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DESIGN AND INVITRO CHARACTERIZATION OF VORICONAZOLE MICROSPHERES LOADED TOPICAL EMULGEL

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ABSTRACT

Voriconazole is used to treat invasive aspergillosis and candidiasis and fungal infections caused by Scedosporium and Fusarium species, may occur in immunocompromised patients including people undergoing allogeneic bone marrow transplant, who have hematologic cancers. The purpose of the present study is to formulate and evaluate microspheres loaded topical gel containing voriconazole as a model drug microspheres were prepared by using aqueous ionotropic gelation method. Different polymers, different drug to polymer(s) ratio(s) and other parameters were screened to study their effects on properties of microspheres and to optimize each parameter. The controlled release emulgel was formulated by changing the polymer ratio. FT-IR study confirmed the purity of drug, concede no interaction between the drug and excipients and analyze the parameters affecting the morphology and other characteristics of the resultant products employing scanning electron microscopy (SEM). Microspheres loaded topical gel has been shown that encapsulation and controlled release of voriconazole could reduce the side effect while also reducing percutaneous absorption when administered to the skin. The microspheres obtained were subjected to preformulation studies like bulk density, tapped density, angle of repose, carr's index, hausner's ratio the results obtained were with in the limit. The microspheres were characterized by percentage yield, drug entrapment efficiency, Particle size analysis, then the optimized microspheres formulation were incorporated into the

gel prepared with various polymer(s) ratio(s) and was evaluated by parameters like Visual inspection, pH measurement, spreadability studies, viscosity and in vitro drug release by using Franz diffusion cell. The result of studied revealed that the optimized batch shows 97.24% release in 12 hours and stable for around there.

KEYWORDS: Voriconazole, Microsphere loaded topical gel, Controlled release, FTIR.

INTRODUCTION

For many decennium, medication of an acute disease or a chronic disease has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. This factor as well as other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems.[1,3] The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue.[4] Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is be filled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.[5] Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin. The synthetic polymers include polylactic acid and polyglycolic acid. Shows two types of microspheres: Microcapsules, where the entrapped substance is completely surrounded by a distinct capsule wall and micromatrices, where the entrapped substance is dispersed throughout the microsphere matrix. Microspheres are small

and have large surface to volume ratios. At the lower end of their size range they have colloidal properties.[6]

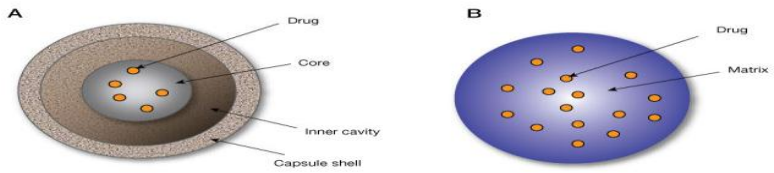

Fig 1: Schematic diagram illustrating microspheres.

Voriconazole [(2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol)] (Voriconazole) is a drastic antifungal drug of the azole family with low aqueous solubility (0.71 mg·mL^{-1}), which classifies it to BCS class II (Biopharmaceutics Classification System). Voriconazole is mainly used for the treatment of several fungi infections, such as invasive aspergillosis, infections from *Candida albicans* and *fusarium* species.[7,8] Its limited solubility in water classified Voriconazole as drug with low bioavailability, which limits its effectiveness. This major problem can be solved only by new clinical trends and new pharmaceutical formulations.

The objective of work was envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating controlled release of Voriconazole microspheres loaded gel for Genital infection. The microsphere loaded gel have advantages such as efficient absorption and more drug retention time.

MATERIALS AND METHODS

Materials

Voriconazole was received as a gift sample from Natco laboratories Ltd., Hyderabad, (India), which is a water-in soluble drug, was chosen as a model drug. EC (Signet Chemical Corporation, Mumbai, India) was used as a matrix-forming agent and HPMC various grades (Merck Specialities Pvt Ltd, Mumbai, India) was used as polymers in this study. Distilled water was used for all experiments. All other chemicals were of analytical pharmaceutical grade.

Methods

Drug – Excipient compatibility studies

Fourier Transform Infrared (FTIR) spectroscopy

FT-IR techniques have been used to study the physical and chemical interaction between drug and excipients used. The physical properties of the physical mixture were compared with those of plain drug. Samples was mixed thoroughly with 100mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 3500 cm to 500 cm. The resultant spectrum was compared for any spectrum changes.

Preparation of Standard Calibration Curve of Vorniconazole

10 mg of voriconazole drug was accurately weighed and dissolved in 10 ml of 6.8 PH in 10 ml volumetric flask, to make (1000 µg/ml) standard stock solution (1). Then 1 ml stock solution (1) was taken in another 10 ml volumetric flask to make (100 µg/ml) standard stock solution(2), then again 1 ml of stock solution (2) was taken in another 10 ml volumetric flask and then final concentrations were prepared 2, 4 ,6, 8, 10, 12, 14, 16, 18 and 20 µg/ml with 6.8 PH. The absorbance of standard solution was determined using UV/ VIS spectrophotometer at 270 nm. Linearity of standard curve was assessed from the square of correlation coefficient (r2) which determined by least-square linear regression analysis. The absorbance so obtained was tabulated as in table2. Calibration curve was constructed and shown in Fig.2.

Batches of microspheres were prepared which involved reaction by using Ethyl Cellulose and HPMC as polymers and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Voriconazole (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The Microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of Microspheres and then air-dried. The composition of all the formulations is shown in Table 1.

Characterization of Microspheres

Percentage yield: The percentage of production yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

% Yield= Practical mass (Microspheres) / Theoretical mass (Polymer + Drug) x 100

Drug entrapment efficiency

Powdered microspheres were suspended in 10 ml of phosphate buffer (pH 6.8). After 24 hrs, the solution was filtered, the filtrate was centrifuged at 2000 rpm for 3 minutes and then analyzed for drug content spectrophotometrially (Shimadzu UV-1800, Japan)) at 272 nm and the concentration of soluble drug was calculated.[9] The amount of drug entrapped in the Microspheres was calculated by the following formula,

Entrapment efficiency during = Weight of drug added formulation -- weight of drug recovered from

Microspheres / Weight of drug added during formulation

Particle size analysis

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticles sizes were calculated under 45x magnification.

