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RESEARCH ARTICLE

Formulation, Evaluation and Release Mechanism of Ketoconazole Microsponge by Liquid-Liquid Suspension Polymerization Method

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Abstract

The aim of the present study is to formulate a microsponge based release system contains ketoconazole for controlled release of drugs for an effective fungal treatment. Compatibility study involving drug and polymer mixture done by FTIR and was initiate to be compatible. Ketoconazole loaded microsponges were prepared by liquid-liquid suspension polymerization method using ethyl cellulose and hydroxypropyl methylcellulose as polymers with different drug polymer ratios. Later, formulated microsponges were characterized. The particle size was measured by SEM, entrapment efficiency by assay and *in vitro* drug release profile by dissolution. The effect of preparation variables such as a drug to polymer ratio and stirring speed on the physical characteristics of microsponges is examined. The formulated microsponges were spherical and porous, with a mean particle size of 100µm at 400 magnifications. 87.6% of drug was entrapped in the system and 89.40% of drug was released at 12th hour from FII. From the above findings, we conclude that microsponges were prepared successfully and evaluated. The evaluation report shows that the sizes of the particle are within the range and controlled release of drug was achieved. This indicates that polymer choosen for the study is appropriate. The mathematical kinetic modeling shows that FII formulation undergoes zero order kinetic and follows Higuchi model, which follows diffusion mechanism in the release of drugs.

Keywords: *Ketoconazole, Microsponges, Ethyl cellulose, Hydroxypropyl methylcellulose, Liquid-Liquid suspension polymerization.*

Introduction

Microsponges are polymeric release systems composed of porous microspheres. They are small sponge-like spherical shaped particles that consist of interconnecting voids within a non-collapsible structure with a large porous surface with particle size ranging from 5-150µm.[1] Ketoconazole is an imidazole derivative used in the systemic treatment of fungal infections and having high lipophilic property, which binds with fatty tissues leads to higher concentration.[2]

Ketoconazole poorly distributes in the cerebrospinal fluid [3] as well as oral absorption and solubility are also poor. [4] Ethyl Cellulose (EC) is insoluble in water but soluble in several organic solvents with good stability. It forms tough tensile films and maintains better flexibility at low temperatures. [5] The EC is non-toxic, strong in antibiotic effects and metabolically inert. It is also capable of withstanding degradation due to oxidation by sun and, or ultraviolet rays.[6] Hydroxypropyl methylcellulose (HPMC) is non-ionic cellulose. HPMC is soluble in cold water as well as in most glacial organic solvents and not soluble in diethyl ether, acetone and anhydrous alcohol.[5] In cold water, it swell into a clear or slightly turbid colloidal solution.[7] Its aqueous solution has surface activity, high transparency and stable properties. Since dissolution of HPMC in water is not affected by the pH value, so it was use in the current study.

The HPMC and EC polymers were selected in the present study to enhance the solubility[8], rate of absorption and bioavailability for the chosen drug.[9] Thus these two polymers provide a coating material around the drug, which acts as a control release drug delivery system.[10] Thereby, Microsponges was used for controlled release system in which active

pharmaceutical ingredients are incorporated in the microporous bead and initiate a reduction in side effects with enhanced therapeutic efficacy.[11] The microsponge drug delivery system has properties like better stability and enhanced flexibility in formulation.[12] The benefits of using microsponge are as follows: (i) absorbs oil up to 6 times its weight without drying (ii) extended release upto 12 hours (iii) good stability at low and high temperature and resistant to many chemical (iv) minimal irritation with better tolerance, hence results in improved patient compliance and (v) improves the product aesthetics and increases bioavailability of the drug (vi) flexibility will improve (vii) non-toxic, non-irritating and non-allergenic. [13,14-15]

Materials and Methods

Ketoconazole was obtained from Life Care Formulation, Pondicherry as gift sample, whereas ethyl cellulose is procured from Hi-Media Laboratories, Mumbai. Hydroxypropyl methylcellulose, dichloromethane, 0.01% Tween 80 was procured from the SD fine chemicals Ltd., Mumbai, while ethanol is procured from the Changshu Hongsheng Fine Chemical Co. Ltd, Changshu. All the materials used were of laboratory grade.

Preparation of Microsponges

Different microsphere formulations were prepared using a liquid–liquid suspension polymerization method. In all the formulations, drug weight was kept as constant (300 mg) while the polymers (EC and HPMC) proportions were varied. The polymers are dissolved in 8 ml of dichloromethane and 8 ml of ethanol at room temperature for 12 hours. Ketoconazole is dissolved to the above polymeric solution. The resulting slurry was gradually introduce as a thin stream into 200 ml of water contain 0.01% tween 80 and stirred with heating magnetic stirrer for 1 hour to let the volatile solvent to evaporate totally. The microsponges were filtered and repetitively washed with distilled water. The obtained microsponges were dried for the night in oven at 40°C.[16]The product is stored in a 40°c ± 2°c.

Evaluation of Microsponges

The Percentage yield of microspheres

The production yield of microsponges of both the batches is calculated by using the following formula:[17]

$$Yield(\%) = \frac{Practical\ mass}{Theoretical\ mass(drug + polymer)} \times 100$$

Scanning Electron Microscopy (SEM)

The obtained microsponges are subjected to the measurement of size and shape by using Scanning Electron Microscope (SEM).The Microsponges was layered with platinum by ion sputtering with autofine coater. The microsponges were kept on the sample holder and scanned for their shapes, and it is captured.

Drug Entrapment Efficiency

The formulated microsponges are evaluated for drug entrapment efficiency. A known quantity of microspheres is dissolved in 10ml of dichloromethane in the separating funnel and the sample was extracted with aliquots of 0.1N NaOH. The extract is transfer to a 100ml volumetric flask and volume is made up using 0.1N NaOH. The solution was filtered, and absorbance is measured at 296nm against the blank. The following formula calculated the total amount of drug entrapped in the microspheres:[18]

$$\mathrm{DEE}(\%) = \frac{\text{Amount of durg actually present in the sample}}{\text{Theoretical drug content in the sample}} \times 100$$

Fourier Transform Infrared (FTIR) Analysis

FT-IR spectra identified the interaction involves between drug and polymer. It was obtained by using Agilent Resolution pro technique and was compared with the spectra available in the official book.

