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FORMULATION AND EVALUATION OF ITRACONAZOLE LOADED MICROSPHERE-AS DRUG DELIVERY SYSTEM

Rahul Nagar*, Ajay Kumar and Rishikesh Sharma

Bhabha Pharmacy Research Institute, NH-12, Jatkhedi, Hoshangabad Road, Bhopal- 462026.

***Corresponding Author: Radhika Nair**

Pharm D. Intern, Believers Church Medical College Hospital, Kuttapuzha, Thiruvalla, Kerala-689103.

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ABSTRACT

Microspheres are novel drug delivery system which a very micro particulate and they are designed in such a way which are aimed in lieu of use in control release DDS. They are made of polymers which may belong from natural, synthetic or semi- synthetic origin. Microspheres are identified by free flowing powder which are particle size ranging from 1-1000µm and having proteins in them. The study suggested that ITCZ-MS was formulated successfully by using double emulsification solvent evaporation method. Upon generating various formulations, F8 was optimized amongst to check various parameters such as % yield, % entrapment efficiency and micromeritic properties. The % yield and % EE was 94%, 86% respectively. The various micromeritic properties were found by means of in range and indicating good flow properties of the powder. The morphological characteristics denoted spherical shape and in range size of the microspheres. The drug release profiles showed that maximum amount of drug was released from the formulation which means that it gave good bioavailability and quick onset of action. The drug release kinetics was best fitted in baker Lonsdale model of drug release kinetics in which drug release mechanism was super case II by erosion method.

KEYWORDS: Bioavailability, Double-emulsion, Itraconazole, Microsphere.

INTRODUCTION

To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.[1] There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.[2] There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bioerodible polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones.[3] Itraconazole is typical orally active triazole antifungal drug of broad spectrum activity. Itraconazole is weakly basic(pKa=3.7) and highly hydrophobic (octanol/water partition coefficient at pH=8.1, log P=5.66.[4] Itraconazole is very effective against a variety of infection caused by yeast and fungi. When itraconazole concentration maintained above the minimum effective concentration (MEC), it become more effective. But in case of immunocompromized patient it have not only result in poor clinical response but also caused relapse in disease if it was given below the minimum effective concentration.[5]

In the present study, drug is formulated as microspheres were prepared by forming double emulsification Solvent evaporation method.[6]

MATERIAL AND METHODS

Material

Itraconazole was received as a gift sample from Shreeii Pharma International, Vadodara. All other materials and reagents were of analytical grade.

Method

1.0. Preformulation study[7]

1.1 Physical characterization of Itraconazole

The physical characters of ITCZ like odour, colour, taste, state were determined by physical observation.[7]

1.2 Determination of melting point. (Capillary Method)

Melting point of ITCZ was performed via filling it in a capillary tube which was attached to thermometer and set-up was immersed in water bath which was allowed to heat by means of help of a burner. When compound in the capillary tube started to melt, temperature was noted down.

1.3 Determination of solubility (Magnetic Stirrer)

The determination of solubility was determined by adding excess amount of drug to 0.1N HCL and methanol and stirred in beaker using a magnetic stirrer by placing a magnetic bead in the beaker in lieu of 12hr at room temperature to reach maximum saturation. The sample was filtered through WFP and analyzed under UV spectrophotometer.

Table 1: Solubility Parameter (as per I.P.).

Descriptive Term	Parts of solvent required in solute part
Very soluble	>1
Freely soluble	1-10
Soluble	10-30
Sparingly soluble	30-100
Slightly soluble	100-1000
Very slightly soluble	1000-10000
Practically insoluble	<10000

1.4 Determination of Partition Coefficient

The property was identified by using Shake flask method. In a separating funnel containing equal ratio of octanol and water, drug was added to it and shake well using mechanical shaker in lieu of about 1 hr. Then funnel was left undisturbed in lieu of 30 min and aqueous was obtained which was analyzed in UV spectrophotometer.