The average particle size was determined by using the Edmondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed

d – Mean size range

Micromeritic properties

Bulk density, Tapped density and Hausner's ratio and Carr's index, were determined to assess the flow ability of the prepared microspheres.

Bulk density

The product was tapped using bulk density apparatus for 1000 taps in a cylinder and the change in volume was measured. Bulk density of the formulations was determined by using the following formula,

Bulk Density = Total Weight / Total bulk Volume

Tapped density

Tapped density is used to investigate packing properties of microcapsules into capsules. The tapped density was measured by employing the conventional tapping method using a 10mL measuring cylinder and the number of tappings was 100 as sufficient to bring a plateau condition. Tapped density was calculated using the following formula:

Tapped Density= Total Weight / Total tapped volume

Hausner's ratio: It is another parameter for measuring flow ability of the microspheres. It is calculated using the following formula,

H = Bulk Density/ Tapped Density

Compressibility index (carr's): It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence the consolidation index. It is also called as compressibility index. It is denoted by CI and is calculated using the formula below.

$$\text{Compressibility index} = (1- Vo/V) * 100$$

Where, Vo = volume of microspheres before tapping

V = volume of microspheres after 100 tappings.

Production yield (%)

The production yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres and % production yields were calculated as per the formula:

$$\% \text{ Yield} = \text{Actual Yield} / \text{Theoritical Yield} \times 100 \%$$

Evaluation of mucoadhesive property

The mucoadhesive property of microspheres was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread on to each

prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test, when the test apparatus was operated, the sample is subjected to slow up and down movement in simulated gastric fluid pH 6.8 at 37^0C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour up to 8 hours the machine is stopped and number of microspheres still adhering to mucosal surface was counted.

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \times 100$$

In-vitro release studies

The in-vitro release of venlafaxine microspheres was done with phosphate buffer pH of 6.8 for 12 hrs by using dissolution apparatus USP I Basket type at a temperature of 37.0±0.5°C. Microsphere equivalent to 10 mg of drug was taken and it was inserted in the basket wrapping it with muslin cloth (900 ml phosphate buffer pH 6.8, at 37±2°C and was adjusted to 100 rpm). The sample was taken for every half an hour for 12 hrs. To maintain the sink condition, the samples withdrawn were replaced with an equal volume of dissolution medium at different time intervals. After suitable dilution, samples were analyzed at ultraviolet visible spectrophotometer at 272 nm.[10]

Formulation of the mucoadhesive microsphere-loaded gel

The gels with varying concentrations of Carbopol 934P were prepared by dispersing required quantity of Carbopol in required quantity of distilled water with continuous stirring and kept overnight for complete hydration. Further appropriate quantities of triethanolamine were added to previous polymeric mixture. Mucoadhesive microsphere of voriconazole was added to the above polymeric mixture with constant stirring. Final pH of the preparation was adjusted to 4.5 with 0.5 M sodium hydroxide solution. The gel was then further modified by the addition of varying proportion of HPMC K4M.[13] The composition of all the formulation is given in Table 2.

Characterization of mucoadhesive microsphere gel

Study of the physical properties

Visual inspection

The organoleptic properties, such as color, texture, consistency, homogeneity and physical appearance of gel containing microspheres were checked by visual observation.

Determination of pH

Determination of pH is done by using Systronic digital pH meter 335. pH meter was calibrated before use by using standard buffer solution.

Viscosity

The viscosity of the formulated gel is determined by DV-E Brookfield viscometer using spindle no 64.

Spreadability

One of the criteria for Emulgel is to meet the ideal quality is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability.

It is calculated by using the formula,

$$S = M. L / T$$

Where

M = wt. tied to upper slide L = length of glass slides. T = time taken to separate the slides

Extrudability study

In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and gel was extrudes until the pressure was dissipated [Table-6].

Drug Content Determination

Drug concentration in emulgel was measured by spectrophotometer. Voriconazole content in emulgel was measured by dissolving known quantity of emulgel in solvent (ethanol) by Sonication. Absorbance was measured after suitable dilution at 256 nm in UV/VIS spectrophotometer.

In Vitro Release Study

Franz diffusion cell was used for the drug release studies. Emulgel (200 mg) was applied onto the surface of cellophane membrane evenly. The cellophane membrane was clamped between

the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 256 nm after appropriate dilutions. Cumulative corrections were made to obtain the total amount of drug release at each time interval. The cumulative amount of drug released across the cellophane membrane was determined as a function of time.

Gel strength study

A sample of 50 gm of microspheres loaded gel was placed in a 100 ml graduated. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel.[12]

Accelerated stability studies of emulgel

Stability studies were performed according to ICH guidelines. The formulations were stored in hot air oven at 37 ± 2° and 45 ± 2° for a period of 3 months. The samples were analyzed for its appearance and drug content every two weeks by UV-Visible spectrophotometer at 256 nm. Stability study was also carried out by measuring the change in pH of gel at regular interval of time.

In-Vitro Drug Release Kinetics

To determine the release mechanism and kinetics, the results of the in-vitro dissolution study of formulated microsphere loaded gel were fitted into various kinetics equations, such as zero-order, first order, Higuchi's model, Korsemeyer-Peppas model and Hixson-Crowell model. Correlation coefficient values (R2) were calculated from the linear curves obtained by regression analysis of the plots.

RESULT AND DISCUSSION

Determination Of λmax

Stock solution (1000µg/ml) of Vorniconazole was prepared in 0.1N HCl. This solution was appropriately diluted with 6.8 phosphate buffer to obtain a concentration of 10µg/ ml. The resultant solution was scanned in the range of 200nm to 400nm on UV-Visible spectrophotometer. The drug exhibited a λmax at 256nm. A solution of 10µg/ml of Voriconazole was scanned in the range of 200 to 400nm. The drug exhibited a λmax at

256nm in 6.8 phosphate buffer and had good reproducibility. Correlation between the concentration and absorbance was found to be near to 0.9999, with a slope of 0.0245. Calibration curve spectrums were shown in Table-2 and in Figures 1.