In-Vitro Drug Release Study

In vitro drug release studies are performed in pH6.8 phosphate buffer (900ml). The buffer is used as dissolution media at temperature 37 ± 0.5˚C and at 100 rpm. The dissolution study is performed in USP apparatus XXIV- Type I. At the intervals of 2, 4, 6, 8,10 and 12 hours, samples (10ml) were withdrawn, and an equal amount of fresh dissolution medium is replaced every time. Withdrawn samples be filtered through a membrane filter and suitably diluted with dissolution media and assayed at 296nm using a UV spectrophotometer.[19]

Kinetic Model and Drug Release Mechanism

In this present study the challenge was made the design for ketoconazole loaded

microsponges and its release profile was interpret with various mathematical models[20]

- Zero order model
- First order model
- Higuchi model
- Korsmeyer - Peppas model
- Hixson – Crowell model

Zero Order Model

Zero order models are the probabilistic model in which the possibility of incident of any given result at an exacting point in the time dose not depends on any outcome of the process. According to the principle of pharmacokinetics, drug release from the dosage form can be representing by the equation:[21]

$$C_0 - C_t = K_0 t$$
$$C_t = C_0 + K_0 t$$

Where
C_t is the amount of drug released at time t,
C_0 is the initial concentration of drug at time t=0,
K_0 is the zero-order rate constant.

Zero order kinetics defines the process of steady drug release from a drug delivery system and the level drug in the blood remains stable throughout the delivery. To study release kinetics the data obtained from *in-vitro* dissolution study is plotted between cumulative drug release (%) and time. The zero-order rate constant and correlation coefficient can be calculated from the graph using the slope, which indicates the order of kinetics. If the correlation coefficient value is > 0.9 then the system follows zero order of kinetics, which shows the best fit model.

First Order Model

The first order model is defined as the process is one the rate is directly proportional to the concentration of drug undergoing reaction i.e., larger concentration quicker the reaction. It is follows linear kinetics. The drug release that follow first order kinetics it is represented by the following equation

$$DC/dt = -K_1 C$$

K_1 is the first order rate constant, expressed in time^{-1} or per hour. The above equation is rearranged and integrating

$$\log C = \log C_0 - K_1 t/2.303$$

To study release kinetics the data obtain from *in-vitro* dissolution study is plotted between log % of drug remaining and time. The zero-order rate constant and correlation coefficient can be calculated from the graph using the slope, which indicates the order of kinetics. If the correlation coefficient value is > 0.9 then the system follows zero order of kinetics, which shows the best fit model.[20]

Higuchi Model

The drug release form the drug delivery system (DDS) involves both dissolution and diffusion. There are different mathematical model illustrate drug release from the DDS. 'Higuchi equation' is the most major kinetic equation in the recent period of controlled-release formulation. Higuchi equation is one of the most extensively used equations for controlled-release formulation. The Higuchi equation is represented by:

$$Q = A\sqrt{D(2Co - Cs)Cst}$$

Where Q is the cumulative amount of drug released in time t per unit area, D is the diffusion coefficient of the drug molecule in the matrix. C_0 is the initial drug concentration and C_s is the drug solubility in the matrix. When the correlation coefficient is superior for the above plot then we can interpret that the major mechanism of drug release is diffusion controlled release mechanism. There are some important assumption are prepared in this Higuchi equation.

They are (i) matrix solubility is much higher than the initial drug concentration in the system (ii) sink condition is maintained perfectly (iii) diffusivity of the drug is constant and (iv) polymer swelling is small. The sink conditions are achieved by ensuring the concentration of the released drug in the release medium never reaches more than 10% of its saturation solubility. This model was useful in the release profile of ketoconazole loaded microsponges. The estimation was done in the graphical presentation by (Cumulative % drug release *vs.* Square root time).[22]

Korsmeyer-Peppas Model

Once it has been conclude that the major mechanism of drug release is diffusion controlled from Higuchi plot then it come the release of drug follow which type of diffusion.

For accommodating the dissolution mechanism form the matrix, the release data were set using the well-known practical equation estimated by korsmeyer and peppas. Korsmeyer and peppas describe an easy connection which described the drug release from a polymeric system follows which type of dissolution and they represented the equation as:

$$\mathbf{Mt/M\infty = K_{kp}t^{n}}$$

Mt/M∞ is a fraction of drug released at time t,

$$\mathbf{\log(Mt/M\infty)=\log K_{kp}+n\log t,}$$

Where
Mt is the amount of drug released at time t,
M∞ is the amount of drug released after time ∞,
N is the diffusional exponent or drug release exponent,
K_{kp} is the Korsmeyer release rate constant.

To study the release kinetic the graph is plotted between log cumulative % drug release vs. log time.[23]

Hixson-crowell Model

The Hixson-crowell model describe that the release from the system may have change in surface area and diameter of particles. The particles in the regular area and they are proportional to the cube root of its volume. From the above idea Hixson-crowell establish a relationship release of the drug and time. It is represented by equation as:

$$\mathbf{W_0^{1/3} - W_t^{1/3} = K_{HC}t}$$

WhereW_0 is the initial amount of drug in the pharmaceutical dosage form (amount of drug remaining at time 0); W_t is the remaining amount of drug in the pharmaceutical dosage format time t; K_{HC} is the Hixson-crowell constant for identifying surface volume relation. This equation is used interpretation of dissolution data of conventional dosage form, dispersible dosage form and immediate release dosage form. If the above equation has higher correlation coefficient then we can interpret the change in surface area during the process of dissolution has a significant effect on release of the drug. To study the release kinetic the graph is plot in between cube root of drug percentage remaining *vs.* time. [23-24]

Results and Discussion

Formulation of Microsponge by Liquid–Liquid Suspension Polymerization Method

In liquid–liquid suspension polymerization technique, the development of the microsponges could be by the fast diffusion of dichloromethane and ethanol (good solvent for the polymer and drug) into the aqueous medium, may decrease the solubility of the polymer in the droplets, since the polymer was insoluble in water.

The immediate mixing of the dichloromethane, ethanol and water at the interface of the droplets induce precipitation of the polymer, thus forming a shell enclosing dichloromethane, ethanol and dissolved drug. The finely isolated droplet of the polymer solution of the drug was solidifying in the aqueous phase via diffusion of the solvent. The percentage yield is calculated and provided in Table No. 1.

Characterization of Microsponges by SEM

The particle sizes of ketoconazole loaded microsponges were analyzed by scanning electron microscopy. The SEM revealed that microsponges had uniform size distribution. The average particle size of ketoconazole loaded microsponges is establish to be 100µm (50µm to 150µm) at 400 magnifications. The SEM image is provided as Fig. 1.