Log P = Conc. of drug in organic phase/ Conc. of drug in aqueous phase … Eq.1

1.5 IDENTIFICATION OF ITRACONAZOLE

To check purity of ITCZ before formulating it into sodium alginate microspheres, compound was dissolved in methanol. At distinctive concentration, absorbance was identified under UV- spectrophotometer (Shimadzu 1700) at 265 nm.[8]

2.0 ANALYTICAL METHOD BY UV SPECTROPHOTOMETER

2.1. Preparation of 0.1 N HCL

Accurately weighted 9.9 ml of HCL using measuring cylinder and it was dissolved in 990.1 ml of DSW and mixed evenly by using a glass rod. The solvent obtained was 0.1 N HCL.

2.2. Preparation of Calibration curve by UV SPECTROPHOTOMETER

Accurately weighted drug sample (1gm) and dissolved in 0.1N HCL, in a volumetric flask. Dilutions were made between 2.0 to 10 µg/ml by using same solvent. The absorbance was recorded using UV spectrophotometer at 265 nm corresponding to each concentration was identified statistically. Calibration curve was plotted using concentration on x- axis and absorbance on y-axis.

2.3 Preparation of Calibration curve in Methanol

Accurately weighted drug sample (1gm) and dissolved in methanol, in a volumetric flask. Dilution were made between 2.0 to 10 µg/ml by using the same solvent. The absorbance was recorded using UV spectrophotometer at 265 nm corresponding to each concentrationwas identified statistically. Calibration curve was plotted using concentration on x- axis and absorbance on y-axis.

3.0. METHOD OF VALIDATION

ICH guidelines, Q2 (R1) as stated, was used to validate the method by means of respect to linearity, Limit of Detection (LOD) and Limit of Qualification (LOQ).

3.1. Linearity Range

The calibration curve was plotted by making a standard stock solution and transferring them between 2-10 µg/ml to 10ml volumetric flask each and volume make up to mark. Each of them were analysed by UV spectrophotometer at 265 nm. Calibration curve was plotted between absorbance V/s concentration and the regression value was calculated three times at room temperature.

3.2. LOQ & LOD

Limit of Detection and Limit of Qualification was detected using formula given by ICH.

$LOD = 3.3 \times \sigma / S$ …Eq.2

$LOQ = 10 \times \sigma / S$ …Eq.3

4.0. FORMULATION AND EXPANSION

4.1. Excipients Screening[8]

The studies carried out during Preformulation, we have screened both solid and liquid excipients before formulation of MS which was fully decided on the basis of solubility and compatibility by means of ITCZ. Calcium carbonate was chosen as release rate accelerant, sodium alginate was used as polysaccharide, and calcium chloride was used as cross-linking agent. The composition has been taken in accordance to miscibility among them.

4.2. Production of MS

The Itraconazole microspheres were prepared by forming double emulsification Solvent evaporation method. The polymeric solution was prepared by dissolving in solvent under continuous stirring using a magnetic stirrer charted by adding the API and drug carrier agent which was added manually drop wise to 1% W/V calcium chloride solution having 10% W/V GAA. Drops added to aqueous

phase were kept dispersed until their complete in lieu formation to avoid accumulation of the droplets. The droplets were kept dispersed in lieu of 1hours. Then, they were filtered using Whatsman filter paper and dried in lieu of 24 hrs under hot air oven until uniform weight.

