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# Formulation and Evaluation of Egg Albumin Based Delayed Release Microspheres of Itraconazole

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## ABSTRACT

The present study deals with the formulation and evaluation of egg albumin based delayed release microspheres of itraconazole, which is drug of choice for systemic fungal infections. The study was undertaken in order to achieve the best possible drug-polymer (egg albumin) ratio to get delayed drug release using natural biodegradable polymer. The preparation of microsphere was done by using heat denaturation technique. The prepared microspheres were evaluated on various parameters like the size, morphology, micromeritic properties, percent drug entrapment, in-vitro dissolution studies and the scanning electron microscopy (SEM).The in vitro dissolution studies were carried out for 10 hrs. at pH 6.8 which showed that the drug release for the batch EA4 (drug: polymer ratio – 1:4) was 87.02 ± 5.89 after 10 hrs. The hypothesis of this research work i.e delayed drug release using natural biodegradable polymer was well supported by results of its evaluation.

**Keywords:** Itraconazole, egg albumin, delayed drug delivery systems, heat denaturation method.

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# INTRODUCTION

Over the past few decades there has been a great stress on the development of oral delayed release dosage forms. The reason for the stress on oral delayed release dosage forms has been due to the ease of administration, patient compliance and the flexibility in drug administration and ease of manufacturing. In this regard, the researchers in past have emphasized more on microspheric drug delivery systems. Microspheres are characteristically free flowing particles made from natural/synthetic polymer and ideally they have a particle size less than 200µm[1-3]. There has been an extensive study on the microspheres as a drug delivery system to protect the sensitive macromolecules from acidic and enzymatic degradation and also to allow delayed release and tissue targeting. The microspheric drug delivery systems by the virtue of their controlled drug release help in reducing the administration frequency of the drug to the patient and also helpful in delayed release of less bioavailable drugs. Itraconazole was invented in the year 1984; it is a triazole antifungal agent which is used for the treatment of fungal infections. It is a highly selective inhibitor acting via inhibiting the cytochrome P-450 dependent enzymes; resulting in the impairment of ergosterol synthesis which is an essential component of the fungal cell membrane. Inhibition of ergosterol synthesis that in turn results in the increased cellular permeability causing leakage of cellular contents.[4] Its oral bioavailability is 55% with a half-life of 21 hrs[5], so due to its bioavailability problems there is a need for the development of drug delivery system that is convenient for patient and improves the bioavailability along with extended release of drug in the body. The use of egg albumin in formulations is due to the fact that it is a naturally obtained polymer and is biodegradable in nature with good aqueous solubility. As well as it has a property of protein binding and physical entrapment. It also supports passive as well as facilitated release of various types of incorporated drugs from the polymer matrix[6]. So the concept for the formulation of egg albumin based microspheres of itraconazole was devised to increase the bioavailability of the drug to get a sustained release of drug, resulting in decrease in the dosing frequency.

# MATERIALS AND METHODS

## Materials

Itraconazole was obtained as gift sample from Ahlcon Parentrals (India) Ltd., Bhiwadi, Rajasthan. Egg albumin was obtained from the hen's egg white. Empty gelatin capsule shells were obtained as gift sample from Aimil pharmaceuticals.

## Evaluation of drug and the drug-excipient compatibility studies

Drug identification was done by physical analysis, melting point, solubility and by spectral analysis.

The evaluation of drug excipient compatibility was carried out by using Infrared spectroscopy on the physical mixture prepared by mixing equivalent amount of drug and polymer.

IR spectra of pure drug itraconazole, polymer (egg albumin), complex of drug and polymer were obtained in the region 4000-600 $cm^{-1}$ with the help of Bruker IR spectrophotometer (Alpha)using ATR technique. (Figure 1 to Figure 3)

The UV spectrum of the pure drug was obtained by scanning the methanolic solution (5ml) of itraconazole in the wavelength range of 700-200 nm, using Shimadzu UV-1800 spectrophotometer. (Figure 4)

Melting point of the drug obtained was determined using 0.2mm capillary sealed at one end and melting point apparatus. The FTIR and the UV spectra obtained were compared with the reference spectra obtained from Japanese Pharmacopoeia, 16$^{th}$ edition.