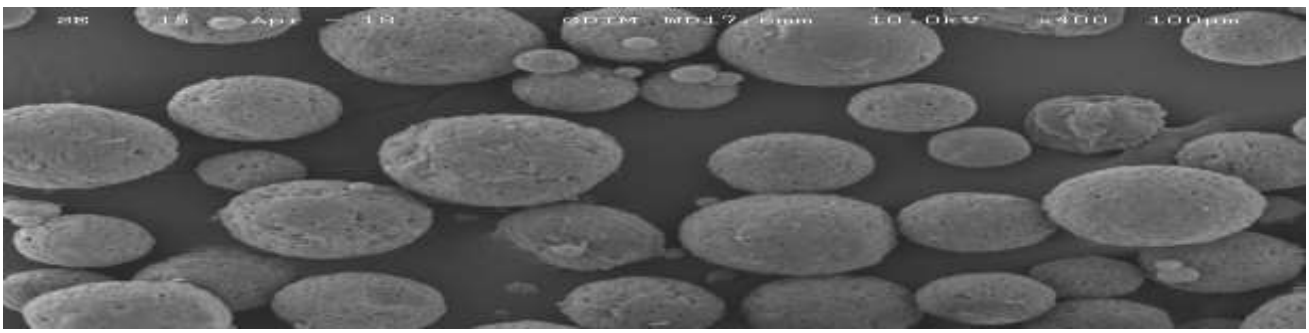

Fig. 1: SEM image of microsponges at magnification 400X

Fourier Transform Infrared (FTIR) Analysis

An FTIR spectrum of pure ketoconazole, hydroxypropyl methylcellulose and ethyl cellulose and mixture was obtained. The fundamental peak of ketoconazole in 1643 (kenotic C=O Stretch) cm-[1]were observed. It indicates that the FTIR spectra of pure ketoconazole were compatible with hydroxypropyl methylcellulose and ethyl cellulose polymer, as shown in Fig. 2, 3, 4 and 5.