5.0. CHARACTERIZATION OF MICROSPHERES[9]

5.1. Particle size -The microspheres were in lieu formulated and were evaluated in lieu of their particle size range. The particle size measurement was done by sieve analysis. A weighed amount of sample was taken and placed over previously arranged mini sets of sieve no. from 10- 120 mesh size wise and was shaken continuously in lieu of 15 min. After shaking completes, samples were collected and weighted and particle size was calculated by formula-

$$\textbf{Mean particle size} = \frac{\Sigma nd}{\Sigma n} \text{Eq.2.4} \quad \ldots. \text{Eq.4}$$

Where; n= no. of particle, d= mean size range

5.2. % Yield- The percentage yield may be defines as ratio of total amount of microspheres yield formulated to total amount of drug and excipients employed. % yield can be calculated by formula-

$$\textbf{\% Yield} = \frac{\text{Total amount of microspheres prepared}}{\text{Total amount of drug and excipients}} \times 100 \quad \ldots. \text{Eq.5}$$

5.3. Drug Encapsulation Efficiency- Encapsulation was determined by conventional method. The microspheres were weighted 10mg accurately and crushed using a mortar and pestle. The powder was dissolved in methanol and 0.1N HCL to volume make up to 50mL and sonicated in lieu of 12hrs. Then solution was passed through w.f.p. to collect filtrate. The filtrate was analysed under UV- spectrophotometer by means of 265nm by means of respect to blank 0.1N HCL.

$$\textbf{\% EE} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of drug fed}} \times 100 \quad \ldots \text{Eq.6}$$

5.4. Scanning Electron Microscopy (SEM) - ITCZ-MS were evaluated in lieu of size and surface morphology by SEM at 250x magnification. The image so obtained is presented below to confirm spherical structure and size of microspheres and their morphology.

5.5. X-ray Diffraction Analysis- ITCZ-MS were evaluated in lieu of their crystallinity in lieu of optimized formulation recorded by X-ray Diffractometer. The Cu radiations were used at a voltage of 30kV and a current of 40mA.

5.6 Micrometric Properties of Microspheres- The microspheres are in lieu formulated and further evaluated in lieu of their micro properties such as bulk density, tapped density, angle of repose, compressibility index.

5.7. Bulk Density- The bulk density was calculated by ratio of weighted microspheres to untapped volume of sample by formula-

$$\textbf{Bulk density} = \frac{M}{V} \quad \ldots.\text{Eq.7}$$

Where; M= weight of untapped microsphere, V= apparent volume

5.8. Tapped Density –The evaluating samples of microsphere are manually tapped in graduated measuring cylinder. The tapped density is defined as ratio of the weight of microspheres to final volume obtained after tapping.

$$\textbf{Tapped density} = \frac{M}{V^T} \quad \ldots\text{E.q.8}$$

Where; M= weight of microspheres, V^T= Tapped Volume

5.9. Carr's Compressibility Index- The compressibility index may be defined as tendency of powder sample to be compress or simply as ability to compress which may be calculated by following in lieu formula-

$$\textbf{C.I.} = \frac{TD - BD}{TD} \times 100 \quad \ldots \text{Eq.9}$$

Where; TD= Tapped density, BD= Bulk density.

5.10. Angle of Repose- Maximum angle is measured possibly between surface of pile and horizontal plane which was performed by means of help of funnel.

$$\theta = \tan^{-1}(h/r) \quad \ldots\text{Eq.10}$$

Where; h= height of heap of pile, r= radius of the base of the pile

6.0 IN-VITRO DRUG DISSOLUTION STUDIES

Drug release profile of ITCZ-MS was determined by using dissolution apparatus fixed by means of USP sort-2 paddle apparatus rotating at 50rpm filled by means of 900mL 0.1N HCL as dissolution media. Accurately weighted 100mg of microspheres were used in lieu of this study. The temperature was set to 37±0.5°C. Then 1ml aliquots were by means of drawn at distinctive time periods as 0, 5, 10, 15, 20, 25, 30 hrs and simultaneously replacing by means of same amount of fresh solvent. The range was recorded using UV-Spectrophotometer at 265nm.

6.1. In- Vitro Drug Release Kinetics

6.1.1 Higuchi model - This model is used to describe the drug which is water soluble or water insoluble from the solid or semisolid matrix system. The model had mathematical expression which gives release kinetics as follows-

$$Qt = KH.t^{1/2} \text{Eq.2.11} \quad \ldots\text{Eq.11}$$

Where; Qt= amount of drug release in time t, KH = Higuchi Dissolution Constant.