FT-IR incompatibility study of drug and excipient

The FTIR spectra of Voriconazole exhibited distinctive peaks at 1718 cm-1 due to C=N stretching, 1437 cm-1 due to because of aromatic C-H stretching and peak at 3227-3327 two weak intensity broad bands OH strechning. The spectrum found that there were no interactions of drug with excipients. Hence it indicates no change in chemical integrity of the drug. FTIR spectrums were shown in Figures 2.

Percentage Yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and Microspheres lost during the washing process. The percentage yield was found to be in the range of 76 to 82% for Microspheres containing HPMC polymer. Results were shown in Tables 5.

Evaluation of the microspheres

Surface morphology and particle size analysis

The average particle size of all the formulations were in the range of 30-120 µm and particle size was increased with increase in concentration of the polymer. The increase in viscosity of polymer solution with increase in polymer concentration produced larger particles in higher polymer formulations. Microspheres were shown in Figurer 5. The microspheres obtained under the conditions were found to be spherical and without aggregation, and median size ranged from 8-19 µm.

Encapsulation efficiency

The encapsulation efficiency ranged from 62.17% to 79.42%. The encapsulation efficiency increased as the concentration of polymer (encapsulating material) was increased.

In vitro drug release study

In vitro release profile of voriconazole -loaded microspheres in phosphate buffer with pH of 6.8 is shown in Fig. 3. Here, sustained release of drug was observed from the formulation in

phosphate buffer (pH 6.8) for a duration of 12 hrs. About 58.09-75.89% of drug release was achieved in less than 7 hrs from the microspheres. The improvement of the dissolution rate of the drug from the microspheres can be also due to their small size and the cross-linking of polymer and drug that lead to the uniform dispersion of the drug into the polymeric network.

Scanning electron microscopy

The microspheres prepared by solvent evaporation method have good spherical shape with smooth surface in its morphology and the particles were distributed uniformly without forming any clumps.

Characterization of Drug loaded Emulgel

Physical appearance

The prepared voriconazole emulgel formulations were white to slighthly yellowish, viscous creamy preparation with a smooth and homogeneous appearance.

pH

The pH values of all formulations were found in the range 6.50 to 6.80. Hence all the formulations are satisfactorily complying with pH values needed for topical application and pH of skin.

Spreadability

The observations for spreadability of all formulations are shown in Table 6. The spreadability of the formulation depends on its viscosity. The greater the viscosity the longer will be the time taken for spreading on the skin. The values of spreadability denote that the gel is easily spreadable by small amount of force. The spreadability of formulation F3 was found to be less as compared to F8 formulations; this indicates high viscosity of carbopol gel rigid to spread on skin. Gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out of the targeted site. The formulations exhibited moderate gel strength shown in table 6.

Viscosity

Viscosity of the gels was determined using DV-E Brookfield viscometer using spindle no 64. The viscosity was found to be 221.66– 223.66 cps, of F6, F7 and F8, respectively. The increase in viscosity of the formulations was directly proportional to the polymer concentration.

Extrudability

The observations for extrudability of all formulations are shown in table 6. F8 formulation shows highest extrudability when compared with other formulations.

Drug content determination

1 g of the prepared microspheres loaded topical gels was mixed with 100 ml of suitable solvent (ethanol). Aliquots of different concentration were prepared by suitable dilution after Sonication and filtering the stock solution and absorbance was measured. Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve. The drug content of microspheres loaded topical gels of Voriconazole formulations are given in table 6.

In vitro Drug Release

Diffusion studies of all the formulations were carried out using franz diffusion apparatus. The diffusion studies were conducted by using diffusion media, pH 6.8. This shows that more sustained release was observed with the increase in percentage of polymers. As the polymer to drug ratio was increased the extent of drug release decreased. The in vitro release profiles of voriconazole from its various microspheres loaded topical gel formulations are represented in Figure 5. It was observed that all the formulation had become liquefied and diluted at the end of the experiments, indicating water diffusion through the membrane. In general, it can be observed from figures that the better release of the drug from all Emulgel formulation. The release of the drugs from its Emulgel formulation can be ranked in the following descending order: F8 > F9> F2 > F4> F5 > F7 > F3 > F1, Where the amounts of the drug release of the drug released after 24 hours were 97.45%, 94.14%, 93.47%, 88.37%, 88.17%, 85.22%, 88.11% and 85.22 respectively. Thus the higher drug release was observed with formulation F8. The lower drug release of formulations may be attributed to the high polymer concentration in the former. With an increase in the polymer concentration, the micelles formed are closely packed on gellation thus resisting the drug release to the external environment.[13]

In-Vitro Drug Release Kinetics

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the in-vitro drug diffusion data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix and Krosmeyer Peppas model. The coefficient of determination (R 2) was used as an indicator of the best fitting for each of the

models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the Higuchi release kinetics model (R 2 = 0.9819 to 0.996) whereas release exponent value (n) ranged from 0.496 to 0.753. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Higuchi release kinetics model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Stability studies

A stability study of the optimized formulation was done for appearance, pH, and viscosity for up to 90 days at a time interval of 30 days. The formulation did not show much variation in any of the parameters. The results obtained were tabulated in Table 7 and 8. From these results, it was concluded that formulation F8 was stable throughout the period.

Table 7: Accelerated stability study of optimized microspheres loaded gel formulation F8 at 25 C°.

Storage Temp. C° 25+ 2	Period of studies in month		
	1 month	2 month	3 month
Appearance	Good	Good	Good
Drug content	95.86 ± 0.05	96.29 ± 0.11	96.19± 0.05
pH	6.77 ± 0.06	6.78 ± 0.018	6.80 ± 0.028

Table 8: Accelerated stability study of optimized microspheres loaded gel formulation F5 at 45 C°.

Storage Temp. C° 25+ 2	Period of studies in month		
	1 month	2 month	3 month
Appearance	Good	Good	Good
Drug content	97.96 ± 0.01	97.89 ± 0.03	97.43 ± 0.02
pH	6.79 ± 0.09	6.79 ± 0.014	6.80 ± 0.013

Table 1: Formulation of Biooadhesive Microspheres.

