Original Article

Formulation and development of tinidazole microspheres for colon targeted drug delivery system

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ABSTRACT

The objective of the present study was to formulate and optimize colon targeted tinidazole microspheres. To achieve these objective nine formulations of microspheres were prepared by emulsion solvent evaporation method using Eudragit polymer. A $3^2$ factorial design was employed in formulating the microspheres with concentration of surfactant ($A$) and stirring speed ($B$) as independent variables. Percent drug release was considered as dependent variable. The effect of drug-polymer concentration, surfactant concentration, cross-linking agent and stirring speed were evaluated with respect to entrapment efficiency, particle size, surface characteristics, micromeritic properties, DSC study and in vitro drug release studies. The particle size and entrapment efficiency were found to be varied by changing various formulation parameters like surfactant concentration and stirring speed etc. IR study confirmed the drug-polymer compatibility and scanning electron microscopy indicates that the microspheres have the rough and porous surface due to arising as a trace of solvent evaporation during the process. The release profile of tinidazole from Eudragit microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit microspheres are promising as a carrier for colon targeted delivery of tinidazole.

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1. Introduction

Drug delivery to the colon is beneficial for the oral delivery of proteins and peptide drugs degraded by digestive enzymes of the stomach and small intestine and for the delivery of low molecular weight compounds. Delivery of drug substances to the colon may improve systemic bioavailability to a level which is not feasible by un-modified oral drug delivery. This may improve efficacy of drug treatment or open up the possibility to switch to oral instead of parenteral administration. Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, cirrhosis disease, amebiasis, colonic cancer and local treatment of colonic pathologies and systemic delivery of protein and peptide drugs. This route may also be useful in
the treatment of diseases susceptible to diurnal rhythm such as asthma, arthritis, etc.\(^3\)

There are several approaches, which is utilized in achieving colon targeting include use of pH-sensitive polymer, time-dependent formulation, bacterial degrading coating material, biodegradable polymer matrix and hydrogels and prodrug.\(^4\)

Microspheres have played a vital role in the development of controlled and or sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release.\(^5\) There are several publications based on drug-containing microspheres using the Eudragit series of polymers as the encapsulating materials.\(^6\) The Eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems. These polymers are available in various grades possessing a range of physicochemical properties.

The objective of the study is to formulate and develop colon targeted drug delivery system of tinidazole microspheres by using Eudragit L 100 and Eudragit S 100 as a pH-sensitive polymer. By directly targeting the drug to colon, the maximum concentration of drug reaches and increases the residence time of drug in colon with an improved patient compliance, lesser side effects and an ideal drug delivery system.

2. Materials and methods

2.1. Materials

Tinidazole was received as a gift sample from Meditab specialities Pvt. Ltd., Daman, India. Eudragit L 100 and S 100 were of Evonik India Pvt. Ltd., Mumbai, India and all the solvents and other reagents used were of the best laboratory reagent (LR) grade.

2.2. Method

2.2.1. Preparation of tinidazole microspheres\(^7\)

Tinidazole microspheres were prepared by emulsification solvent evaporation method. Accurately weighed EL 100 and ES 100 in 1:2 ratios were dissolved in ethanol and acetone in 1:2 ratios to form a homogenous polymer solution. Tinidazole was added into the polymer solution and mixed thoroughly. Plasticizer (dibutyl phthalate 50% w/v) was added to above solution. The above organic phase was slowly poured at 30 °C into liquid paraffin (15 mL) containing span 80 of different concentrations with stirring speed at different rpm to form a smooth emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone and ethanol evaporated and smooth walled, rigid and discrete microspheres were formed. The microspheres were collected by decantation and the product was washed with petroleum ether (40–60 °C), three times and dried at room temperature for 3 h. The microspheres were then stored in a desiccator over fused calcium chloride for further use. Nine batches were performed with optimization (Tables 1 and 2).

2.3. Evaluation

2.3.1. Drug-polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan).

2.3.2. Particle size analysis\(^8\)

The particle size analysis was used to found the particle size of microspheres. The particle size analysis study was performed by using Malvern, ZS-90 particle size analyzer.