**Preparation of microspheres**

Microspheres were prepared by using heat denaturation method; in this method egg white containing egg albumin equivalent to 500 mg, 1000 mg, 1500 mg, and 2000 mg were studied for their effectivity. The four different formulations were studied using 1:1, 1:2, 1:3 and 1:4 drug is to polymer ratio. Drug was added to the egg white to prepare a tick paste and then water was added to dilute the solution up to 50 ml. The above content was then added in a stream to the non-aqueous phase prepared by mixing 0.5 ml span 80 as emulsifying agent in 50 ml liquid paraffin and then stirred for 10 min.; to prepare primary emulsion. The primary emulsion formed was then added drop wise to 100 ml of preheated ($80^0$C) liquid paraffin to form a secondary emulsion which was stirred for 1 hr. The temperature of the secondary emulsion was then reduced to $40^0$C by cooling for the hardening process and maintained for 25 minutes. After 25 minutes, the emulsion was stabilized with glutaraldehyde solution (25% v/v) (0.5ml) for 15 min. Separation was done in order to separate liquid phase from the solid phase. Then the resulting solid phase was washed several times with n-hexane and then dried at 40±2 $^0$C for 72 hours in the hot air oven.

**Table 1: Composition of Microspheres**

| S. No. | Formulation Code | Drug | Egg albumin (Polymer) | Drug:Polymer ratio |
|---|---|---|---|---|
| 1. | EA1 | 500 mg. | 500 mg. | 1:1 |
| 2. | EA2 | 500 mg. | 1000mg. | 1:2 |
| 3. | EA3 | 500 mg. | 1500 mg. | 1:3 |
| 4. | EA4 | 500 mg. | 2000mg. | 1:4 |

## EVALUATION OF MICROSPHERES

### Micromeritic properties of microspheres[7]

The prepared microspheres were evaluated for micromeritic properties like bulk density, tapped density, Carr's index, Hauser's ratio and angle of repose. (Table 2)

**Table 2: Micromeritic properties of microspheres**

| Sr no. | Property | EA1 | | EA2 | | EA3 | | EA4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Observation | Inference | Observation | Inference | Observation | Inference | Observation | Inference |
| 1 | Bulk density | 0.31 $g/cm^3$ | ----- | 0.28 $g/cm^3$ | ----- | 0.33 $g/cm^3$ | ----- | 0.3 $g/cm^3$ | ----- |
| 2 | Tapped density | 0.41 $g/cm^3$ | ----- | 0.35$g/cm^3$ | ----- | 0.43 $g/cm^3$ | ----- | 0.37 $g/cm^3$ | ----- |
| 3 | Hausner's ratio | 1.2 | fair | 1.25 | fair | 1.3 | passable | 1.2 | fair |
| 4 | Carr's index | 22.5% | passable | 20% | fair | 23.2% | passable | 18.9% | fair |
| 5 | Angle of repose | $31.42^O$ | good flow | $38.04^0$ | fair | $33.69^0$ | good flow | $35.9^0$ | fair |

### Apparent bulk density

Bulk density was determined by measuring the volume of 30 g of powder sample taken in a 100 ml graduated cylinder and then tapped it on a hard surface 3times from a height of 1 inch using a tapped density apparatus.

$$U = M/V_b$$

Where,

M = Mass of microspheres (g)

$V_b$ = volume of microspheres (after three tapping)

### Tapped density

Tapped density was determined by mechanically tapping a 100 ml measuring cylinder containing the 30 g powder sample. After observing the initial volume, the cylinder was tapped 100 times from a height of 1inch on a hard surface using a tapped density apparatus.

$$b = m/v_t$$

Where, m = mass of microspheres (g)

$V_t$= volume of microspheres (final tapped volume)

### Carr's Index

The Carr index(Carr's Compressibility Index) is an indication of the compressibility of a powder.

It is named after the pharmacologist Charles Jelleff Carr(1910–2005). The Carr's Index is calculated by the formula

$$\text{Carr}'\text{s Index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \text{X100}$$

**Hausner's ratio**

The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. It is named after the engineer Henry H. Hausner (1900–1995). The Hausner ratio is calculated by the formula

$$\textit{H}\text{ausner}'\text{s ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

**Angle of repose**

Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles. The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of material formed while falling from the funnel.

$$\theta = \text{Tan}^{-1}(h/r)$$

Where,

θ = Angle of Repose

h= height of the heap

r= radius of the heap formed

From the study of micromeritic properties of the microspheres, conclusion was drawn regarding the nature of the flow of the microspheres prepared.

