ARTICLE INFO

ABSTRACT

In previous few decades, many research study have been carried out related to the drug delivery system (DDS) in the human physiology to achieve the most appropriate bio-availability in desirable range within the therapeutic window to get quick onset of action. In conventional era, we saw most prescribed mode of medication were oral. As compared to the present situation, drug are prescribed as per convenience of the patient which is most orally administration. Thus, above all reliable advantages, oral formulations have been reported in earlier decline in reaching the MSC (Maximum Safe Concentration). Thus, to reduce concentration problems, a novel DDS has been designed as microsphres (MS), which can be loaded to preferable formulation for administration.

Itraconazole Microspheres (ITCZ-MS) are the triazole, a class of anti-fungal drug which has 99.8% PPB. In market, generally the prescribed dosage forms are oral with 200mg dose for acute infection and 400mg dose for the chronic infections.

ITCZ MS were made by encapsulation of the API within the polymeric film separately and this was further transferred to another aqueous system with help of a needle syringe drop wise at 40°C at 100 rpm. Varying polymeric concentration formulation of ITCZ MS were made, collected and washed followed by air drying for 24 hrs and evaluated by SEM, XRD, FTIR, entrapment efficiency etc.
INTRODUCTION:
In the present times now and pandemic situation, the prevalence of the infection have attained the rapid on growth. ITCZ is class of antifungal drug which is hydrophobic in nature. The most preferred route of administration is oral route which helps the patient to maintain the patient-compliance. Since the employed API lipophilic, it will also help in improving the drug solubility. Generally the prescribed dosage forms are oral with 200mg dose for acute infection and 400mg dose for the chronic infections. Thus, to reduce concentration problems, a novel DDS has been designed called as MS which can be loaded to preferable formulation for administration. The aim of the research is to maintain drug concentration in the plasma for longer activity of the drug. When the formulation are administered at regular intervals, they may cause dose-dumping which often leads to serious toxicity. To reduce such toxicity related problems, the development of the MSs loaded drug delivery systems will reduce the dosing criteria and the subsequent loaded formulation will be enabled to prolonged action. In this study, lipophilic drug will be encapsulated by the polymeric solution mixture under miscible solvent system to generate the immiscible system which upon being added drop wise to a separate surfactant mixture will cause the formation of the MS. The resultant product was observed for its characteristic properties by performing evaluation studies. (Kumar N. et. al. 2008).

MATERIALS
ITCZ was a sample product from Theon Pharmaceuticals Ltd. Baddi, Himanchal Pradesh, India. Polymer, calcium carbonate, calcium chloride, glacial acetic acid other excipients were of analytical grade and used as received.

METHOD
Preparation of ITCZ MS
The ITCZMSs were prepared by performing double emulsification Solvent evaporation method. The polymeric solution was prepared by dissolving in the solvent under continuous stirring followed by adding the API and drug carrier agent which was added manually drop wise to 1% W/V calcium chloride solution containing 10% W/V glacial acetic acid. The drops added to the aqueous phase were kept dispersed until their complete formation to avoid accumulation of the droplets. The droplets were kept dispersed for 1 hours. Then, they were filtered using Whatmann filter paper and dried for 24 hrs under hot air oven until uniform weight (Kanamura K, Makino K et. al. 2008).

<table>
<thead>
<tr>
<th>Trail No.</th>
<th>Formulation No.</th>
<th>Polymer (g)</th>
<th>Drug (g)</th>
<th>CaCO₃ (g)</th>
<th>CaCl₂ (g)</th>
<th>Aqueous phase containing 10% glacial acetic acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>0.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>0.7</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>1.0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>1.5</td>
<td>0.3</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>1.7</td>
<td>0.7</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>2.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>
CHARACTERIZATION STUDIES

Assay of the drug-
The assay of the drug is necessary to be primarily evaluated which help to confirm the purity of the drug after receiving from warehouse.

The drug was dissolve in a solvent which was further used to make the stock solution. The stock solution was pipetted out for different concentration in volumetric flask and marked up to 10mL volume. The absorbance was recorded in UV- spectrophotometer at 265nm using the solvent as reference solvent.

