Formulation and evaluation of trimetazidine hydrochloride and clopidogrel bisulphate multi-unit solid dosage forms

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ABSTRACT

Trimetazidine HCl is the first cytoprotective anti-ischemic agent which, in contrast to classical antanginal drugs, is effective to ward off heart attack by enhancing the heart’s energy producing function rather than weakening the heart act. Clopidogrel bisulfate plays a central role as a platelet inhibitor in acute coronary syndromes, interventional cardiology and in secondary prevention of ischemic events in patients with myocardial infarction. The aim of this work was to develop a sustained release formulation of trimetazidine hydrochloride microspheres and immediate release formulation of clopidogrel bisulphate layer–tablet which were planned to be used for future preparation of bilayer tablets of the two drugs. Trimetazidine HCl microspheres were prepared by the emulsification-solvent evaporation technique using ethyl cellulose as retardant polymer at different drug: polymer ratio while clopidogrel layer-tablet was prepared and optimized by using different concentrations of crospovidone as disintegrant by direct compression method using a compression force of 15 kg/cm² while. The 1:2 drug: polymer microspheres formulation showed the highest entrapment efficiency and production yield and a sustained drug release for up to 12 hr. On the other hand, the clopidogrel layer-tablet formulation containing 4% crospovidone showed the highest rate and extent of drug release.

Keywords: Trimetazidine, microspheres, Clopidogrel, layer-tablet

INTRODUCTION

Trimetazidine hydrochloride \([1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride]\)[1] is the first cytoprotective anti-ischemic agent [2]. It functions via unique mechanisms enhancing the coronary blood flow by shifting energy substrate utilization to glucose through inhibition of fatty acid metabolism [3]. Unlike conventional anti anginal drugs including calcium channel blockers and β-blockers, trimetazidine offers aging individuals a complementary and potentially more effective way to ward off heart attack by enhancing the heart’s energy producing function rather than weakening the heart [4]. Clopidogrel bisulphate \([\text{Methyl (S)-2-chlorophenyl(4,5,6,7-tetrahydrothieno[3,2-c]pyridin-5-yl)acetate bisulphate}]\) [1] plays a central role as a platelet inhibitor in secondary prevention of ischemic events in patients with myocardial infarction[5]. A clinical study concluded that trimetazidine combined clopidogrel treatment of unstable angina pectoris has positive clinical effects and high security and thus it is worthy of clinical application [6].

Microspheres are homogeneous or monolithic structures made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed throughout the matrix. Emulsification-solvent evaporation technique is the oldest and most widely used method to accomplish encapsulation [7]. It allows for the
creation of microparticles or nanoparticles that have a more optimized release of the encapsulated material [8]. Due to the unavailability of products containing both trimetazidine HCl and clopidogrel bisulphate as a dosage unit, this preliminary work was undertaken for the favor of future preparation of the two drugs as bilayer tablets.

EXPERIMENTAL SECTION

2.1. Materials

2.1.1. Apparatuses
U.V spectrophotometer and FTIR spectrophotometer (Shimadzu Corporation, Japan); Hydraulic pellet press (KP–587, PCI Services, India); Differential scanning calorimeter (Mettler – Toledo Star 822 system, Switzerland); USP dissolution tester apparatus and disintegration Tester apparatus (Pharma test, India); Friabilator and hardness tester (Techno Scientific, India).

2.1.2. Materials and Reagents
Trimetazidine Hydrochloride (Niveditha chemicals, India), clopidogrel bisulphate (Zydus –Cadila Healthcare Ltd, India), ethyl cellulose and crospovidone (Shreji chemicals, India), acetone, Methanol, ethanol, light liquid paraffin, hydrochloric acid, microcrystalline cellulose, lactose, magnesium stearate , and talc (Sd fine chemical Ltd . India), potassium dihydrogen phosphate, sodium hydroxide, potassium bromide and potassium chloride (Qualigens Fin chemicals, India).

2.2. Methods

2.2.1. Ingredients compatibility
FTIR spectrophotometry of trimetazidine HCl, clopidogrel bisulphate and physical mixtures of the two drugs, clopidogrel bisulphate /ethyl cellulose and trimetazidine HCl /ethyl cellulose were carried out. The spectra were compared to evaluate drug/drug and drug/polymer incompatibilities. The pellets of drug (or mixture) and potassium bromide were prepared by compressing the powders at 20 psi for 10min on KBr-press and the spectra were scanned in the wave number range of 600 - 4000cm⁻¹.