S. no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Drug	100	100	100	100	100	100	100	100	100
2	HPMCK4M	100	200	300						
3	HPMCK15M				100	200	300			
4	HPMCKK100M							100	200	300
5	Methanol	5	5	5	5	5	5	5	5	5
6	Water	10	10	10	10	10	10	10	10	10
7	Calcium chloride(5%)	QS	QS	QS	QS	QS	QS	QS	QS	QS

Table 2: Preparation of Emulgel Formulations.

S. no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Carbopol 940(mg)	100	200	300	-	-	-	-	-	-
2	Carbopol 934(mg)	-	-	-	100	200	300	-	-	-
3	Carbopol 974(mg)	-	-	-	-	-	-	100	200	300
4	Triethanolamine(v/v)	2%	2%	2%	2%	2%	2%	2%	2%	2%
5	PEG(%V/W)	30%	30%	30%	30%	30%	30%	30%	30%	30%
6	Methyl parabben(mg)	750	750	750	750	750	750	750	750	750
7	Distilled water(ml)	QS	QS	QS	QS	QS	QS	QS	QS	QS

Table 3: Calibration curve data for Voriconazole in simulated fluid pH 6.8 buffer at 261nm.

Concentration [µg/1 ml]	Abs
0.5	0.155
1	0.301
1.5	0.441
2	0.621
2.5	0.744
3	0.971

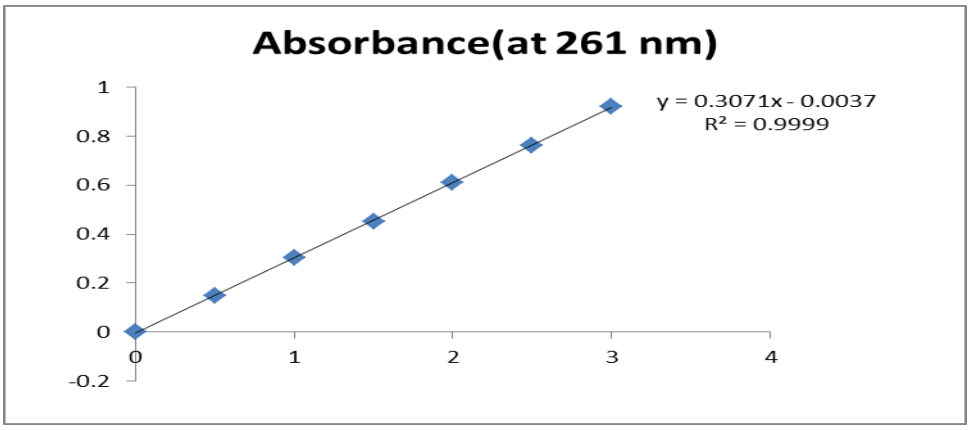

Figure 1: Standard graph Of Voriconazole in simulated gastric fluid pH 6.8.

DRUG AND EXCIPIENT COMPATABILITY STUDIES

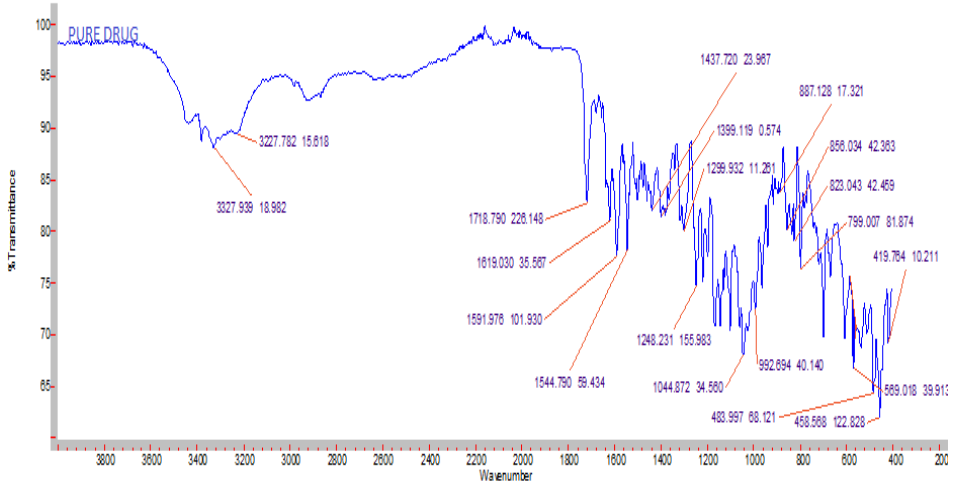

Figure no. 2: FTIR spectrum of pure drug.

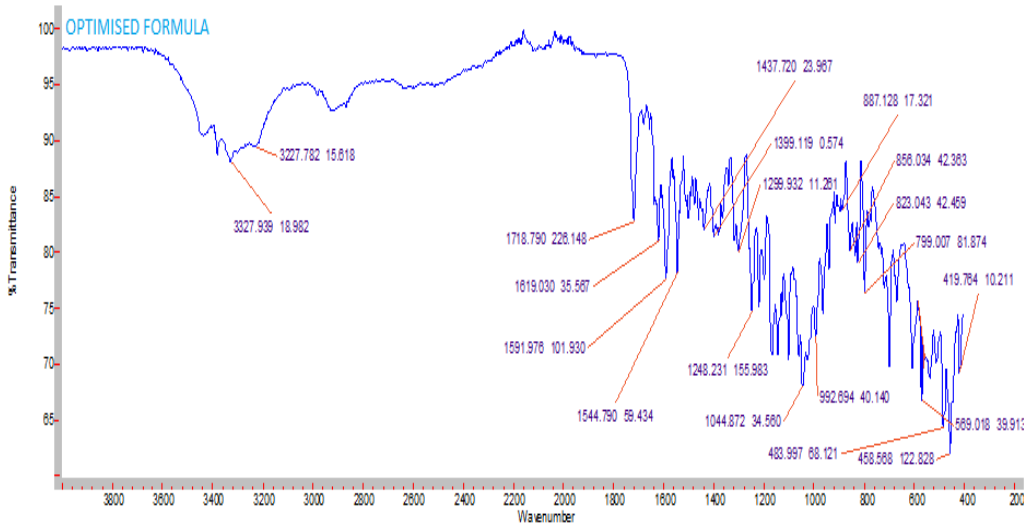

Figure 3: FTIR spectrum of optimised formulation.