2.3.3. Percentage yield\(^9\)

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres (equation):

\[
\text{% yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100
\]

2.3.4. Surface morphology (SEM)\(^8\)

Scanning electron microscopy has been used to determine the surface morphology and texture. SEM studies were carried out by using JEOL Model JSM-6390LV scanning microscope.

2.3.5. Micromeritic properties of microspheres

The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr’s and Hausner’s ratio. The angle of repose was determined by the fixed-based funnel method. Bulk and tapped densities were measured in 10 mL of a graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. The volume occupied by the sample...
after tapping was recorded and bulk density, tapped density, Carr’s index and Hausner’s ratio was calculated.

2.3.6. **Entrapment efficiency**

Microspheres containing equivalent to 10 mg of drug was allowed to equilibrate in 100 mL of phosphate buffer pH 7.4 for 24 h. The solution was filtered using Whatman filter paper (44). The resulting solution was analyzed using a UV spectrophotometric method at 318 nm in the presence of a blank prepared from microspheres containing all materials except the drug.

\[
\% \text{ Drug entrapment} = \frac{\text{calculated drug concentration}}{\text{theoretical drug concentration}} \times 100
\]

2.3.7. **Differential scanning colorimetry (DSC)**

DSC studies were performed using a DSC METTLER Switzerland with thermal analyzer. Accurately weighed samples (about 5 mg) were placed in a sealed aluminum pan, before heating under nitrogen flow (20 mL/min) at a scanning rate of 20 °C per min from 40 to 300 °C. An empty aluminum pan was used as reference. DSC thermograms of pure substances, their physical mixtures and drug-loaded micro-particles were recorded.

2.3.8. **In vitro drug release study**

In vitro release study of microspheres was performed in pH progression medium at 37 °C ± 0.5 °C. The drug dissolution test of microspheres was performed by the paddle method (USP dissolution apparatus Type II, Electrolab Limited, India). Microspheres equivalent to 100 mg were weighed accurately and put in muslin cloth and tied this to paddle over the surface of 900 mL of dissolution medium. The content was rotated at 100 rpm. The pH of the dissolution medium was kept 1.2 for 2 h using 0.1 N HCl. After 2 h, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintained up to 8 h. The samples were withdrawn from the dissolution medium at various time intervals using a pipette. The rate of drug release was analyzed using UV spectrophotometer (JASCO, Ahmadabad, India).

2.3.9. **Statistical design**

Design-Expert software (Design Expert trial version 8.0.7.1; State-Ease Inc., Minneapolis, MN, USA) was used. A two-factor three-level full factorial design was used for systemic study of combination of polymers. Polynomial models including interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers. Polynomial models including three-level full factorial design was used for systemic study of the interaction and quadratic terms were generated for the entire combination of polymers.

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_12X_1X_2
\]

Where **Y** is the dependent variable; **b**<sub>0</sub> is the arithmetic average of all the quantitative outcomes of nine runs. **b**<sub>1</sub>, **b**<sub>2</sub>, **b**<sub>12</sub> are the estimated coefficients computed from the observed experimental response values of **Y** and **X**<sub>1</sub> and **X**<sub>2</sub> are the coded levels of the independent variables. The interaction term (**X**<sub>1</sub>**X**<sub>2</sub>) shows how the response values change when two factors are simultaneously changed.

**Table 1** summarizes the translation of the coded levels to the experimental units used in the study and **Table 2** summarizes the experiment runs used. In this study factorial design based on the response surface method was adopted to optimize effective factors for the release of the drug from the microspheres.

Analysis of variance (ANOVA) and all statistical analysis were also performed using the software. Calculation of the effects was performed. The significant effects would constitute the model. The F-value was then calculated by comparing the treatment variance with the error variance. The multiple correlation co-efficient was calculated which is a measure of the amount of variation about the mean, which is explained by the model. The main effects and interactions are plotted and results interpreted. All assumptions underlying the ANOVA are checked. For statistical purposes, the assumption is made that residuals are normally distributed and independent with constant variance.