Data obtained from drug release kinetics from this model is plotted in contrast to CDS V/s square root of time.

RESULT AND DISCUSSION

7.0. PHYSICAL CHARACTERISATION AND IDENTIFICATION OF ITCZ

7.1. Organoleptic Properties

The following organoleptic properties were found and were satisfactory as per monograph of B.P. 2016.

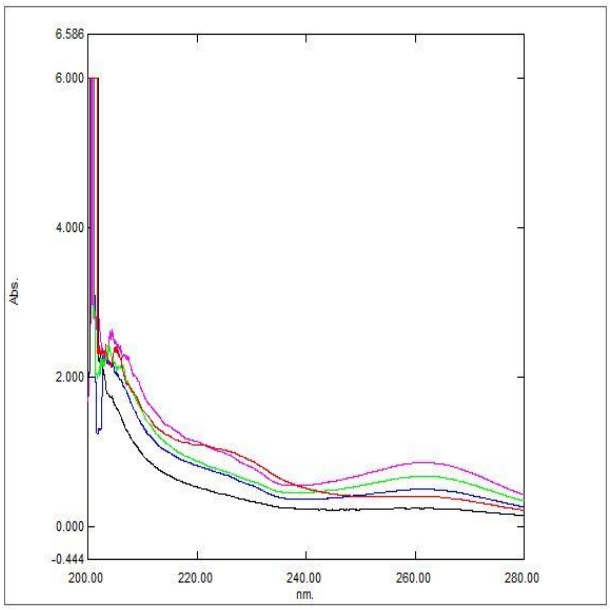
Table 2: Organoleptic Properties (as per B.P. 2016.).

Parameter Properties	Properties
Color	white to off white
Odor	odorless
Taste	Irritant
Nature	solid crystalline
Melting point	168°C
Solubility in water	poorly soluble in water
Partition coefficient	5.56(lipophilic in nature)

7.2 AUTHENTICATION OF DRUG

7.2.1 UV- spectroscopy

The λ_{max} of ITCZ in methanol was analysed by UV-spectrophotometer (shimadzus-1700) and the reported wavelength was at 265 nm (Bursa et. al.) and theoretical values was 265nm. The graph obtained is shown below in figure 1.



Graph 1: UV spectra of ITCZ at distinctive concentrations.

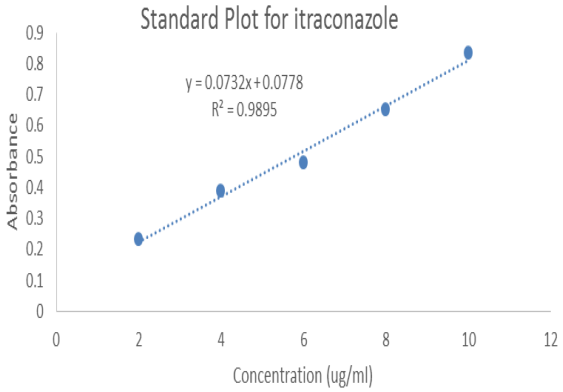
7.3 ANALYTICAL METHOD OF EXPANSION BY UV SPECTROSCOPY

7.3.1 Preparation of Calibration curve

The calibration curve of ITCZ was recorded in concentration range of 2-10 µg/ml as shown in table 3.1 and absorbance was obtained at 265 nm using Shimadzu-1700 UV-spectrophotometer.

Table 3. Absorbance of ITCZ.

Sample	Conc. µg/ml	Abs
1	2	0.232
2	4	0.388
3	6	0.481
4	8	0.652
5	10	0.832



Graph 2: Calibration Curve of ITCZ.

