Formulation Development and In Vitro Release Studies of Tenofovir-containing Microsponges

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Abstract:

Background. Microsponges are intended to carry drugs with minimum dose, while delivering beneficial effects such as better drug stability and reduced adverse effects in recipients. The present investigation was aimed to develop and characterize microsponges containing tenofovir (TNF).

Methods. Quasi-emulsion diffusion technique was used to prepare TNF-containing microsponges. Eudragit L-100 was used as a polymer, whereas glycerol and dibutylphthalates were used as plasticizers. Drug and polymer were experimented in five formulations (F1-F5) of different ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5. They were characterized for various physical parameters such as size, crystallinity, and interactions.

Results. Fourier-transform infrared spectroscopic results showed that there was no incompatibility between the drug and excipients. Drug entrapment efficacy was found between 47% and 67%, and the particle size ranges were between 4.52 µm and 8.98 µm. Cumulative drug diffusion of formulation F2 (drug:polymer ratio = 1:2) was found to be 84.15% in 180 min for an X-ray diffraction studies of pure drug and microsponges formulation clearly indicated the reduction in the crystallinity of the drug which could be the reason for the improved solubility of the drug. Scanning electron microscopy analysis results indicated that the formulations have excellent structure and almost all formulations exhibited in spherical shape.

Conclusion. Based on all the evaluation parameters, formulation F2 was concluded as the best formulation, and it is an alternative approach for conventional therapy with better patient compliance.

Keywords: Microsponges, EudragitL-100, Cumulative drug release, X-ray diffraction studies, Scanning electron microscopy

1 Introduction

Tenofovir (TNF) is an antiviral drug that inhibits the viral reverse transcriptase enzyme, and thus, it inhibits the replication of retrovirus. The bioavailability of TNF is only 25% and this could be caused by its low permeability. TNF is classified as a Class III drug according to the biopharmaceutical classification system [1].

Microsponges are a type of drug delivery systems that are microscopic, polymeric, and sponge-like. The microsponges are non-collapsible micropores with large porous surfaces that are mostly used for extended topical modified drug releases. The sizes of microsponges can range from 0.007 µm to 0.2 µm through which the bacteria cannot penetrate the tunnel of microsponges [2-4]. Microsponges are designed to deliver the dose efficiently, enhance
drug stability, reduce the side effects in recipients and improve patient compliance. Various active ingredients such as substances that are antifungal, anti-infective, and anti-inflammatory, as well as essential oils, emollients, fragrances, sunscreens agents can be entrapped in microsponges. These porous microspheres can be used in various formulations such as gels, ointments, lotions, creams, and powders [5].

The concept of microsponges for drug delivery was introduced by Won in 1987 and patented by advance polymer systems Inc. [6]. They have developed a wide variety of microsponges and used them in cosmetics, over-the-counter drugs, and prescription drugs. A minimum dose can be efficiently delivered by the microsponges. Some of the other applications of microsponges include enhanced stability, reduced side effects, and tailor-made release profiles [7-9].

2 Materials and methods

2.1 Materials

TNF sample was gifted by Sai Mirra Innopharm Limited, Chennai, and other required chemicals were procured from different sources as listed in the following. Eudragit L-100, glycerol, dichloromethane, and polyvinyl alcohol were obtained from Qualikam, Nagpur. Sodium alginate was obtained from Essel Lab, while dichloromethane (DCM) was from Finar reagents. All other excipients used are of analytical grade.

2.2 Characterization of drug

2.2.1 Melting point

Using a melting point apparatus (SMP10/1, Stuart, UK), melting point of TNF was noted. The sample was introduced into a capillary tube in which one end was closed. This capillary was then inserted into a bath of silicone oil and heated in a controlled manner with the help of an electric heating coil.

2.2.2 Construction of calibration curve

TNF was dissolved in 0.1 N hydrochloric acid (HCl) with pH 1.2 by keeping the concentration range from 2 to 20 μg/ml. It was analyzed by ultraviolet-visible spectrophotometer (Shimadzu, Japan) at 276 nm for absorption.

2.2.3 Drug-excipient compatibility study

Using KBr press, Fourier-transform infrared spectroscopic (FTIR) spectra were recorded using FTIR 8400S spectrophotometer (Shimadzu, Japan) from wave number 4000 to 400 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. Samples were compressed as pellets using hydraulic press by applying 5 tons of pressure for 5 min and were kept under the path of light, and spectra were recorded.