Table 3: Peak results for FTIR Studies of Voriconazole with the excipient.

S.No.	Functional groups	Present peaks	Actual frequency range
1	C=N Streching (Aromatic)	1437-1718	2260-2210
2	2 weak intensity broad bands-OH	3227-3327	3300-2500
3	C-H strech	1437	1470-1450
4	C-N Medium Streching	1248	1250-1020
5	Medium Strech of NH2	1544-1591	1650-1580
6	Various NH Wagging bonds	419-589	919-665
7	Various out of CH plan deformation	992-1044	900-1050

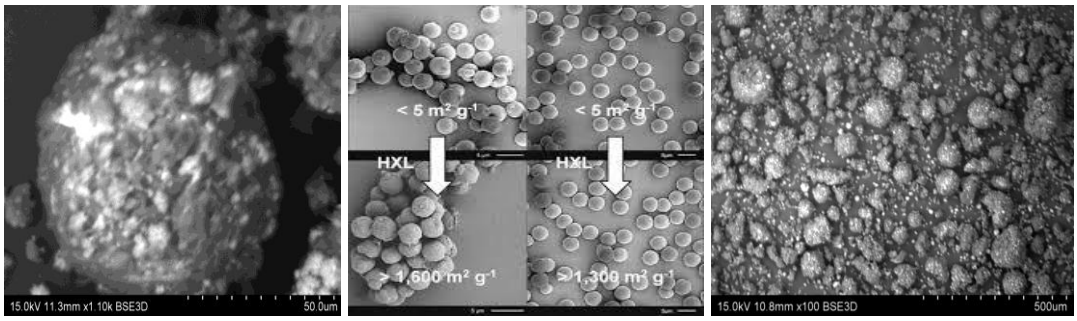

Figure 4: Scaning electron microscopy

Table 5: % yield of the formulations.

Formulation	% yield
F1	**76.25**
F2	**58.16**
F3	**78.43**
F4	**79.51**
F5	**68.24**
F6	**80.16**
F7	**78.54**
F8	**80.51**
F9	**79.42**

Table 6: Pre formulation parameters.

S.No	Formulation Code	Mean particle size (µm)	Bulk density Gm/cm^3	Tapped density	Hausner's Ratio	Carr's index	Angle of repose
1	F1	650	0.49±0.04	0.54±0.04	16.21±0.06	0.86±0.06	25.11
2	F2	670	0.52±0.09	0.52±0.04	16.87±0.05	0.98±0.05	25.67
3	F3	720	0.50±0.05	0.58±0.05	17.11±0.01	0.64±0.03	25.54
4	F4	690	0.51±0.06	0.54±0.07	17.67±0.08	1.12±0.04	25.43
5	F5	780	0.52±0.03	0.57±0.03	16.92±0.04	6.8±0.08	27.34
6	F6	770	0.53±0.04	0.56±0.06	17.65±0.09	1.06±0.09	26.22
7	F7	760	0.43±0.04	0.53±0.04	16.24±0.06	0.84±0.06	27.11
8	F8	690	0.51±0.09	0.54±0.04	16.67±0.05	0.87±0.05	26.67
9	F9	700	0.53±0.05	0.55±0.05	17.73±0.01	0.67±0.03	28.54

Table 7: Percentage entrapment efficiency.

Formulation	Percentage Entrapment efficiency
F1	62.17
F2	42.64
F3	59.43
F4	62.45
F5	58.41
F6	67.42
F7	57.42
F8	60.51
F9	79.42

Table 8: Invitro release studies.

TIME (h)	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
0	0	0	0	0	0	0	0	0	0
1	24.88	21.11	18.66	15.88	27.77	22.44	18.44	17.11	25.77
2	31.55	31.55	25.11	24.22	36.44	32.22	29.33	26.44	35.33
3	42.44	39.77	35.44	32.66	43.77	40.88	39.55	37.55	43.55
4	53.55	47.77	40.66	39.33	54.66	48.66	45.55	46.88	54
5	62	56.66	52	47.55	64.01	57.55	57.33	55.77	63.55
6	74.66	62.44	57.33	55.77	75.77	63.55	65.33	63.55	75.33
7	83.55	69.55	63.11	61.77	84.65	70.44	71.55	71.33	84
8	89.33	75.33	69.11	69.55	90	76.55	77.56	75.77	89.77
9	92.66	84.66	75.33	77.55	92.22	85.55	81.55	79.77	92.66
10	85.55	90.66	82.66	85.55	84.88	91.33	83.33	82.44	85.11
11	80.22	84.22	90.66	90.66	79.55	85.77	89.55	86.88	80.66
12	78.88	80.88	89.55	94.66	77.55	81.11	87.55	90.66	78

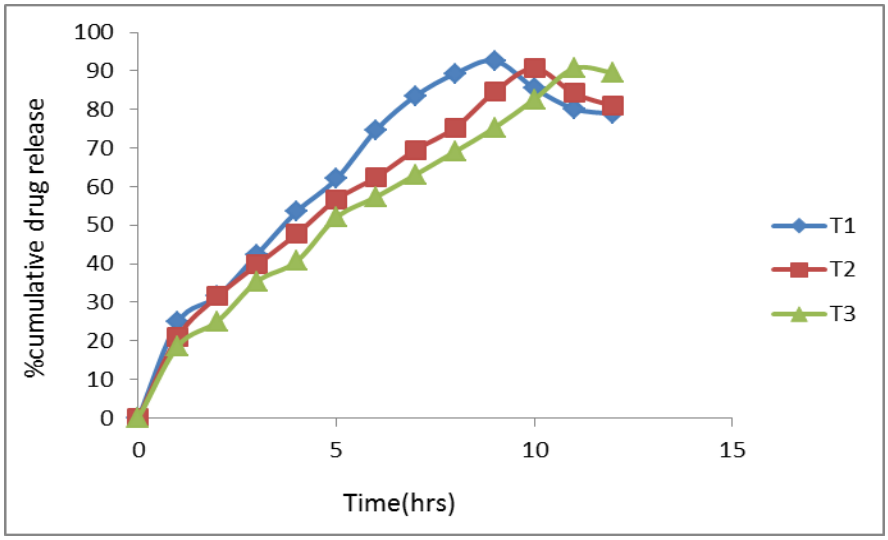

Figure 5: Dissolution profile of T1, T2, T3 formulations.