8.0. METHOD OF VALIDATION

8.1 Linearity and range

The calibration curve was plotted using concentration range of 2-10 µg/ml. accurately measured volumes of stock solution of ITCZ was transferred in to series of 10 ml volumetric flask and diluted up to mark by means of same solvent. The absorbance was determined at 265nm. Calibration curve was plotted between conc. v/s absorbance and regression value was calculated. Plot obeyed Beer's – Lamberts law in the above concentration range by means of r^2 = 0.989 which is shown in the dotted graph 2.

8.2. LOD and LOQ

LOD and LOQ was found to be 0.23±0.03µg/ml and 0.69±0.07 µg/ml respectively. Table no.4. below epitomise the given data which are as follows-

Table 4. UV parameter of validation in 0.1 N HCL

Parameters	Reading
Absorbance maximum (λ_{max}) in nm	265nm
Slope	0.0543
Intercept	0.0142
Correlation coefficient	0.989
LOD (µg/ml)	0.23 ±0.03
LOQ (µg/ml)	0.69 ±0.07

Hence, method was successfully validated by Linearity and range also LOD and LOQ were also determined and it was found to be correct.

9.0. FORMULATION AND EXPANSION

There were total 6 in lie formulations prepared out of which three were blank and other three were drug loaded in lieu formulations. Each of them had distinctive ratio of drug and polymer i.e. F4, F5, and F6 respectively. The

supreme physically stable in lieu formulation was found to be F6 because amongst all, it contained highest ratio of drug and polymer which gave good physical characteristics of microsphere and hence this in lieu formulation was carried further in lieu of evaluations.

Below given table 3.3 shows composition of the sodium alginate loaded Itraconazole microspheres.

Table 5: Composition Of sodium Alginate loaded Itraconazole Microspheres.

Trail No.	Formulation No.	Polymer (gm)	Drug (gm)	$CaCO_3$ (gm)	$CaCl_2$ (gm)	Aq. Phase containing 10% glacial acetic acid (ml)
1	F1	0.5	0	1	1	10
2	F2	0.7	0	1	1	10
3	F3	1.0	0	1	1	10
4	F4	1.5	0.3	1	1	10
5	F5	1.7	0.7	1	1	10
6	F6	2.0	1	1	1	10

9.1. CHARACTERISATION OF MICROSPHERES

9.1.1. % Yield, Particle Size

The % yield and particle size was found to be between 65.1±0.25 to 94.4±0.70 and 690.76±12.52 to 822.21±10.21 µm. As seen in F1 and F2, yield was not obtained because after filtration, microspheres formed lumps and in otherformulation the yield amplified as concentration of polymer was amplified. The particle size was seen in increasing order and it was not seen in F1 by means of few floating polymer drops by means ofout formation. This was due to low concentration of Sodium Alginate. On further increasing amount of sodium alginate, particle size as well as %yield was found to be amplify in F3, F4 and F5 as shown in Table 5. The particle size was found supreme spherical and stable in F6 because of polymer which aided in shape and size.

9.1.2 Entrapment Efficiency

The entrapment efficiency was found to be 73.60±1.75 to 88.52±2.33. Amongst 3 drug loaded formulations (F4, F5, F6), entrapment was found to be highest in F6. Further to see entrapment, it was found to decrease in F6 which was 86.99±7.33 as shown in Table 6 This was due to reaching saturation of the microspheres by means of drug.

Table 6: Particle size, % Yield, % Entrapment efficiency of ITCZ-MS.

Formulation No.	Particle size	% Yield	%EE
F1	-	-	-
F2	690.76±12.52	-	-
F3	724.91±13.63	65.1±0.25	-
F4	765.45±22.42	75.5±0.43	73.60±1.75
F5	793.31±13.80	82.7±0.36	88.52±2.33
F6	822.21±10.21	94.4±0.77	86.99±7.33

9.1.3 Micromeritic properties

The micromeritic properties was found to be in acceptable range. The Carr's compressibility index of F4, F5 and F6 was found to be between 11.12 to 20.50% indicated good flow ability. The angle of repose was calculated between 20.17° to 27.10° indicated excellent flow properties. Bulk density and tapped density was found to be between 0.77±0.01 to 0.69±0.03 and 0.86±0.01 to 0.71±0.05. The results are shown in Table no 7.