2.2.2. Standard calibration curves
A stock solution of 100mg/100ml of trimetazidine HCl in phosphate buffer pH6.8 was prepared. Then a serial dilution were done to obtain solutions in the concentration ranging from 10 to 100µg/ml. The absorbance of those solutions was measured at 269nm using UV spectrophotometer. Separately, a stock solution of 50mg/100ml of Clopidogrel bisulphate in (10:90) methanol: pH2 HCl buffer. A serial dilution was carried out to obtain standard solutions of concentration ranging from 10 to 50µg/ml. Absorbance of each solution was then measured at 220nm. The standard calibration curves of each drug were constructed and the regression equations of those curves were determined.

2.2.3. Preparation and evaluation of trimetazidine HCl microspheres
(a) Preparation
Trimetazidine HCl microsphere formulations (T1, T2 and T3) were prepared by emulsification-solvent evaporation technique [9]. The drug: polymer ratios were 1:1, 1:1.5 and 1:2, respectively. T1 formulation was prepared by dissolving of 6 g of the polymer ethyl cellulose and 6 g of trimetazidine HCl in acetone. The solution was then added dropwise, under mechanical stirring at 500 rpm, to a beaker containing Span 80 in 150 ml of light liquid paraffin. After the emulsion was prepared, stirring continued for 2 hours to facilitate solvent evaporation. The obtained microspheres were separated and washed with n- hexaneand then dried at room temperature for 12 hours [10].

(b) Evaluation
(i) Microspheres size and morphology
The particle size of microspheres was measured using an optical microscope. The average diameter of microspheres was calculated and the frequency of distribution of their particle size was also studied [11]. Differential scanning calorimetry (DSC) thermogram of the drug-loaded microspheres was compared to those of the drug and blank microspheres in order to determine crystalline or amorphous forms of microspheres. Besides, scanning electron microscopy (SEM) was used to examine the microspheres shape and surface morphology [12].
(ii) Production yield, drug content & entrapment efficiency

Production yield of each microsphere formulation was determined by the weight ratio of the dried microspheres to the loading amount of the drug and polymer. To determine the entrapment efficiency, a quantity of 100mg of the microspheres was thoroughly triturated in 100 ml of phosphate buffer pH 6.8. 1 ml of this solution was diluted to 10 ml with the same solvent. This solution was kept overnight for complete dissolution of trimetazidine HCl. The absorbance of the solution was then measured at 269nm. Production yield, entrapment efficiency and drug content were calculated using the following formulae [13-15]

\[
\text{Production Yield(%) } = \frac{\text{Total mass of microspheres}}{\text{Total mass of raw material}} \times 100
\]

\[
\text{Drug content (%) } = \frac{\text{Actual drug content}}{\text{Weight of microspheres}} \times 100
\]

\[
\text{Entrapment efficiency } = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]

(iii) In vitro drug release from microspheres

The release of trimetazidine HCl from microspheres was determined by using USP basket type dissolution apparatus. Accurately weighed amount of microspheres were taken for dissolution study. The microspheres were placed in a non-reacting muslin cloth that had a smaller mesh size than the microspheres. The mesh was tied with a nylon thread to avoid the escape of any microspheres. Phosphate buffer pH 6.8 was used as the dissolution medium and maintained at 37°C and at a rotation speed of 100 rpm. Samples were withdrawn at 1 hour intervals for up to 12 hours and analyzed spectrophotometrically at 269 nm. The drug release kinetics were studied by fitting the dissolution profile of each microsphere formulation to zero order. First order, Higuchi and Koresmeyers Peppa’s models. Equations used to express those orders and models, respectively were:

\[ Q_t = Q_o + K_o t, \quad \log Q_R = \log Q_o - \frac{kt}{2.303}, \quad Q_t = K_H t^{0.5} \quad \text{and} \quad Q/Q_o = K_p t^n \]

where \(Q_t, Q_o\) were the cumulative % drug released at time and the initial % drug released, respectively; \(Q_R\) was the cumulative % drug remaining to be released at a time. \(Q_o\) was the maximum % drug released, \(K_o, K_H\) and \(K_p\) were the equations constants, \(t\) was the drug release time and \(n\) was the diffusional exponent [16-18].

2.2.4. Preparation and evaluation of Clopidogrel bisulphate layer

(a) Preparation

200mg clopidogrel bisulphate tableted-layer was prepared by direct compression on hydraulic pellet press with compression force 15kg/cm². Three formulations (C1, C2 & C3) of that layer were prepared with different percentage of the disintegrant crospovidone of 2%, 4% and 6%, and slightly different lactose content of 37.5%, 35.5% and 33.5%, respectively. The drug content in all formulation was 37.5%. Other ingredients included formulation were microcrystalline (20%), magnesium stearate (1%) and talc (2%).