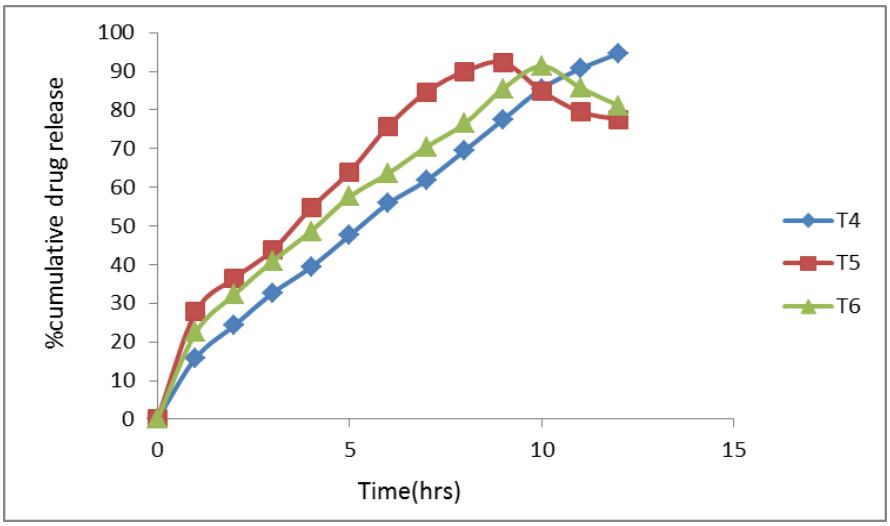

Figure 6: Dissolution profile of T4, T5, T6 formulations.

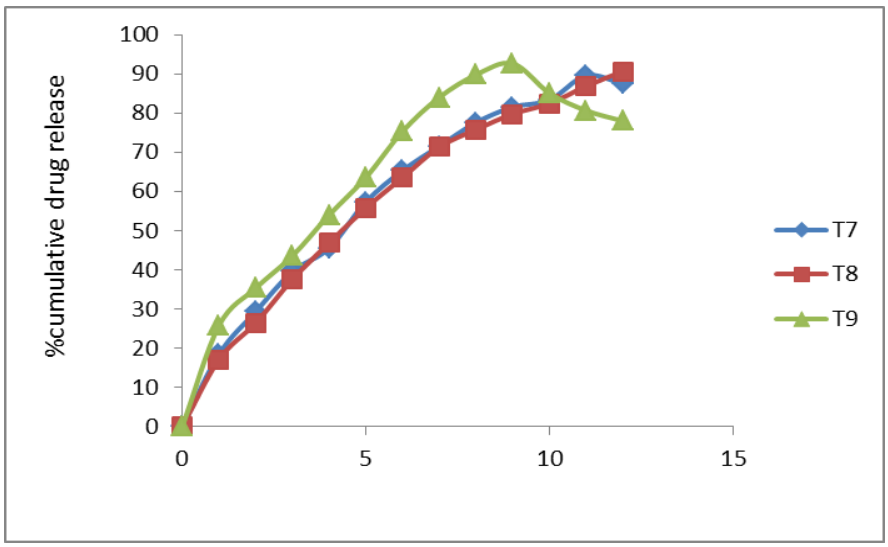

Figure 7: Dissolution profile of T7, T8, T9 formulations.

Table no: 9 Physical parameters of microspheres loaded topical gels of Voriconazole.

S.NO	Formulation code	% Drug content (mg)	Spreadability (gm.cm\sec)	Viscosity(cps)	PH	Extrudability study
1	F1	92.37	11.75	210.4	6.82	95.67
2	F2	91.07	11.17	217.6	6.85	96.73
3	F3	91.48	11.25	214.56	6.87	94.55
4	F4	91.88	12.55	207.55	6.88	97.62
5	F5	92.57	11.89	218.98	6.81	95.39
6	F6	93.46	12.82	221.66	6.80	95.78
7	F7	92.55	11.79	223.54	6.82	96.43
8	F8	92.87	12.55	213.42	6.88	97.78
9	F9	93.46	11.87	212.99	6.87	97.68

IN-VITRO RELEASE STUDIES

Table 9: In-vitro cumulative percentage drug release profile for Voriconazole microspheres loaded gels.

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	9.77	12.14	6.12	14.21	8.11	5.12	2.11	9.21	18.51
1	17.51	23.16	15.45	21.55	17.5	10.22	8.15	21.8	22.15
2	23.4	28.77	20.47	22.47	22.85	18.21	15.21	29.21	28.55
3	28.14	32.47	25.44	35.11	28.14	28.77	29.54	35.11	32.14
4	32.11	38.64	30.14	40.94	33.14	34.57	33.47	39.45	38.74
5	36.97	42.97	35.79	45.71	36.47	36.11	37.64	48.21	42.44
6	41.97	46.12	40.78	55.87	42.64	39.14	42.55	53.77	48.71
7	48.79	50.77	45.88	59.44	49.66	42.15	48.31	59.34	58.64
8	52.77	52.74	60.22	61.47	52.77	46.78	53.11	65.77	66.54
9	55.94	58.44	63.48	69.14	56.52	49.14	56.47	72.64	71.24
10	61.87	75.32	69.47	73.41	62.99	59.14	62.44	89.54	82.14
11	69.77	85.11	73.44	79.41	68.14	69.14	68.21	93.11	86.14
12	85.22	93.47	88.14	88.37	88.17	85.22	88.54	97.45	94.14