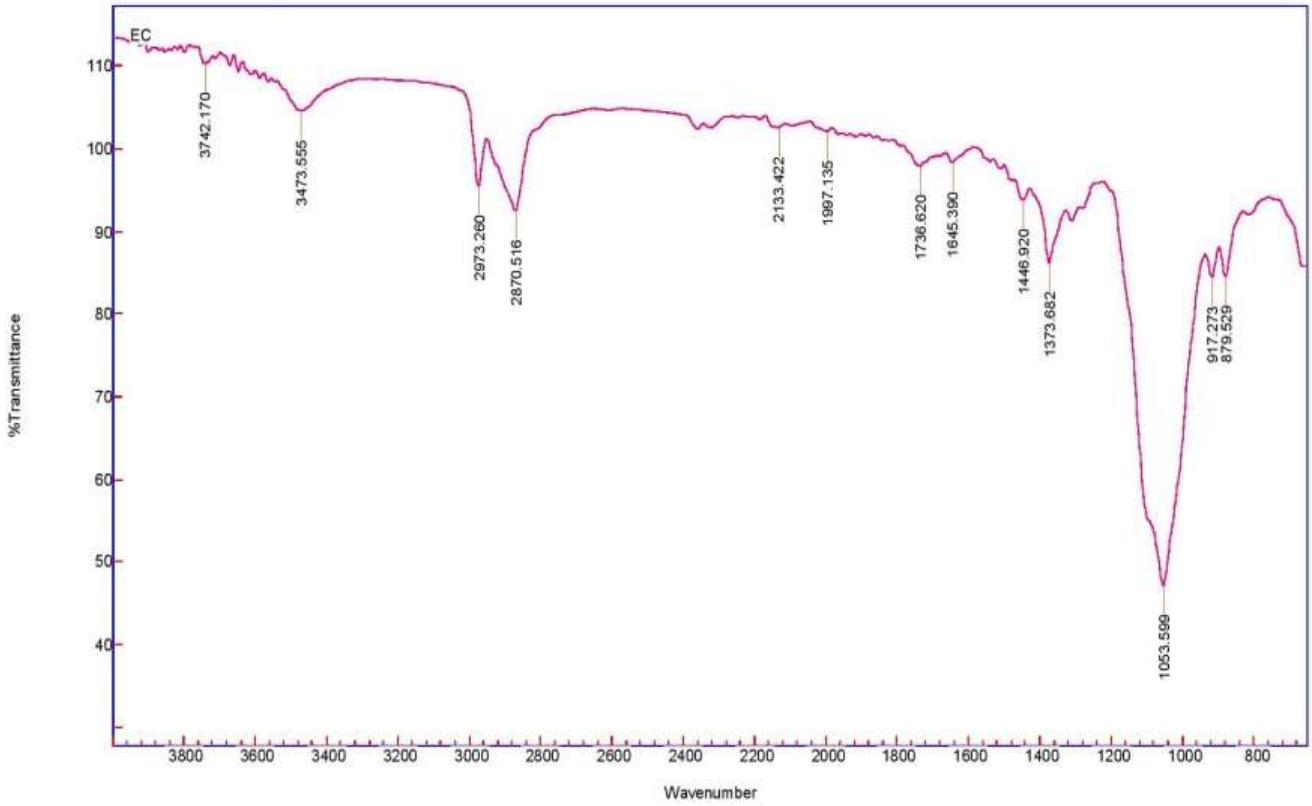

Fig. 2: FTIR Spectra of Ethyl Cellulose

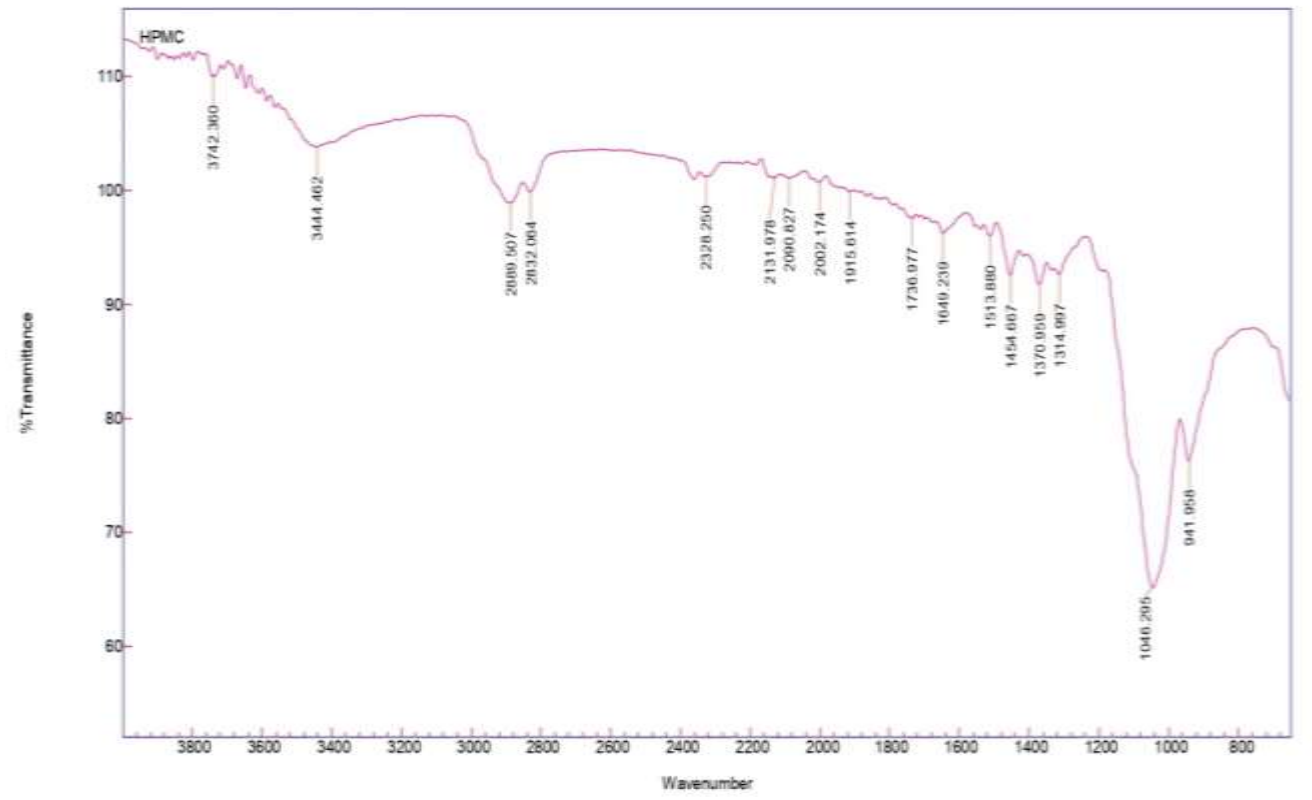

Fig. 3: FTIR Spectra of Hydroxypropyl methyl cellulose

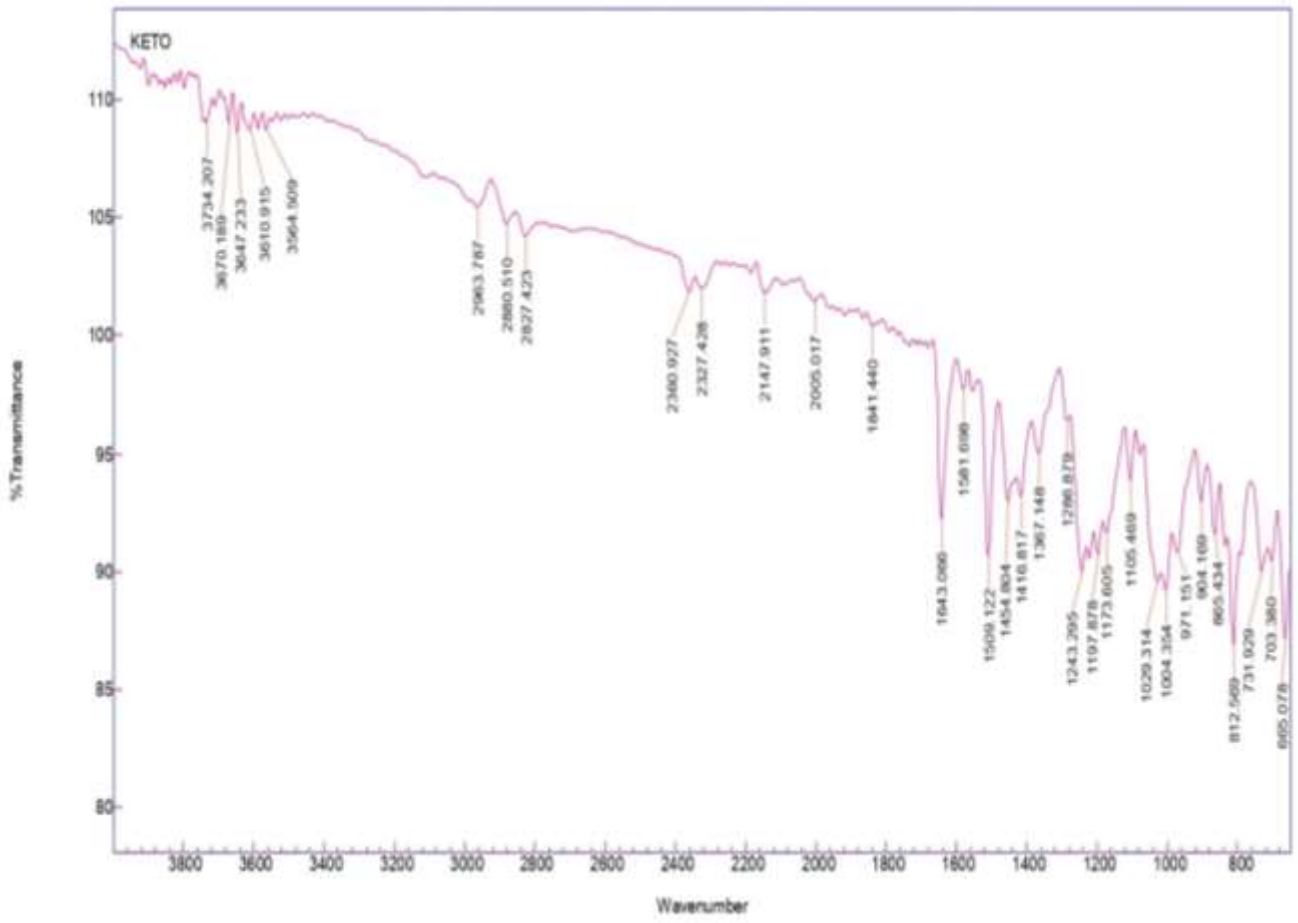

Fig. 4: FTIR Spectra of Pure Ketoconazole

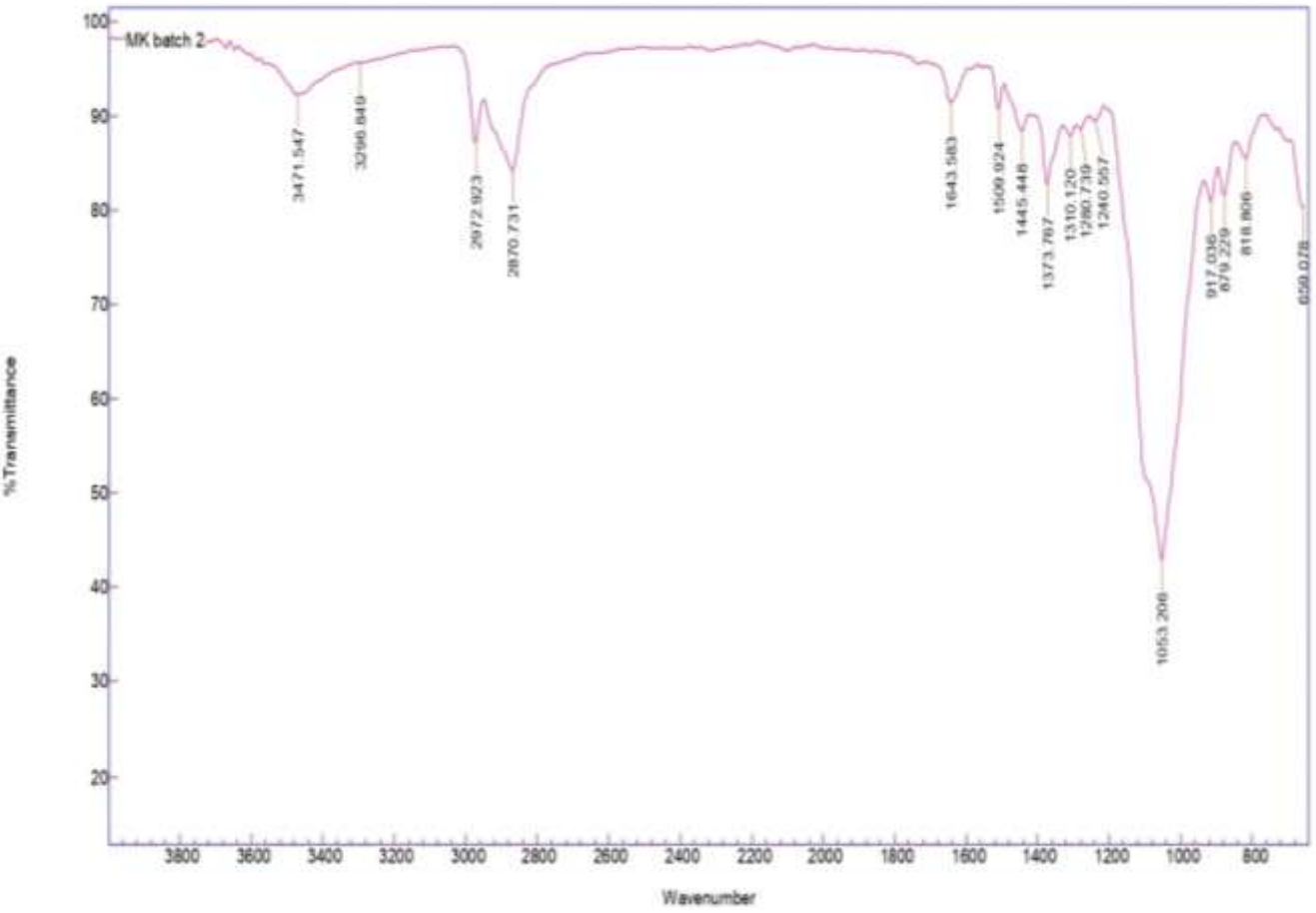

Fig. 5: FTIR Spectra of Formulation II ketoconazole loaded microsponge

Drug Entrapment Efficiency

The entrapment efficiency was observed maximum in the formulation, FII, containing an equal proportion of ethyl cellulose and HPMC. The highest entrapment efficiency means more considerable the quantity of drug encapsulated in the polymer coating. The encapsulation efficiency of the drug ketoconazole was found to be 74.4% for FI while for FII 87.6%, whereas for FIII 61.8%. The data is represented in Table 1.

Table No. 1: Evaluation of physicochemical characteristics of prepared microsponges at various levels of process and formulation variables

F.No	Drug (mg)	EC/HPMC	Stirring rate (rpm)	Temperature	Yield (%)	DEE (%)
I	300	2:1	500	60ºC±0.5ºC	94.34	74.4
II	300	1:1	500		99.13	87.6
III	300	1:2	500		91.30	61.8

In vitro Release Study

The drug release was established to be enhanced depending upon the polymer: drug ratio. The drug released is measured at 2, 4, 6, 8and 12 hours and the data was shown in Fig. 3