Particle size –The MS were formulated and were evaluated for 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 for 15 min. After shaking completes, the samples were collected and weighted and particle size was calculated by the formula-

\[
\text{Mean particle size} = \frac{\sum nd}{\sum n}
\]

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

% Yield – The percentage yield may be defined as the ratio of the total amount of the MSs formulated to the total amount of the drug and excipients employed. % yield can be calculated by the formula-

\[
\% \text{ Yield} = \frac{\text{Total amount of MS prepared}}{\text{Total amount of drug and excipients}} \times 100
\]

1. Drug Encapsulation Efficiency-
Encapsulation was determined by conventional method. The MSs 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 for 12h. Then the solution was passed through whatsmann filter paper to collect the filtrate. The filtrate was analysed under UV-spectrophotometer with 265nm with respect to blank 0.1N HCL.

\[
\% \text{ EE} = \frac{\text{Weight of drug in MSs}}{\text{Weight of drug fed}} \times 100
\]

2. Micromeritic Properties of MSs-The MS are formulated and further evaluated for their micro properties such as bulk density, tapped density, angle of repose and compressibility index.
**Bulk Density**—The bulk density was calculated by the ratio of the weighted MSs to the untapped volume of the sample by the formula:

\[
\text{Bulk density} = \frac{M}{V}
\]

Where; \(M\) = weight of untapped MS, \(V\) = apparent volume.

**Tapped Density**—The evaluating samples of the MS are manually tapped in the graduated measuring cylinder. The tapped density is defined as the ratio of the weight of the MSs to the final volume obtained after tapping.

\[
\text{Tapped density} = \frac{M}{V_T}
\]

Where; \(M\) = weight of MSs, \(V_T\) = Tapped Volume.

**Carr’s Compressibility Index**—The compressibility index may be defined as the tendency of the powder sample to be compress or simply as the ability to compress which may be calculated by the following formula:

\[
\text{C.I.} = \frac{\text{TD} - \text{BD} \times 100}{\text{TD}}
\]

Where; \(\text{TD}\) = Tapped density, \(\text{BD}\) = Bulk density.

**Angle of Repose**—The maximum angle is measured possibly between the surface of the pile and the horizontal plane which was performed with the help of the funnel.

\[
\theta = \tan^{-1}(h/r)
\]

Where; \(h\) = height of heap of pile, \(r\) = radius of the base of the pile.

**Morphological Characteristic of ITCZ-MS**

**Scanning Electron Microscopy (SEM)**

ITCZ-MS were evaluated for the size and surface morphology by SEM at 250x magnification. The image so obtained are presented below to confirm the spherical structure and size of the MSs and their morphology.

**Drug Polymer Compatibility studies**

- **X-ray Diffraction Analysis**

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

- **FTIR- Analysis**

The chemical interaction between the drug and polymer was checked by performing FTIR spectroscopy. The blank and drug loaded formulations were checked for any possible interaction in the frequency range of 4000-400 cm\(^{-1}\).

**In-Vitro drug Dissolution studies**

The drug release profile of the ITCZ-MS was determined by using dissolution apparatus fixed with USP type-2 paddle apparatus rotating at 50rpm filled with 900mL 0.1N HCL as dissolution media. Accurately weighted 100mg of MSs were used for this study. The temperature was set to 37±0.5°C. Then 1mL aliquots were withdrawn at different time periods as 0, 5, 10, 15, 20, 25, 30 h and simultaneously replacing with the same amount of fresh solvent. The spectrum was recorded using UV-Spectrophotometer at 265nm.

**In-Vitro Drug Release Kinetics**

- **Higuchi Model**

This model is used to describe the drug which are water soluble or water insoluble from the solid or semisolid matrix system. The model had mathematical expression which give the release kinetics as follows:

\[
\text{Qt} = \text{KH} \times t^{1/2}
\]

Where; \(\text{Qt}\) = amount of drug release in time \(t\), \(\text{KH}\) = Higuchi Dissolution Constant.

The data obtained from the drug release kinetics from this model is plotted against CDS V/s square root of time.

- **Baker Lonsdale Model**

This model is used to linearization of drug release kinetics especially from the spherical formulation.
such as MS or microcapsules. The mathematical equation for this model is given as-
\[ Kt = \frac{3}{2} \left[ 1 - \left(1 - \frac{Mt}{M\infty} \right)^{\frac{2}{3}} \right] - \frac{Mt}{M\infty} \]

Where; Mt = amount of drug release in time t, M∞ = drug release at infinite time and K is release constant

RESULT AND DISCUSSION

Assay of pure ITCZ
The purity of the drug sample obtained from the warehouse were tested immediately after receiving.