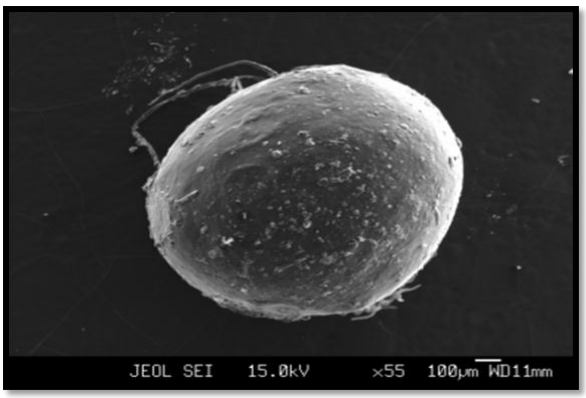
Table 7: Micromeritic properties of ITCZ-MS.

F. No.	Bulk Density	Tapped Density	Angle of Repose	Carr's Index
F1	-	-	-	-
F2	-	-	-	-
F3	-	-	-	-
F4	0.77±0.01	0.86±0.01	20.17	11.12
F5	0.73±0.04	0.81±0.03	26.12	15.26
F6	0.69±0.03	0.71±0.05	27.10	20.50

9.2 MORPHOLOGICAL CHARACTERISTIC

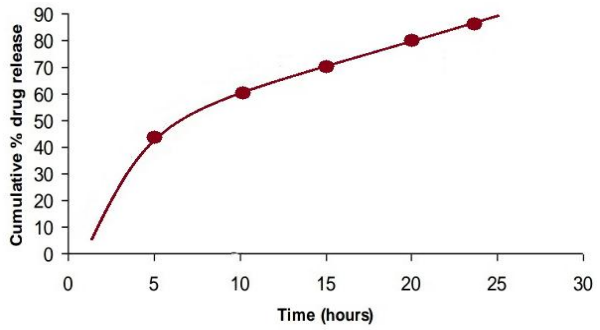
9.2.1. Scanning Electron Microscopy

The size and shape of the ITCZ-MS were determined by SEM. The size was found to be spherical by means of little roughness without any porous deformity in morphology. The agglomeration was not seen because of rigidity. The images obtained from SEM is shown in fig 1.



9.2.2. In-Vitro Drug Release

The dissolution media was freshly prepared just before use. To study drug release, (Shimadzu, Japan) apparatus was used. The optimized sample F6 was tested in lieu of drug release in lieu of 1hr at distinctive time interval. It was found that nearly 90% of drug was released by 60min as shown in graph 3. In control release formulation and gave high bioavailability.



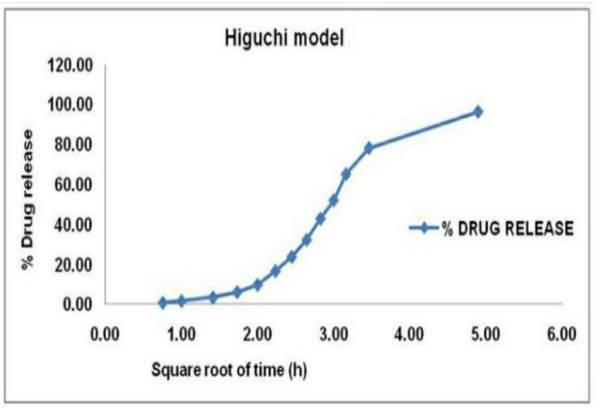
Graph 3: In-Vitro Drug Release from Microsphere optimized formulation (F6).