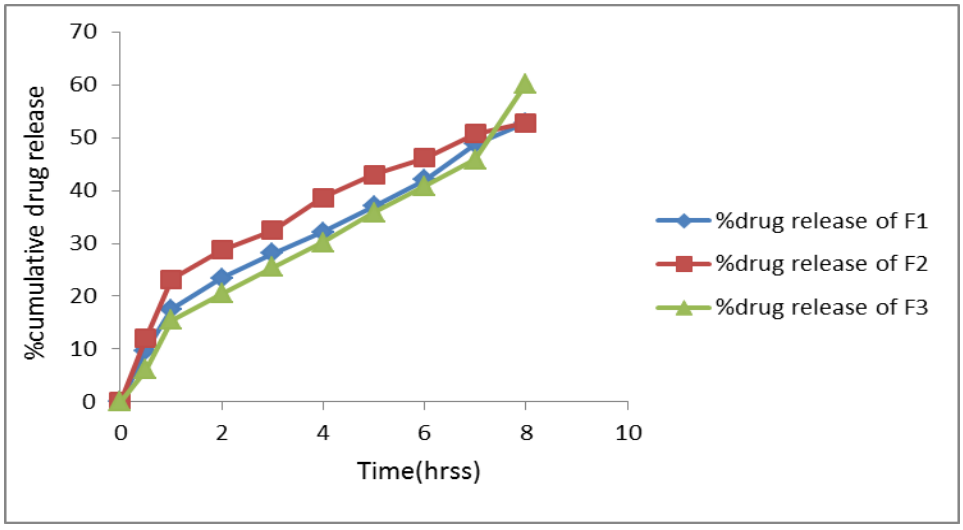

Fig. 8. Dissolution profile of F1, F2, F3 formulations.

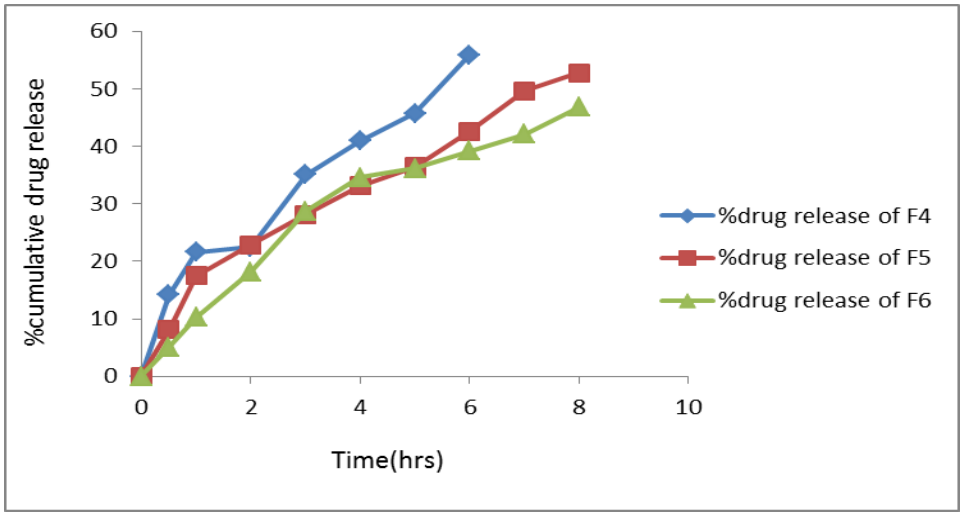

Figure 9: Dissolution profile of F4, F5, F6 formulations.

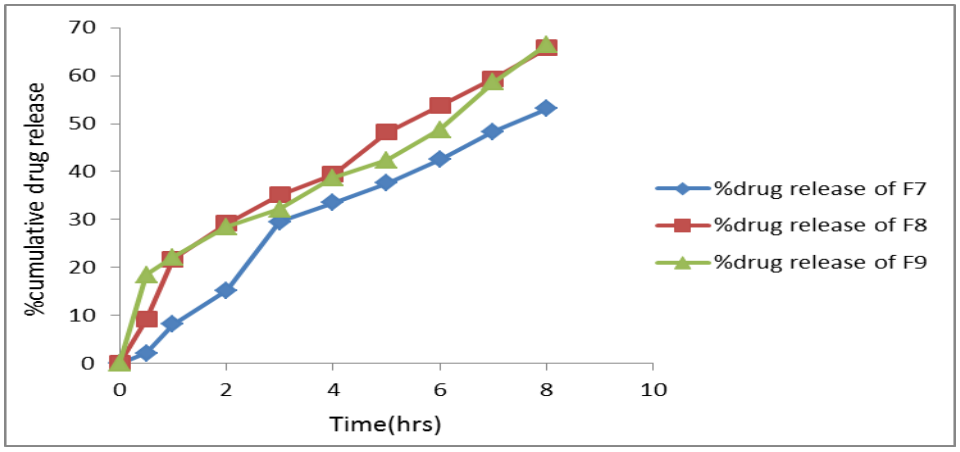

Figure 10: Dissolution profile of F7, F8, F9 formulations.

Table 11: Release kinetics data for optimised formulation.

Cumulative (%) Release Q	Time (T)	Root (T)	Log(%) Release	Log (T)	Log (%) Remain
0	0	0			2.000
9.21	30	5.477	0.964	1.477	1.958
21.8	60	7.746	1.338	1.778	1.893
29.21	120	10.954	1.466	2.079	1.850
35.11	240	15.492	1.545	2.380	1.812
39.45	360	18.974	1.596	2.556	1.782
48.21	480	21.909	1.683	2.681	1.714
53.77	600	24.495	1.731	2.778	1.665
59.34	720	26.833	1.773	2.857	1.609
65.77	780	27.928	1.818	2.892	1.534
72.64	840	28.983	1.861	2.924	1.437
89.54	900	30.000	1.952	2.954	1.020
93.11	960	30.984	1.969	2.982	0.838
97.45	1020	31.937	1.989	3.009	0.407