**Particle size analysis of microspheres**[8]

The particle size analysis was done with the help of optical microscope using calibrated ocular micrometer. The mean particle size was calculated by measuring the diameter of 50 particles. The average particle size was determined using Edmondson's equation. (Table 3)

$$D = \sum nd / \sum n$$

Where,

n =number of microspheres

d= mean of the size range

D = average particle size (in µm)

From the particle size analysis it was inferred that the microspheres were uniform in size and the size of the microspheres increased with the increase in the polymer concentration.

**Drug entrapment efficiency of microspheres**[9]

For the drug entrapment efficiency of microspheres, 50 mg of microspheres were accurately weighed and dissolved in 50 ml of methanol in a volumetric flask to get a solution containing one mg drug per ml. The resulting solution was filtered through whatman filter paper and then suitably diluted to check for the absorbance on the UV spectrophotometer. The absorbance was measured at 262 nm using UV spectrophotometer (Table 4).

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical weight of the drug}} \text{ X } 100$$

From the percentage drug efficiency study it was inferred that the entrapment of the drug in the microspheres was variable and it was changed with the change in the polymer concentration. It was also observed that in the batches EA3 and EA4 least amount of drug was wasted as most of it was entrapped.

**In vitro dissolution studies of microspheres**

The in vitro release of drug from the micro particles filled in enteric coated gelatin capsules was carried out in basket type dissolution apparatus for all the batches. In the dissolution test the micro particles were firstly subjected to a pH 1.2 buffer for 2 hours and then to a pH 6.8 for next 10 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (100 rpm) and the temperature was maintained at $37\pm0.5^0$C. After a time interval of 1 hr. sample were withdrawn and replaced with fresh media immediately after sampling. The samples withdrawn were analyzed for the drug content by scanning the sample at 262 nm using UV spectrophotometer (Shimadzu UV1800). (Table 5)

From the in-vitro dissolution studies it was inferred that the release of the drug from the microsphere was for a period of 10 hours and it was almost constant throughout that release period.

From all the evaluations carried out i.e micromeritic properties, particle size analysis, drug entrapment efficiency and the in vitro dissolution studies the microspheres of batch EA4 were selected for the scanning electron microscopy (SEM).

**Scanning electron microscopy of microspheres of formulation EA4**[10]

Scanning electron microscopy has been widely used to determine the particle size distribution, texture and the surface morphology of the microspheres. SEM of microspheres was performed at NIPER, Mohali. (Figure 5).The SEM photographs showed that the microspheres formed were spherical in shape with depression marks on its surface.

## RESULTS AND DISCUSSIONS

Four batches with different drug is to polymer ratios (itraconazole : egg albumin: 1:1, 1:2, 1:3, & 1:4) were prepared and evaluated using various analytical techniques like FTIR, SEM, particle size, percent drug encapsulation efficiency and in vitro dissolution studies.

The FTIR spectral studies conducted for the drug excipient interaction study revealed that there was no interaction between the drug and the polymer (egg albumin) as no significant shift was observed in the principle peaks (fingerprint region) of itraconazole (figure 1) and even the comparative spectrum (figure 3) obtained by using the spectrum evaluation feature of the OPUS software; provided along with the instrument, has shown a correlation of 100% against a threshold of 95% when the spectra of drug-polymer mixture, pure drug and the pure polymer were compared; which evidently supported that there is no interaction of drug and the polymer.