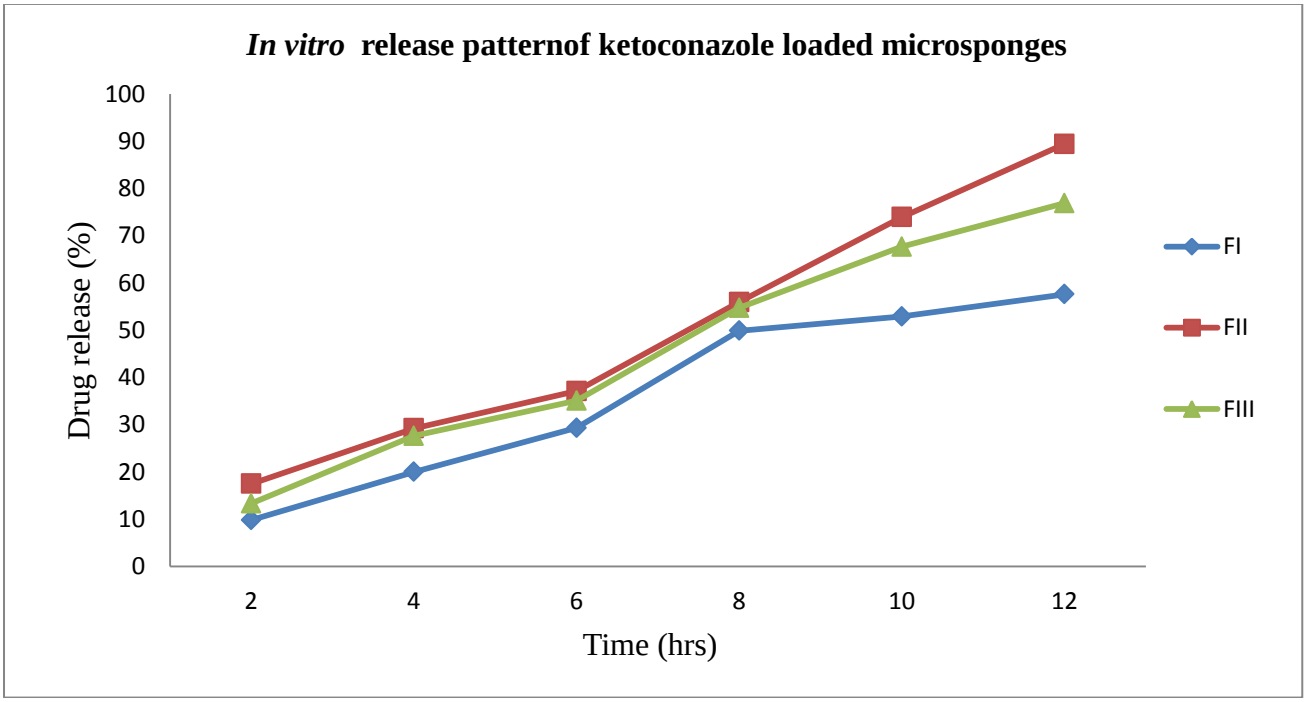

Fig. 4: *In vitro* release pattern of ketoconazole loaded microsponges

From the FTIR spectra is observed that here is no interaction between the drug and polymers. The procedure employed to formulate the microsponges were highly productive with minimal loss during the process in all the three formulations. In the current study, we could bale to get an average production yield of about95%. By varying the polymer ratio, the drug entrapment efficiency and *in vitro* drug release was high in the FII when compared with the other two formulations (FI & FIII). The addition of more amounts of polymers has led to decrease in a drastic change in the DEE and *in vitro* release patterns. The polymer concentration, either at lower or higher concentration has to an aggregation of microsponges.

Pharmacokinetic Parameters for Three Formulations of Microsponges Loaded With Ketaconazole

The pharmacokinetic parameters such as Area under the Curve, Volume of Distribution and Clearance are computed for all three formulations. The FII has low volume of distribution thus, the release will be controlled. When comparing three formulations for clearance FII was optimal. The data are represented in the Table. 2.

Table 3: Result of different pharmacokinetic parameters for all three formulations

Parameters	FI	FII	FIII
k (hr-1)	0.03	0.12	0.12
AUC (mg.hr/ mL)	23089.67	10687.08	9434.94
VD (L)	133.39	69.20	79.15
Cl (L/ hr)	3.90	8.42	9.54

Drug Release Mechanism and Kinetics Models of Ketoconazole Loaded Microsponges

The mathematical order and kinetic models for ketoconazole loaded microsponges is carried for the formulation FII, because the other formulations have low dissolution profile when compared with FII. Also, the zero order kinetic model shows that the higher correlation coefficient then the first order kinetic model and it is determined by the graphical representation. Hence, ketoconazole loaded microsponges follows zero order kinetics. The data are represented in the Fig. 5, 6.

The drug release mechanisms were computed for FII, because the other two formulations (FI and FII) are having low dissolution profile when compared with FII. The *in vitro* drug release profile was applied in the different drug release mechanisms and interpreted from of graphical presentation and determined by the correlation coefficient (r^2).

The data are represented in Table 2 and Fig. 7, 8, 9. The correlation coefficient (r^2 = 0.983) is high for the Higuchi model when compared with other release kinetic models for the FII formulation, which indicates that the FII formulation follows release kinetics of ketoconazole loaded microsponges follows diffusion mechanism.

Table 3: Result of different models in the terms of r^2

Model name	r^2
Higuchi model	0.983
Korsmeyer-peppas model	0.981
Hixson-crowell model	0.928