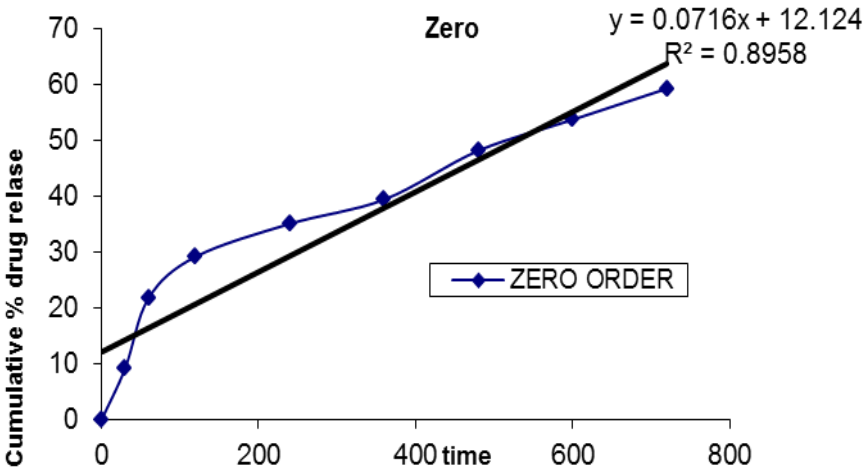

Figure 11: Zero order release kinetics graph.

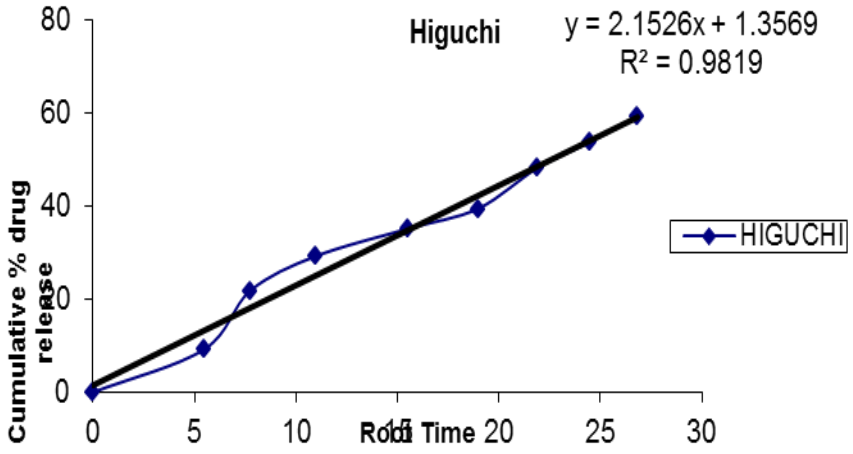

Figure 12: Higuchi release kinetics graph.

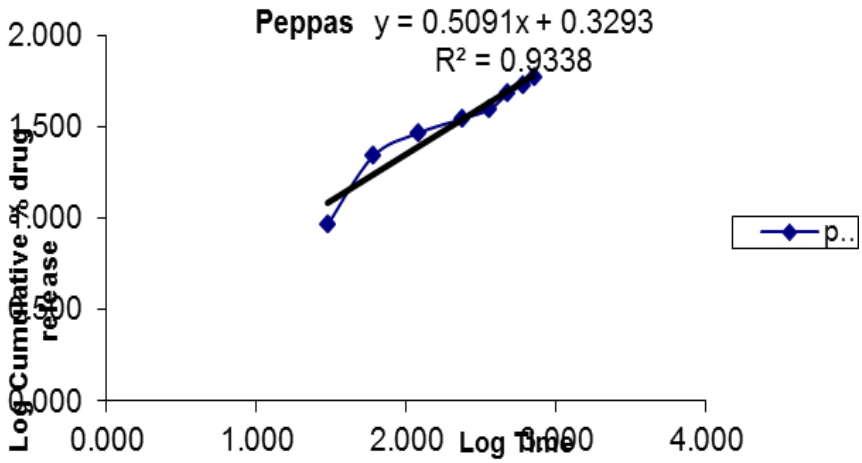

Figure 13: Kars mayer peppas graph.

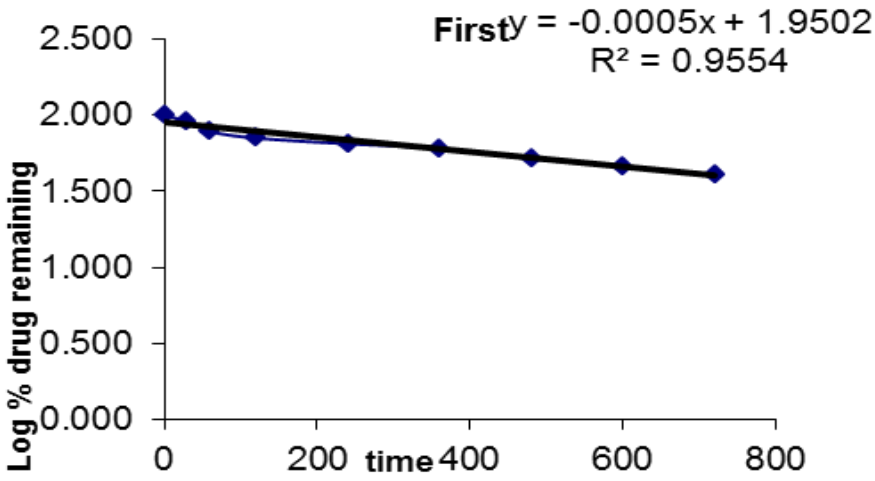

Figure 14: First order release kinetics graph.

CONCLUSION

In the present work, bioadhesive microspheres of Voriconazole using different polymers were formulated to deliver Voriconazole via topical route. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size. The invitro drug release decreased with increase in the polymer. Analysis of drug release mechanism showed that the drug release from the formulations followed diffusion and the best fit model was found to be Higuchi release kinetics. FT-IR studies were carried out to find out the possible interaction between the selected drugs and polymer. FT-IR studies revealed that there was no interaction between the selected drugs and polymer. Among the different batches, Formulation F8 was selected as the ideal formulations, after considering their mean particle size, free flowing nature, better drug loading capacity and in vitro drug release. The percentage drug release microspheres was found to be 97.45%.

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