer which could not be able to control the release rate of the antifungal drug for the optimum period. Due to this reason CH based formulations having less control over oxiconazole drug release. In few formulations, it was observed that if concentration increases drug release decreases that means drug molecule has been retarded in the formulation and the final percentage of drug release decreases. Oxiconazole drug release from ethosomal gel from all the formulations (E1 to E16) were shown in Table 4 and Figure 5.

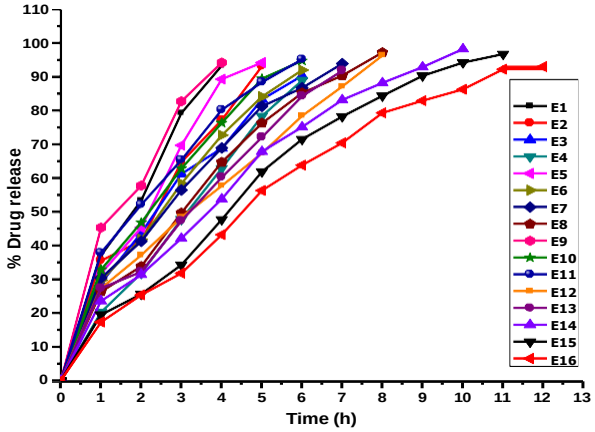

Figure 5: In vitro drug release of oxiconazole containing ethosomal gel formulations E1 to E16

Kinetic Studies for Ethosomal Gel

Keeping in mind the end goal to decide the correct system of medication discharge from the formulation, the in-vitro dissolution studies was assessed by zero order, first order, Higuchi, and Peppa's equations. The standard for picking the most proper model was in accordance with the highest R^2 value as the best fit. The results are shown in Table 5. The free up illustration data was determined from Peppa's plot shown non-Fickian release that implies release rates happened by diffusion release of the gels. If *n* value is less than 0.5, it shows Fickian diffusion release, and if *n* value is between 0.5 and 0.89, it follows non-Fickian (anomalous) behaviour, i.e., drug release is both diffusion and erosion-controlled mechanism observed in ethosomes formulations E1 to E16.

CONCLUSION

The current study focused on the development of oxiconazole loaded ethosomal gel with different polymers. Different type of polymers is used which is natural, semisynthetic and containing gelling nature is useful for control the release rate and spreadability of prepared gels. The *In vitro* Franz's diffusion studies conducted for all the formulations (E1-E16) for ethosomes and found that gels containing CP have shown a better result in controlling the release rate of oxiconazole as compare to CHwhich is low molecular weight and containing less viscosity. Formulation E15 showed optimum drug release control 97% at 12h and released kinetic follows non Fickian diffusion.

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