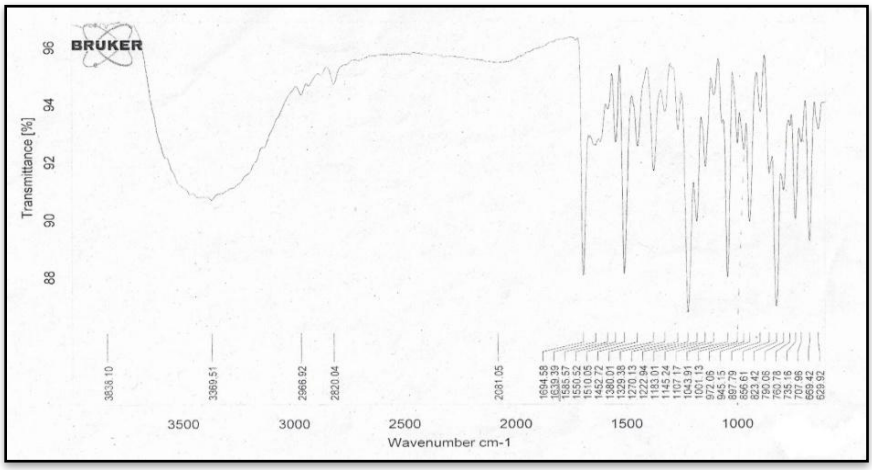



**Figure 1: FTIR of itraconazole**

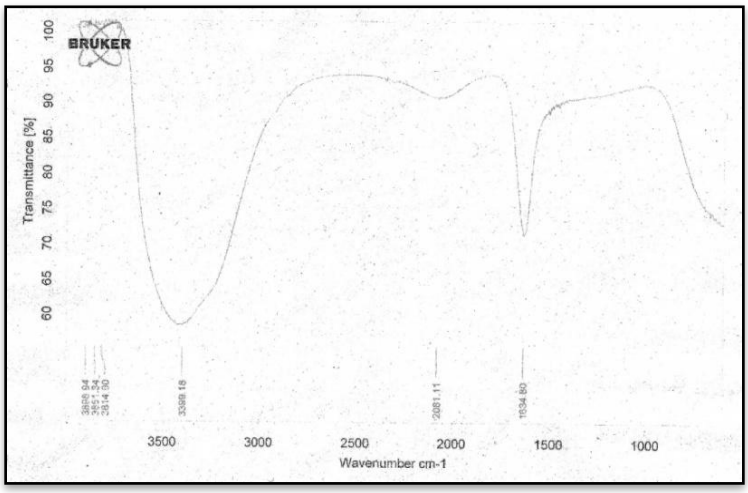



**Figure 2: FTIR of egg albumin**

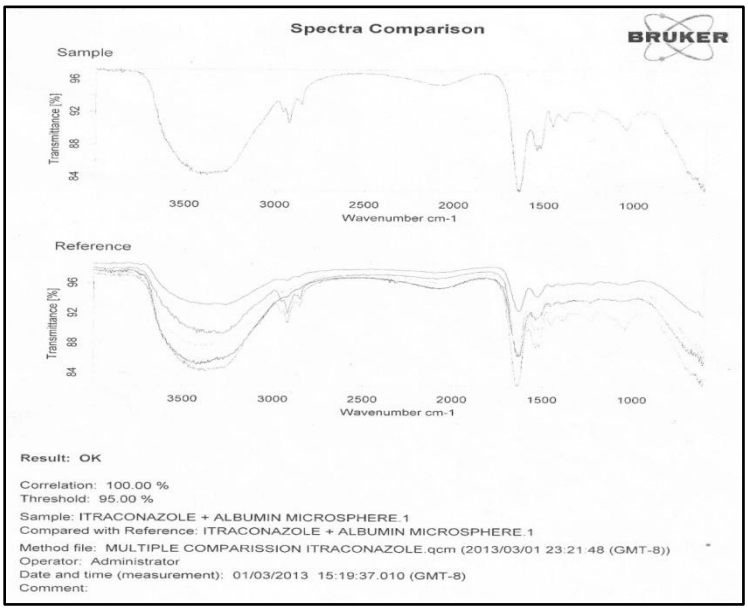



**Figure 3: FTIR Multiple Comparison Spectra**

(The FTIR Comparison spectra of the itraconazole loaded egg albumin with itraconazole shows a correlation of 100% against a threshold of 95%.)

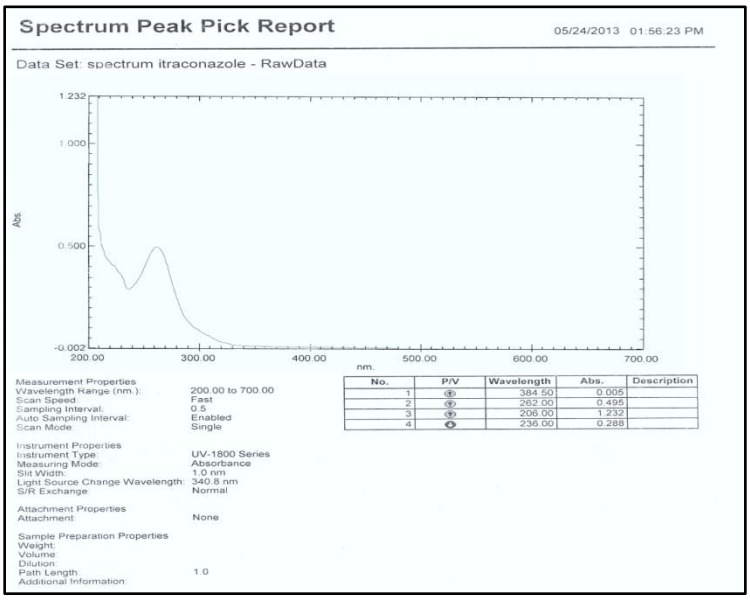
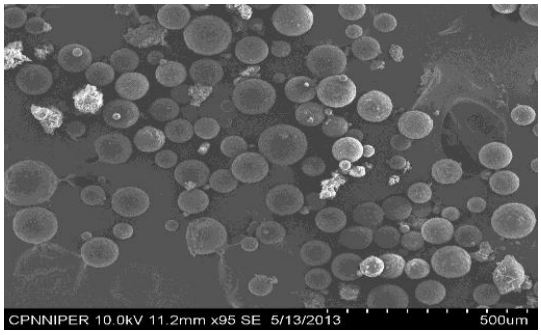