The stock solution was prepared in methanol and different dilutions as 2, 4, 6, 8, 10 µg/mL were made under experimental conditions all throughout. The dilution were checked for absorbance in Shimadzu 1700 UV- Spectrophotometer at 265nm. The results obtained are as follows-

Linearity Data of ITCZ

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.232</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.388</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.481</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.652</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.832</td>
</tr>
</tbody>
</table>

(Fig. 1A: Linearity graph of ITCZ)

\[ y = 0.0732x + 0.0778 \]
\[ R^2 = 0.9895 \]

% of 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, the MSs formed lumps and in other formulation the yield increased as the concentration of polymer was increased. The particle size was seen in increasing order and it was not seen in F1 with few floating polymer drops without formation. This was due to low concentration of Sodium Alginate. On further increasing the amount of sodium alginate, the particle size as well as %yield was found to be increase in F3, F4 and F5 as shown in Table 2. The particle size was found most spherical and stable in F6 because of polymer which aided in shape and size.

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),the entrapment was found to be the highest in F6. Further to see entrapment, it was found to decrease in F6 which was 86.99±7.33 as shown in
Table 2. This was due to reaching the saturation of the MSs with the drug.

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

**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. The 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 3.

<table>
<thead>
<tr>
<th>Form No.</th>
<th>Bulk Density</th>
<th>Tapped Density</th>
<th>Angle of Repose</th>
<th>Carr’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>F3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>0.77±0.01</td>
<td>0.86±0.01</td>
<td>20.17</td>
<td>11.12</td>
</tr>
<tr>
<td>F5</td>
<td>0.73±0.04</td>
<td>0.81±0.03</td>
<td>26.12</td>
<td>15.26</td>
</tr>
<tr>
<td>F6</td>
<td>0.69±0.03</td>
<td>0.71±0.05</td>
<td>27.10</td>
<td>20.50</td>
</tr>
</tbody>
</table>

**Morphological Characteristic- Scanning Electron Microscopy**

The size and shape of the ITCZ-MS were determined by the SEM. The size was found to be spherical with little roughness without any porous deformity in the morphology. The agglomeration was not seen because of rigidity. The images obtained from the SEM is shown in fig.2.
Drug- polymer Compatibility Studies

XRD Analysis

The XRD study was done to check the crystallinity of optimized formulation was recorded by using X-Ray Diffractometer. The diffract gram give the intensities of the constituents of the formulation which confirms that ITCZ gave intense peak which indicated crystalline nature of the drug. The diffract gram of ITCZ loaded MSs show diminished peaks indicating that matrix may have undergone amorphization during the formulation. The study confirms that ITCZ was compatible with other excipients and did not cause any change to formulation. The results are shown in fig.3.

FTIR study

The spectra for FTIR was recorded to assess the compatibility of the pure drug with other excipients present in the formulation. The FTIR spectrum of the optimized formulation F6 was recorded separately and compared the compatibility of the pure drug and excipients between characteristic peaks. The main of ITCZ was obtained at 1510 cm\(^{-1}\) C-H phenyl in plane, 1800 cm\(^{-1}\) C=O carbonyl stretching and 762 cm\(^{-1}\) C-H phenyl out- plane was present in the drug spectra but absent in blank formulations because drug was absent in the blank formulations and the absorption spectra exhibited all the characteristics similar to drug without any prominent changes in the formulations and the FTIR spectra shows that the drug has not gone any possible changes as well as other constituents during the development of the formulation. The FTIR spectrum is shown in fig 4.
In-vitro drug release

The dissolution media was freshly prepared just before use. To study the drug release, (Shimadzu, Japan) apparatus was used. The optimized sample F6 was tested for drug release for 1h at different time interval. It was found that nearly 90% of drug was released by 60 min in control release formulation and gave high bioavailability.

In-Vitro drug release kinetics

The data obtained were fitted to the mathematical model to check drug release from the optimize formulation (F6). The drug release kinetics model used were Higuchi model and Baker Lonsdale model.

The R² value for Baker Lonsdale model was 0.976 which was near to 1 than in higuchi model where R² was 0.871.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Higuchi model R² value</th>
<th>Baker Lonsdale model R² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8</td>
<td>0.871</td>
<td>0.976</td>
</tr>
</tbody>
</table>

(Fig 4. FT-IR spectrum of Sodium alginate, CaCl₂, CaCO₃, Blank formulation and optimized microsphere F6).
CONCLUSION

The study suggested that the sodium alginate blended ITCZ-MS was formulated successfully by using double emulsification solvent evaporation method. Upon generating various formulation, F8 was optimized amongst to check the various parameters such as % yield, % entrapment efficiency and micromeritic properties. The % yield and % EE was 94%, 86% respectively. The various micromeritic properties was found within range and indicating good flow properties of the powder. The morphological characteristics denoted spherical shape and in range size of the MSs. The compatibility studies showed that drug and polymer and other constituents did not interacted to each other which aided in smooth processing. 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


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