### Table 3 – Formulation of tinidazole microspheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (gm)</th>
<th>Polymer ratio EL:ES</th>
<th>Surfactant concentration (80%)</th>
<th>Stirring speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.9</td>
<td>1:2</td>
<td>0.5</td>
<td>2000</td>
</tr>
<tr>
<td>F2</td>
<td>0.9</td>
<td>1:2</td>
<td>0.5</td>
<td>2500</td>
</tr>
<tr>
<td>F3</td>
<td>0.9</td>
<td>1:2</td>
<td>0.5</td>
<td>3000</td>
</tr>
<tr>
<td>F4</td>
<td>0.9</td>
<td>1:2</td>
<td>1.0</td>
<td>2000</td>
</tr>
<tr>
<td>F5</td>
<td>0.9</td>
<td>1:2</td>
<td>1.0</td>
<td>2500</td>
</tr>
<tr>
<td>F6</td>
<td>0.9</td>
<td>1:2</td>
<td>1.0</td>
<td>3000</td>
</tr>
<tr>
<td>F7</td>
<td>0.9</td>
<td>1:2</td>
<td>1.5</td>
<td>2000</td>
</tr>
<tr>
<td>F8</td>
<td>0.9</td>
<td>1:2</td>
<td>1.5</td>
<td>2500</td>
</tr>
<tr>
<td>F9</td>
<td>0.9</td>
<td>1:2</td>
<td>1.5</td>
<td>3000</td>
</tr>
</tbody>
</table>

### Table 4 – Characterization of tinidazole microspheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Entrapment efficiency (%)</th>
<th>Percentage yield (%)</th>
<th>Average particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>51.18 ± 0.03</td>
<td>69.7 ± 0.02</td>
<td>782.6</td>
</tr>
<tr>
<td>F2</td>
<td>54.35 ± 0.02</td>
<td>68.6 ± 0.04</td>
<td>794.5</td>
</tr>
<tr>
<td>F3</td>
<td>45.75 ± 0.01</td>
<td>74.76 ± 0.03</td>
<td>852.7</td>
</tr>
<tr>
<td>F4</td>
<td>61.48 ± 0.04</td>
<td>75 ± 0.02</td>
<td>829.2</td>
</tr>
<tr>
<td>F5</td>
<td>66.51 ± 0.02</td>
<td>76.93 ± 0.02</td>
<td>817.7</td>
</tr>
<tr>
<td>F6</td>
<td>58.50 ± 0.03</td>
<td>77.15 ± 0.01</td>
<td>731.3</td>
</tr>
<tr>
<td>F7</td>
<td>44.28 ± 0.03</td>
<td>72.55 ± 0.04</td>
<td>986.4</td>
</tr>
<tr>
<td>F8</td>
<td>49.87 ± 0.02</td>
<td>69.9 ± 0.06</td>
<td>585.6</td>
</tr>
<tr>
<td>F9</td>
<td>40.56 ± 0.04</td>
<td>68.44 ± 0.08</td>
<td>975.3</td>
</tr>
</tbody>
</table>
for particle size analysis within range of 585.6 μm–986 μm (Table 4).

The FTIR spectra of pure drug, Eudragit and tinidazole microspheres were shown in (Fig. 1). It shows that no incompatibility reactions took place between drug and excipients.

The value of angle of repose of formulation within the range of 17°.97° ± 0.51–26°.22° ± 0.22 indicating good flow properties for the microspheres. The bulk density values ranged between 0.148 ± 0.001 and 0.278 ± 0.004 gm/cm³. The tapped density values ranged between 0.206 ± 0.002 and 0.401 ± 0.03 (gm/cm). The Carr’s index values ranged between 17.55 ± 3.0 % and 42.80 ± 1.2% and Hausner’s ratio values ranged between 1.2140 ± 0.04 to 1.7148 ± 0.08 which can described by Table 5.