2.3 Preparation of TNF-containing microsponges using a quasiemulsification-diffusion method

Microsponges were prepared using the emulsification and evaporation method [10]. The internal phase of the emulsion of formulations F1, F2, and F3 consists of TNF, Eudragit L-100, DCM, and glycerol, whereas the internal phase of formulations F4 and F5 consists of drug, Eudragit L-100, dibutylphthalate, and DCM. The individual formulation was sonicated at 35°C for 15 min. The external phases for formulations F1, F2, and F3 as well as F4 and F5 were prepared using polyvinyl alcohol and sodium alginate, respectively. The solvent (external phase) used was water in all the formulations. The internal phase was slowly added to the external phase with continuous mixing. The final solution obtained was homogenized for 1 – 4 h, filtered and dried at room temperature for 24 h to collect the microsponges. Glycerol and dibutylphthalate were used as plasticizers. Table 1 shows the composition of the five formulations of TNF-containing microsponges and Figure 1 shows the steps involved in their preparation.

Table 1. Composition of tenofovi-containing microsponges

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Tenofovir (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Eudragit L (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Dichloromethane (ml)</td>
<td>30</td>
</tr>
<tr>
<td>Dibutylphthalate (ml)</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol (ml)</td>
<td>1</td>
</tr>
<tr>
<td>Sodium alginate (gm)</td>
<td>0.5</td>
</tr>
<tr>
<td>Polyvinyl alcohol (gm)</td>
<td>-</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>100</td>
</tr>
</tbody>
</table>
2.4 Characterization of prepared formulations

2.4.1 Visual inspection

The organoleptic properties including color, texture, consistency, and homogeneity of microsponges were examined by visual observation.

2.4.2 Production yield

The production yield of TNF microsponges was determined by the formula below [11].

\[
\text{Production yield (PY)} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass (Polymer+Drug)}} \times 100
\]

2.4.3 Encapsulation efficiency

The TNF-containing microsponges were weighed, and 100 mg of the microsponges were placed in 100 ml of 0.1 N HCl solution (pH 1.2) for 12 h while stirring continuously. Then, the samples were filtered using 0.45-µm membrane filter and analyzed at 260 nm with an UV-visible spectrophotometer (Lab India Instruments, India). Encapsulation efficiency was calculated by the following equations:

\[
\text{Actual drug content (\%)} = \frac{M_{\text{act}}}{M_{\text{ms}}} \times 100
\]

\[
\text{Encapsulation efficiency} = \frac{M_{\text{act}}}{M_{\text{the}}} \times 100
\]

where \(M_{\text{act}}\) = actual drug content in microsponges, \(M_{\text{ms}}\) = mass of microsponges and \(M_{\text{the}}\) = theoretical drug amount in microsponges.

2.4.4 In vitro drug release

In vitro release studies were carried out using a 0.45-µm cellophane membrane which had been pre-soaked in the dissolution medium. These cellophane membranes were placed in between

Figure 1. Flow chart for the preparation of microsponges. DCM, dichloromethane; PVA, polyvinyl alcohol.
the donor and acceptor compartments of the Franz diffusion cells. Franz diffusion cell with 15-ml receptor compartment and 2.84-cm$^2$ successful diffusion areas were used for this study. The \textit{in vitro} release of all formulations was studied using Franz diffusion cell system which was maintained at 37 ± 1°C under constant stirring. The 1-ml volume of aliquots was withdrawn at specific time intervals (15, 30, 45, 60, 90, 120, 150, and 180 min) by maintaining sink conditions. These aliquots were then diluted using receptor medium and analyzed by UV spectrophotometer (Lab India Instruments, India) at 260 nm. In this experiment, the release kinetics was analyzed using mathematical models.

2.4.5 Determination of morphology and structural surface topology

For the determination of morphology and structural surface topology, TNF-containing microsponges were examined at 5 kV using Scanning Electron Microscope (SEM) S-3700N (Hitachi, Japan).

2.4.6 X-ray diffraction study

X-ray diffraction patterns were recorded using the X-ray diffractometer Model D5000 (Siemens, Germany) which runs at 5 – 10°/min in terms of 2θ. The instrument was operated at 45 mV voltage and 20 A current.