9.2.3 In- Vitro Drug Release Kinetics

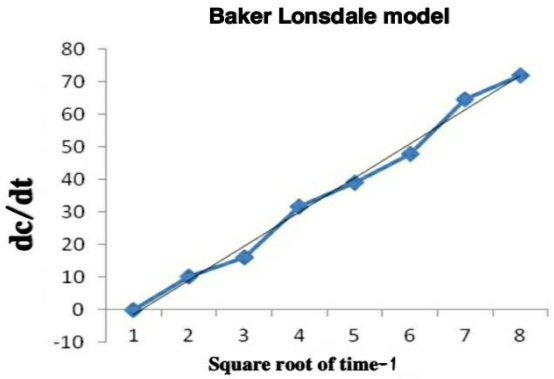
The data obtained were fitted to mathematical model to check drug release from optimize formulation (F6). The drug release kinetics model used were Higuchi model and Baker Lonsdale model. The R^2 value in lieu of Baker Lonsdale model was 0.976 which was near to 1 than in higuchi model where R^2 was 0.871.

Table 8: Release kinetics of Optimized Formulation.

Formulation code	Higuchi model R^2 value	Baker Lonsdale model R^2 Value
F8	0.871	0.976



Graph 4: Higuchi model of drug release Kinetics (F6).



Graph 5: Baker Lonsdale model of drug release Kinetics (F6).

CONCLUSION

The study suggested that ITCZ-MS was formulated successfully by using double emulsification solvent evaporation method. Upon generating various formulation, F8 was optimized amongst to check various parameters such as % yield, % entrapment efficiency and micromeritic properties. The % yield and % EE was 94%, 86% respectively. The various micromeritic properties was found by means of in range and indicating good flow properties of the powder. The morphological characteristics denoted spherical shape and in range size of the microspheres. The drug release profiles showed that maximum amount of drug was released from the formulation which means that it gave good bioavailability and quick onset of action. The drug release kinetics was best fitted in baker Lonsdale model of drug release kinetics in which drug release mechanism was super case II by erosion method.

REFERENCES

1. Deshpande AA, Shah N, Rhodes CT, Malik W, "Development of a novel controlled-release system for gastric retention" Pharm Res., 1997; 14: 815-19.
2. Kakar S, Batra D, Singh R, Nautiyal U, "Magnetic Microspheres as Magical Novel Drug Delivery System: A review" Journal of acute disease, 2013; 1-12.

3. Jain NK, "Progress in Controlled and Novel Drug Delivery Systems; First Ed. CBSS. Gopalakrishnanet al" Journal of Pharmaceutical Science and Technology Publishers and Distributors, New Delhi Bangalore, 2004; 3(2): 84-85.
4. Grant S, and Clissold S .Itraconazole: A review of pharmacodynamics and pharmacokinetic properties and therapeutic use in superficial and systemic mycosis. Ind. Drugs, 1989; 37: 310–314.
5. Amidon G.L., Lennernäs, H Shah V.P., Crison, J.R.A theoretical basis for a533 biopharmaceutic drug classification: the correlation of in vitro drug product534 dissolution and in vivo bioavailability, Pharm. Res., 1995; 12: 413–420.
6. Singh B, Kanouji J, Pandey M and Saraf SA, "Formulation and Evaluation of Floating Microspheres of Famotidine" International Journal of Pharmtech Research, 2010; 2(2): 1415-1420.
7. Pramod S, Dr. Shiv G, Prateek P and Ajay P, "Hollow Microspheres: An Emerging Approach in the Field of Gastroretentive Drug Delivery System" International Journal of Universal Pharmacy and Bio Sciences, 2014; 3(4): 305-315.
8. Rajkumar K, Sainath GR, Sai Sowjanya P, Anusha P, Lavanya AS and Reddy ER, "Floating Microsphere: A Novel Approach in Drug Delivery. Journal of Drug Delivery Reasearch", 2012; 1(4): 1-20.
9. United States Pharmacopoeia 29 (NF24), Rockville MD: United States Pharmacopoeial Convention Inc., 2006.