**Table 5 – Micromeritical properties of different batches of microspheres.**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Angle of repose (°)</th>
<th>Bulk density (gm/cm³)</th>
<th>Tapped density (gm/cm³)</th>
<th>Carr’s index (%)</th>
<th>Hausner’s ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>26°.22 ± 0.22</td>
<td>0.189 ± 0.002</td>
<td>0.286 ± 0.002</td>
<td>33.62 ± 2.3</td>
<td>1.5112 ± 0.1</td>
</tr>
<tr>
<td>F2</td>
<td>24°.06 ± 0.80</td>
<td>0.218 ± 0.005</td>
<td>0.374 ± 0.008</td>
<td>41.59 ± 2.7</td>
<td>1.7148 ± 0.08</td>
</tr>
<tr>
<td>F3</td>
<td>20°.89 ± 1.23</td>
<td>0.228 ± 0.005</td>
<td>0.400 ± 0.016</td>
<td>42.80 ± 1.2</td>
<td>1.7489 ± 0.03</td>
</tr>
<tr>
<td>F4</td>
<td>22°.54 ± 0.99</td>
<td>0.278 ± 0.004</td>
<td>0.401 ± 0.03</td>
<td>30.07 ± 4.6</td>
<td>1.4341 ± 0.09</td>
</tr>
<tr>
<td>F5</td>
<td>24°.05 ± 1.29</td>
<td>0.209 ± 0.002</td>
<td>0.326 ± 0.01</td>
<td>35.70 ± 3.1</td>
<td>1.5578 ± 0.07</td>
</tr>
<tr>
<td>F6</td>
<td>20°.11 ± 0.90</td>
<td>0.274 ± 0.004</td>
<td>0.379 ± 0.008</td>
<td>27.58 ± 0.4</td>
<td>1.3809 ± 0.009</td>
</tr>
<tr>
<td>F7</td>
<td>21°.00 ± 0.5</td>
<td>0.263 ± 0.007</td>
<td>0.319 ± 0.01</td>
<td>17.55 ± 3.0</td>
<td>1.2140 ± 0.04</td>
</tr>
<tr>
<td>F8</td>
<td>23°.47 ± 0.24</td>
<td>0.158 ± 0.001</td>
<td>0.225 ± 0.002</td>
<td>30.45 ± 1.4</td>
<td>1.4384 ± 0.02</td>
</tr>
<tr>
<td>F9</td>
<td>17°.97 ± 0.51</td>
<td>0.148 ± 0.001</td>
<td>0.206 ± 0.002</td>
<td>28.21 ± 1.1</td>
<td>1.3933 ± 0.02</td>
</tr>
</tbody>
</table>

Average of three preparation ± S.D.

- a The angle of repose was calculated with the formula: tan θ = H/R where, H = pile height, R = radius of pile. Therefore; θ = tan⁻¹(H/R).
- b Bulk density = mass of sample / apparent unsettled volume.
- c Tapped density (g/mL) = weight of sample / volume occupied by the sample.
- d Carr’s index = (tapped density - bulk density) / tapped density × 100.
- e Hausner’s ratio = tapped density / bulk density.
The in vitro release study was carried out by buffer change method to mimic the GIT environment. Drug release for the initial 2 h i.e. in 0.1 N HCL, the drug release was found to be low in all cases. Then drug release is found 92.74% at the end of 8 h in pH 7.4 phosphate buffer, shown in Fig. 2.

The produced microspheres were spherical, non aggregated with rough and porous surface, as shown in scanning electron micrographs (Fig. 3). The surface of microspheres was rough due to arising as a trace of solvent evaporation during the process.

Fig. 3 – Scanning electron microphotograph.

The produced microspheres were spherical, non aggregated with rough and porous surface, as shown in scanning electron micrographs (Fig. 3). The surface of microspheres was rough due to arising as a trace of solvent evaporation during the process.