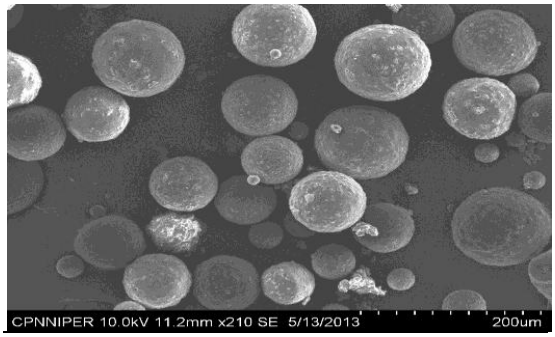

**Figure 4: UV Spectrum of itraconazole in methanol**

The micromeritic studies carried out and the results of the carr's index, hausner's ratio as well as angle of repose of the EA4 batch were 18.9%, 1.2 and $35.9^0$ respectively, which were better than the results of other three batches i.e EA1, EA2, EA3. The results of the batch EA4 were found to be in the acceptable range (table 2) and indicate that the batch was having fair flow properties.

The SEM photographs showed that the microspheres were spherical in shape (figure 5a and 5b) with depression marks on its surface (figure 5c).




(a)




(b)


(c)

**Figure 5: SEM images of itraconazole loaded microspheres of formulation EA4.**

The percent drug efficiency of the all the experimental batches were found to be in the range 38.6 ± 0.95% to 87.15 ± 0.75% (Table 4). The in vitro dissolution studies were conducted to check for the rate and time of release of drug (Table 5). From the data obtained it could be inferred that the release of the drug from the microspheres were almost uniform and the release was found to be 82.85 ± 2.46 and 87.02±5.89% for EA3 and EA4 respectively over a period of 10 hrs.

**Table 3: Particle size analysis**

| S. No. | Formulation Code | Average Particle size (µm) |
|---|---|---|
| 1 | EA1 | 104.49± 2.24 |
| 2 | EA2 | 110.9 ± 1.29 |
| 3 | EA3 | 130.1± 1.31 |
| 4 | EA4 | 136.5± 1.29 |

**Table 4: Percent drug entrapment efficiency**

| S. No. | Formulation Code | Drug Entrapment Efficiency (%)±SD |
|---|---|---|
| 1. | EA-1 | 38.6 ± 0.95 |
| 2. | EA-2 | 50.7 ± 1.00 |
| 3. | EA-3 | 83.9 ± 0.55 |
| 4. | EA-4 | 87.1 ± 0.75 |

**Table 5: In-vitro dissolution study**

| S. No. | Formulation code | In-vitro release (%)±SD |
|---|---|---|
| 1. | EA1 | 93.74 ± 3.05 |
| 2. | EA2 | 90.01 ± 2.13 |
| 3. | EA3 | 82.85 ± 2.46 |
| 4. | EA4 | 87.02 ± 5.89 |

## CONCLUSION

The egg albumin based microspheres of itraconazole were prepared by heat denaturation method in order to improve the oral bioavailability with prolonged drug release. The concentration of the polymer influenced the particle size as well as the in vitro release. The in vitro release studies showed that the drug release was prolonged for more than 10 hrs. So from this it could be concluded that the prepared microspheres can be used for the controlled drug delivery of the drug for a prolonged period.

## FUTURE PROSPECTS

The current work establishes egg albumin as a polymer which could be used to enhance the bioavailability of the drugs with poor bioavailability. There is a need for more intense studies to be conducted on egg albumin to develop products for the common public with better bioavailability and less toxic effects and can be used as a natural biodegradable alternative to the synthetic polymers.

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