2.4.7 Particle size analysis

Particle size analysis was carried out using a microscope (eyepiece and stage micrometers). The eyepiece micrometer is calibrated using the stage micrometer, and the formula for calibration is given below:

\[
\text{Number of stage micrometer division} = \frac{\text{Number of eyepiece division}}{\text{Least count}} \times \text{Number of eyepiece division}
\]

2.4.8 Drug content

Accurately weighed 50 mg of TNF-containing microsponges were transferred to a 50-ml volumetric flask. A small quantity of 0.1N HCl was then added to the flask which was shaken well to dissolve the drug completely. Finally, 0.1 N HCl was added to make up the volume to 50 ml. To know the drug content, the solution is further diluted, and the final solution was filtered using a membrane filter with 0.45-µm pore size, and the absorbance was taken at 260 nm using UV-visible spectrophotometer.

2.4.9 Kinetic modeling

The results of \textit{in vitro} release studies obtained for all the formulations were plotted in models of data release as follows:

a. Cumulative percentage drug released versus time (Zero-order kinetic model)
b. Log cumulative percent drug remaining versus time (First-order kinetic model)
c. Log \(M_t/M\infty \) versus log time (Peppas model)
d. The square root of time versus cumulative percentage drug release (Higuchi model).

3 Results and discussion

3.1 Drug-excipient interaction study

FTIR results revealed that there were no new peaks and disappearance of peaks from the FTIR spectrum of drug and polymer combinations, indicating the absence of significant interaction between the polymer and the drug. All characteristic peaks of TNF were observed in the physical mixture and microsponge formulations. The recognized peaks that are characteristic of

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>Assessment peak of pure drug (cm$^{-1}$)</th>
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<tbody>
<tr>
<td>C=N stretching (aromatic)</td>
<td>1410 – 1450</td>
</tr>
<tr>
<td>Two weak intensity broad bands of O-H bonds</td>
<td>3200 – 3300</td>
</tr>
<tr>
<td>Aromatic CH stretching</td>
<td>3110</td>
</tr>
<tr>
<td>P=O stretching</td>
<td>1680</td>
</tr>
<tr>
<td>C-N medium stretching</td>
<td>1250</td>
</tr>
<tr>
<td>Medium stretch of NH2 scissoring</td>
<td>1550 – 1570</td>
</tr>
<tr>
<td>Various NH wagging bonds</td>
<td>660 – 690</td>
</tr>
<tr>
<td>Various CH out-of-plane deformations</td>
<td>900 – 600</td>
</tr>
<tr>
<td>CH bands</td>
<td>2995 – 2855</td>
</tr>
<tr>
<td>C-N stretch</td>
<td>1300</td>
</tr>
<tr>
<td>NH2 wagging</td>
<td>600</td>
</tr>
<tr>
<td>C=O stretch due to CH bands</td>
<td>2925 – 2855</td>
</tr>
<tr>
<td>O-H weak broad band stretch</td>
<td>3180 – 3450</td>
</tr>
</tbody>
</table>
each ingredient in the mixture are characterized in Table 2, and their FTIR spectra are shown in Figure 2.

3.2 Physical appearance

The desiccated sponges should possess soft, thin, flexible structure, physical integrity, mechanical stability, uniform texture, and thickness with no cracks. Compared with the pure drug, the microsponges obtained by the method as described in this study are fairly white (Figure 3) and thought to have good flow properties [12]. These microsponges carried pores and are clearly visible in the scanning electron microscopic images.

Figure 2. Fourier-transform infrared spectroscopic spectrum of (A) tenofovir drug, (B) excipients, and (C) drug with excipients.
3.3 Production yield

The production yield of all batches of microsponges ranged from 64.32% to 78.92%. The drug-polymer ratio and concentration of sodium alginate seem to have an influence on the production yield. Low production yields were detected in formulation F4 which has a drug-polymer ratio of 1:4 and the yield was 64.32%. A high production yield of about 78.92% was achieved in formulation F3 with a drug-polymer ratio of 1:3. In our observation, the higher the drug and polymer ratio, the higher the production yields. This was probably caused by the high diffusion rate of DCM from high concentrations to low concentrations (i.e., aqueous phase). With this high diffusion rate, additional time is available for the formation of the droplet, thereby improving the yield [12]. Production yields of formulations F1-F5 are shown in Table 3.

3.4 Encapsulation efficiency

High encapsulation efficiency indicates that a higher percentage of TNF can get entrapped in the microsponges if higher drug-polymer ratios are used. The encapsulation efficiency of formulations F1-F5 is shown in Table 3. The encapsulation efficiency was found to be lower than the theoretical value for all the formulations studied. This is because some amount of drug gets dissolved in the aqueous phase or the solvent used in the process.