Fig. 4 – Effect of variables on drug release response (a) effect of variable A (b) effect of variable B.
interaction plot between surfactant and stirring speed on the response

Contour plot shows the effect of surfactant and stirring speed on the % drug release

3D response surface plot for % drug release with respect to surfactant and speed

Fig. 5 – (a): Interaction plot between surfactant and stirring speed on the response. (b) Contour plot shows the effect of surfactant and stirring speed on the % drug release. (c) 3D response surface plot for % drug release with respect to surfactant and speed.
ANOVA results indicated that concentration of surfactant and stirring speed showed individual effect on % drug release. There is no significant interaction between surfactant and stirring speed.

The polynomial equation obtained is as follows:

\[
\text{Drug release} \left( \% \right) = +89.53 - 2.98 \times A - 3.99 \times B + 0.58 \times A \times B - 26.24 \times A^2 - 6.55 \times B^2
\]

The model F-value of 9.99 with probability \( P > F \) of 0.05 implies that the model is significant with only a 4.35% chance that this \( F \) value could have occurred due to noise. The correlation coefficient \( R^2 = 0.9433 \). Precision is a measure of signal-to-noise ratio. F-test used to check the statistical significance of equation 1 shows that the fitted model is strongly significant at 95% confidence level (\( P \)-value < 0.05). In this case \( A^2 \) is significant model term. Values greater than 0.1000 indicate the model terms are not significant. The “Adeq Precision” measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 8.442 indicates an adequate signal. This model can be used to navigate the design space.

Fig. 4(a) shows that as surfactant concentration increases up to optimum limit (i.e. 1%), % drug release was found to be increased where as the concentration of surfactant increases beyond optimum level, % drug release was found to be decreased. The graph concluded that the variable A alone might have significant effect on the drug release.

Fig. 4(b) shows the drug release increases with increasing the stirring speed up to certain limits (i.e. 2500 rpm) and increasing the stirring speed above 2500 rpm then % drug release get decreases. The graph concluded that variable B in the formulation might have individual effect on the increase in % drug release. From Fig. 4(a) and (b) it could be concluded that variable A showed more significant effect than variable B.

Interaction plot and contour plot for drug release are shown in Fig. 5(a) and (b). From the Fig. 5(a), red line represents high level of the variable \( A \) and the black line refers to the low level. There is no significant interaction between variable A and B indicates that variables show individual effect on % drug release.

Fig. 5(b) shows the contour plot of effect of surfactant and speed on drug release. It represented that when the concentration of surfactant and stirring speed was less than the % drug release was minimum and when the surfactant concentration and stirring speed was high then also drug release was in minimum range. It increases when the surfactant concentration and stirring speed was in optimum range.

Fig. 5(c) shows the resulting response surface plot for % drug release. It is demonstrated that the % drug release depends both on the surfactant and the stirring speed. The highest drug release was obtained at optimum level of surfactant and stirring speed.

DSC thermograph of tinidazole, Eudragit L 100, Eudragit S 100, tinidazole microspheres are shown in Fig. 6.

The pure drug tinidazole Fig. 6(a) gives rise to a sharp peak that corresponds to melting point at 126 °C, indicates its crystalline nature. The pure polymer Eudragit L 100 and Eudragit S 100 exhibits a peak at 223 °C and 222 °C respectively, referring to the relaxation that follows the glass transition in Fig. 6 (b) and (c). The peak of drug did not appear in the thermogram of prepared microspheres, it may indicate the drug was uniformly dispersed at the molecular level in the microspheres in Fig. 6 (d).

4. Discussion

From the result of present study, it can be concluded that Eudragit based tinidazole microspheres offer a high degree of
protection from premature drug release in simulated upper GIT conditions and deliver most of the drug load in the colon and allow drug release to occur at the desired site by emulsion solvent evaporation system. A factorial method was used in the study. Based on the results of the physicochemical characterization and in vitro drug release studies, it possessed all the required physicochemical characters and with drug releases up to 8 h where it released 92% of the tinidazole. Thus, Eudragit based tinidazole microspheres are a potential system for colon delivery of tinidazole for chemotherapy of amoebic infection.

Conflicts of interest

All authors have none to declare.

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References