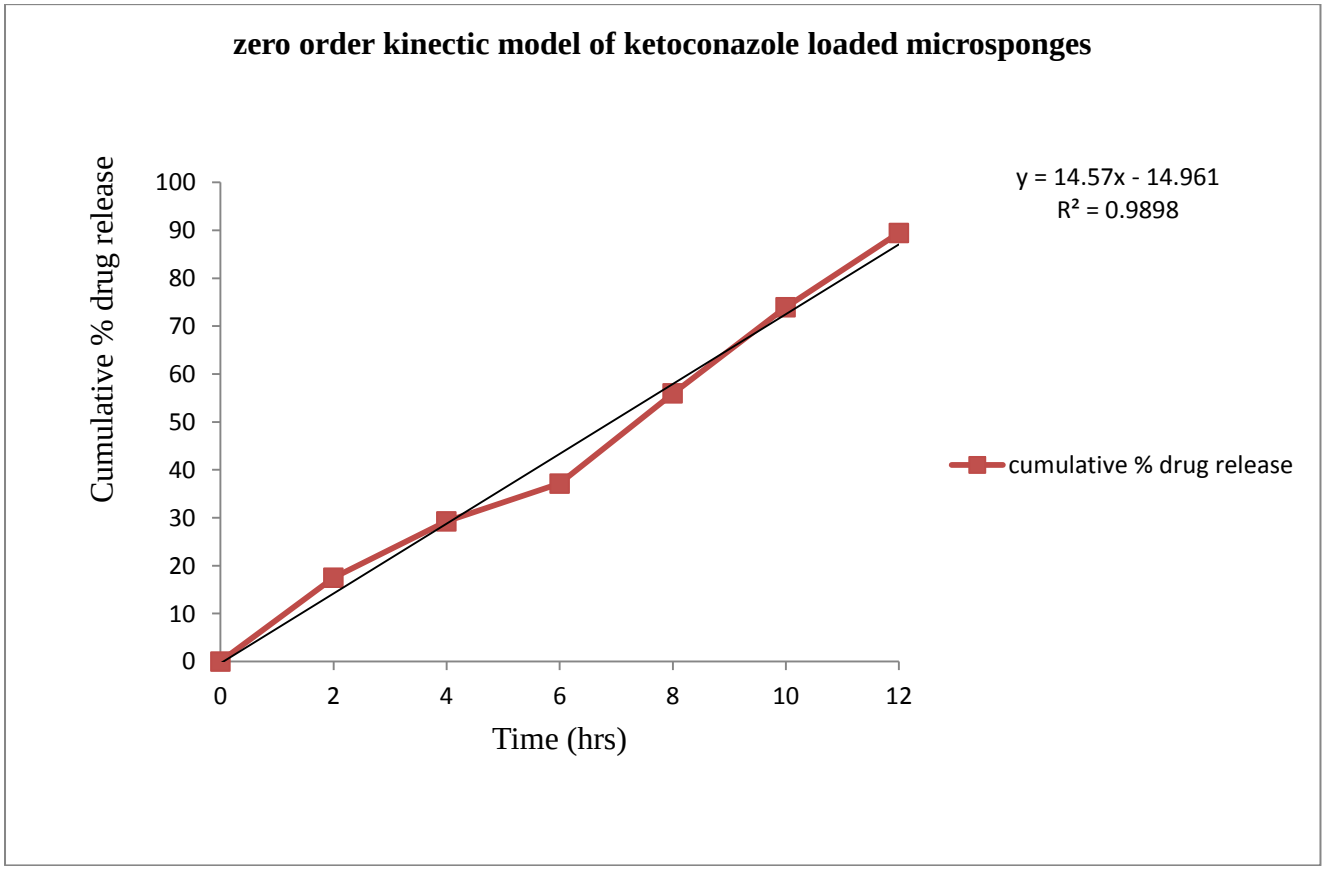

Fig. 5: Zero order kinetic model of ketoconazole loaded microsponges

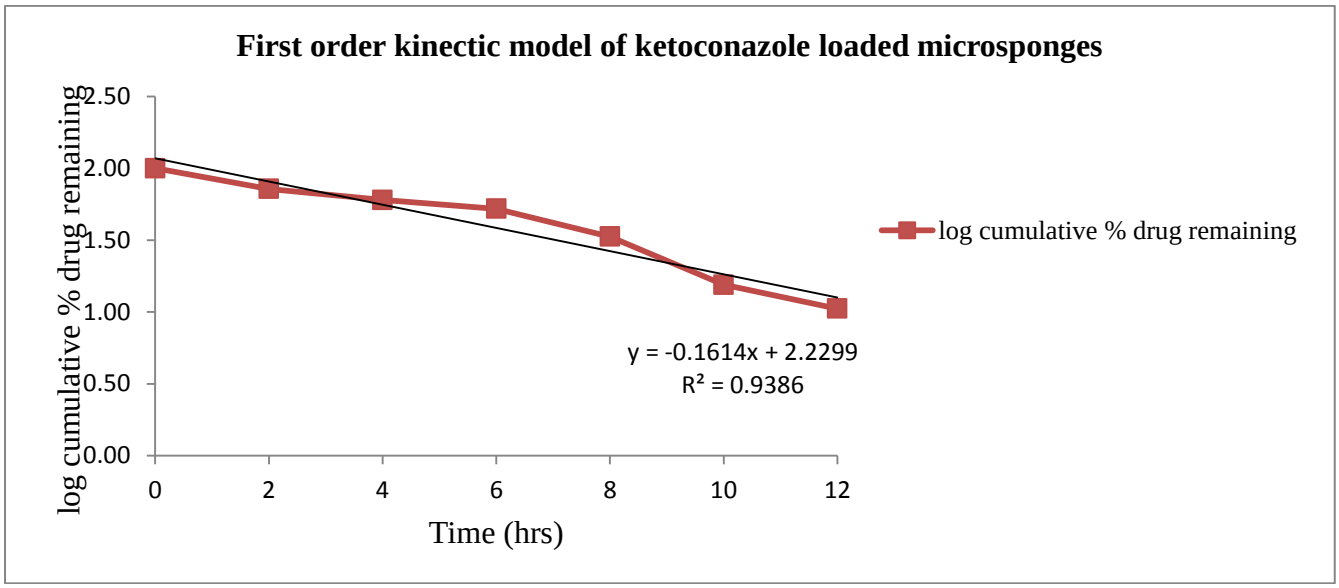

Fig. 6: First order kinetic model of ketoconazole loaded microsponges

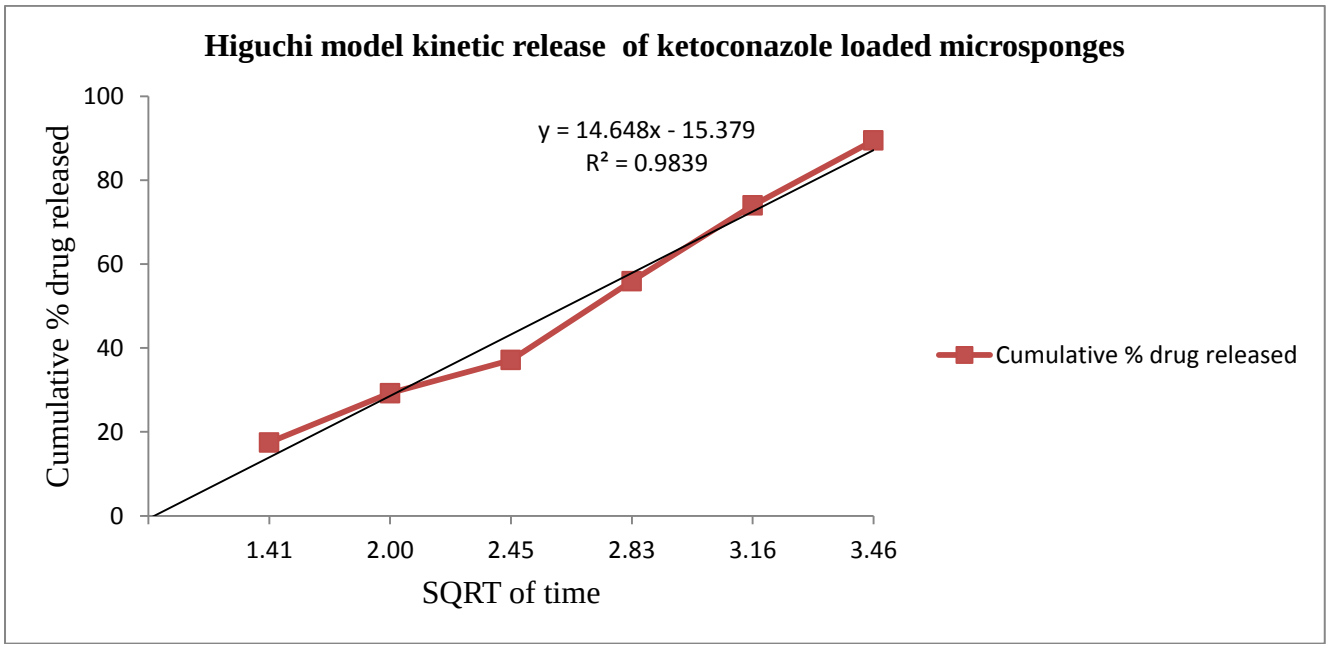

Fig. 7: Higuchi model kinetic release of ketoconazole loaded microsponges

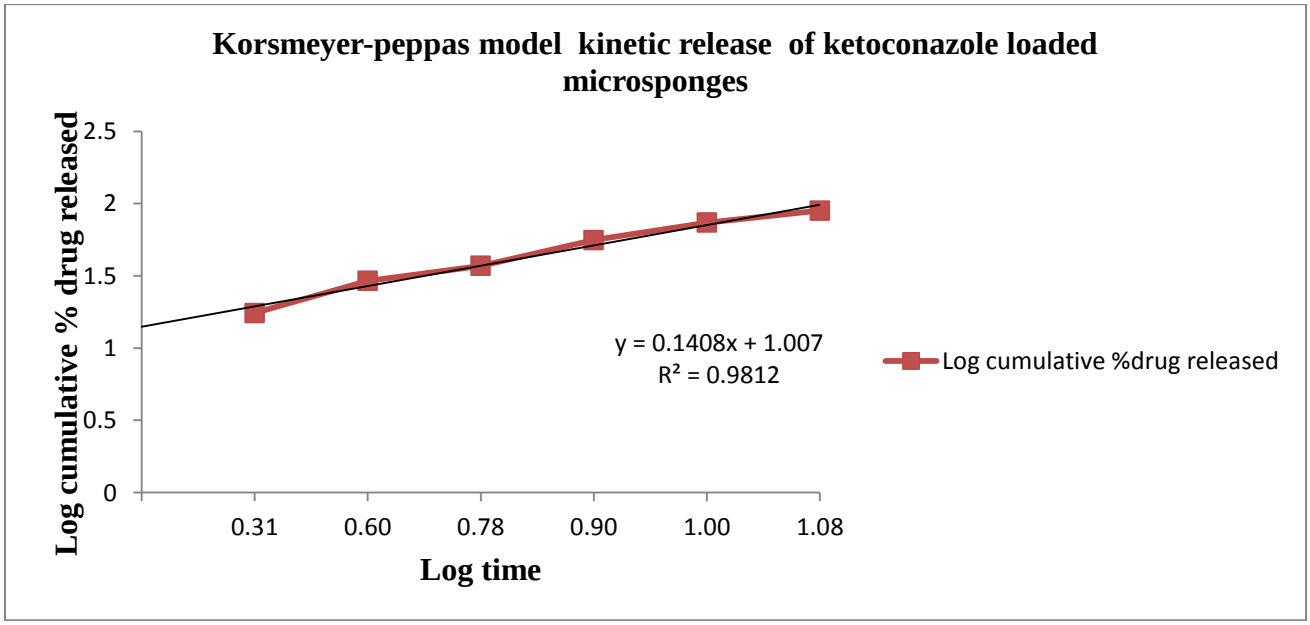

Fig. 8: Korsmeyer-peppas model kinetic release of ketoconazole loaded microsponge

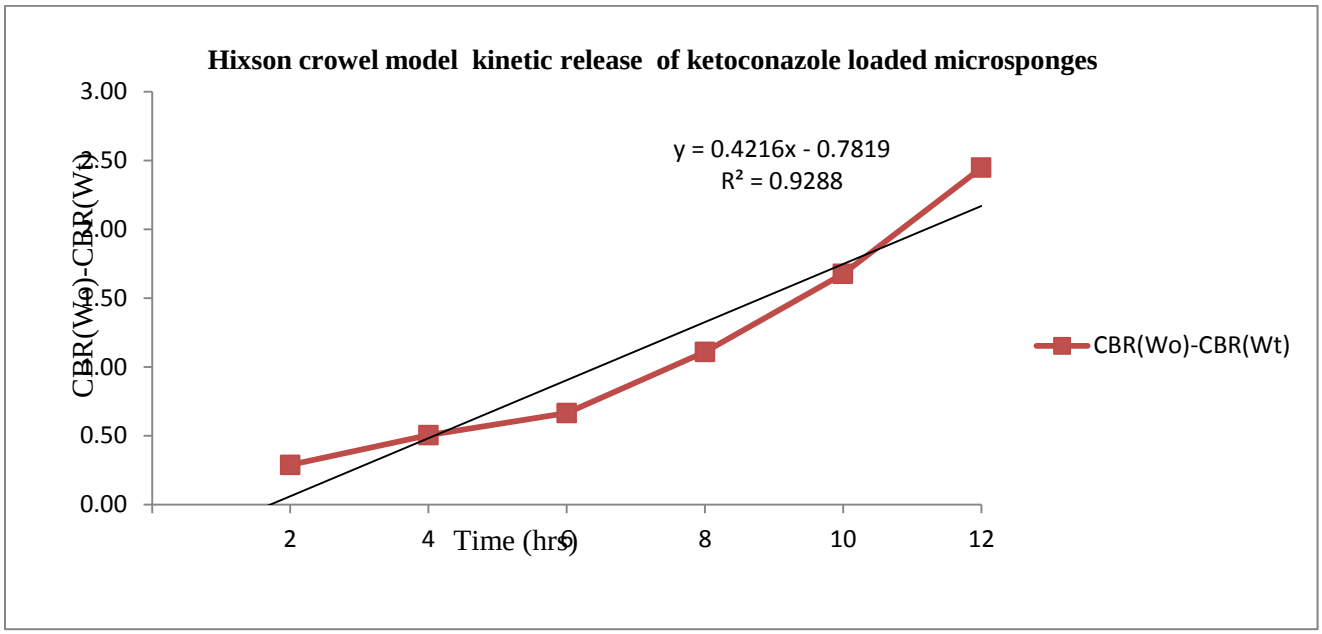

Fig. 9: Hixson crowell model kinetic release of ketoconazole loaded microsponges

Conclusion

From the above study conducted, it was concluded that microsponges of ketoconazole were prepared successfully by liquid-liquid suspension polymerization technique, which is a simple and faster technique. Further, it is observed that as the drug/ polymer ratio increases, the particle size was increased. This is probably because at advanced virtual drug content, the amount of polymer presented per microsponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence, smaller microsponges. Thus, a successful attempt was made to formulate microsponges of BCS–class II drug, which resulted in enhancing the dissolution properties of the drug, which ultimately increase its bioavailability. The drug release pattern of mathematical models was performed and FII formulation follows zero order kinetics with better release mechanism and Higuchi model with higher correlation and it shows diffusion mechanism.

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