Among the five tested formulations, F2 appears to have the highest encapsulation efficiency of 67.21 ± 4.32% while F4 has the lowest efficiency at 47.89 ± 4.32%. In the present study, drug entrapment was found between 47% and 67%. This indicates that the optimum entrapment efficiency can be achieved by changing the drug: polymer ratios and process parameters in future studies [13].

3.5 In vitro drug release

The dissolution studies were performed for formulations F1-F5 up to 180 min. As shown in Figure 4, the changes of the cumulative percent of drug release from 15 to 180 min for formulations F1, F2, F3, F4, and F5 were 52.78 ± 0.25% to 79.71 ± 0.26%, 72.19 ± 0.25% to 84.15 ± 0.13%, 37.41 ± 0.28% to 51.52 ± 0.29%, 68.25 ± 0.25% to 82.58 ± 26%, and 36.55% to 52.81 ± 0.26%, respectively.

Formulation F2 that has the drug and polymer in the ratio of 1:2 showed the highest release. This formulation achieves the maximum dissolution rate because of the reduction in the particle size and porous nature of the microsponges which provides channels for drug release.

3.6 Morphology and structural surface topology

Highly porous interconnecting networks of elongated and circular pores were revealed at different magnification powers (i.e., ×100 and ×300) in all formulations. The closely

![Figure 3. Microscopic pictures of formulations (A) F1, (B) F2, (C) F3, (D) F4, and (E) F5. The microsponges were pictured at ×100.](image)

Table 3. Production yields and encapsulation efficiencies of the prepared formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Production yield (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
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<tbody>
<tr>
<td>F1</td>
<td>71.64</td>
<td>54.53 ± 3.23</td>
</tr>
<tr>
<td>F2</td>
<td>76.98</td>
<td>67.21 ± 4.32</td>
</tr>
<tr>
<td>F3</td>
<td>78.92</td>
<td>56.81 ± 2.35</td>
</tr>
<tr>
<td>F4</td>
<td>64.32</td>
<td>47.89 ± 4.32</td>
</tr>
<tr>
<td>F5</td>
<td>65.90</td>
<td>49.05 ± 2.89</td>
</tr>
</tbody>
</table>
arranged layers as shown in the SEM images of microsponges showed that the sponges do not have damages in their structural framework. The SEM analysis showed that the microsponges have an excellent structure and are almost spherical in nature (Figure 5).

3.7 X-ray diffraction study

According to the X-ray studies, numerous sharp and distinct diffraction peaks of high intensity were observed for TNF in pure form, indicating the crystalline nature of the drug. The microsponges

Figure 5. Scanning electron microscopic images of microsponges in formulations (A) F1, (B) F2, (C) F3, (D) F4, and (E) F5. The microsponges were pictured at ×300.

Figure 6. X-ray diffraction patterns of (A) free drug, (B) drug in microsponges of formulation F2, and (C) drug in microsponges of formulation F2 after 2 h.

Figure 7. Particle size distribution of formulations F1, F2, F3, F4, and F5.
showed typical signals of TNF but with low intensity. The decrease in the intensity of the sharp peaks indicates that there was a reduction in the crystalline nature of the drug when formulated into microsponges. Figure 6 shows X-ray diffraction patterns of free TNF and the drug in formulation F2.

3.8 Particle size analysis

The determination of the average particle size of TNF-containing microsponges was carried out using eyepiece and stage microscopes. The particle size distribution for TNF-containing microsponges of formulations F1-F5 is shown in Figure 7. The typical particle size of microsponges was found in the range of 4.52 – 8.98 µm [14].

3.9 Kinetic modeling

The drug release data of formulation F2 was fitted with release kinetics with zero-order, first-order, Higuchi, and Peppas models. Table 4 shows the release exponent of formulation F2 in different release models. The maximum release exponent of formulation F2 was 0.961 for Peppas model, and its minimum release exponent was 0.063 for Hixson Crowell model. This finding, along with Figure 8, indicates that the mechanism of drug release is simple diffusion.

4 Conclusion

Based on the X-ray diffraction and scanning electron microscopy results, the TNF-containing microsponges were highly porous in nature. Cumulative percent of drug diffusion for formulation F2 (drug: polymer ratio = 1:2) was found to be 84.154% in 180 min. The present study on developing formulation of TNF-containing microsponges achieved a favorable enhancement of drug dissolution from approximately 10% to 84%, which may result in enhanced bioavailability. Further studies are needed to confirm the therapeutic potential of these microsponges.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest in any kind.
Author contributions

R.N.E. conceived, designed, and performed the experiments. R.K.J. analyzed and wrote the paper. V.B. reviewed and verified draft of the paper.

References