(b) Evaluation

Weight variation, hardness, friability and disintegration tests were carried out on each formulation [19]. In addition, the in vitro drug release in HCl buffer pH2 for 1 hour at 37°C and at a rotation speed of 50 rpm was also evaluated using USP dissolution basket type apparatus [20]. The content amount of the drug released was determined spectrophotometrically at 220 nm.

RESULTS AND DISCUSSION

3.1. Ingredients compatibility

The FTIR spectra showed no interaction between the two drugs, trimetazidine HCl/ ethyl cellulose and between clopidogrel/ethyl cellulose as the characteristic peaks of each substance were present and no shift of the peaks was observed.

3.2. Standard calibration curves

Standard calibration curves at 269 nm of trimetazidine HCl (10-100 µg/ml) in phosphate buffer and that of clopidogrel bisulphate (10-50 µg/ml) in HCl buffer pH 2 at 220 nm as shown in Fig.1 were both linear with
linearity $R^2$ of 0.999 for each curve. The regressions equations for the two curves respectively were $y=0.003x+0.006$ and $y=0.023x-0.001$

![Graph of standard calibration curves of trimetazidine HCl in phosphate buffer pH 6.8 at 269 nm (left) and clopidogrel bisulphate in HCl buffer pH 2 at 220 nm (right)](image)

3.3. Evaluation of trimetazidine HCl microspheres formulations

3.3.1. Microspheres size and morphology

The average size (in $\mu$m) of microspheres in the three formulations T1, T2 and T3 were $(40.1 \pm 2.21)$, $(76.04 \pm 4.06)$ and $(76.46 \pm 5.31)$ $\mu$m, respectively. There were also normal distributions of particle sizes in the three formulations which ensured the uniformity of particle sizes necessary for operations like mixing and tabletting and also provided more uniform drug release. The DSC thermogram of trimetazidine HCl microsphere showed absence of endothermic peak of the trimetazidine HCl at 225.7°C (drug melting point) which was observed in thermogram of the drug alone and in the physical mixtures of the drug and polymer. These findings indicated that trimetazidine HCl existed in disordered crystalline phase as a molecular dispersion in polymeric matrix. They also revealed that the physical mixture of trimetazidine HCl and polymer showed the same thermal behavior 225.5°C as the individual component, indicating that there was no interaction between the trimetazidine HCl and the polymer in the solid state. Results of SEM, as shown in Fig.2, revealed that microspheres had spherical shape and showed smooth surface and that would be advantageous to provide good flowability necessary for proper tableting operation during future preparation of bilayer tablets while crystalline character of microspheres would support the stability of the drug in tablets.

![SEM of Trimetazidine HCl/ethyl cellulose microspheres (left X 100; RIGHT X 1400)](image)

3.3.2. Production yield, drug content & entrapment efficiency

The results obtained indicated that the increase of drug:polymer ratio was associated with increase in both the entrapment efficiency of the drug into the microspheres and the microspheres production yield as shown in Table 1.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Production Yield (%)</th>
<th>Drug content (%)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>80.00</td>
<td>42.29</td>
<td>63.74</td>
</tr>
<tr>
<td>T2</td>
<td>87.00</td>
<td>39.96</td>
<td>76.12</td>
</tr>
<tr>
<td>T3</td>
<td>92.33</td>
<td>35.01</td>
<td>93.22</td>
</tr>
</tbody>
</table>

3.3.3. In vitro drug release from microspheres

The in vitro drug release profile of trimetazidine HCl from the three formulations was found to be biphasic with an initial burst effect as shown in Fig.3.
In the first hour, the drug release was 17.93%, 12.18% and 10.63% for T1, T2 and T3, respectively. The reason for the burst release could be attributed to the drug loaded on the trimetazidine HCl microspheres or imperfect entrapment of the drug. Table 2 demonstrates the overall cumulative % release for T1, T2 and T3 at the end of 12th hour which were 81.38%, 79.36% and 71.8%, respectively. Results of analyzing of kinetics of the drug release orders and models (presented in table 2) showed that regression coefficient ($R^2$) of the three formulations on zero order were greater than those on first order which indicated that the drug release of all formulations obeyed zero order kinetic. Evidences of diffusion controlled mechanism was obtained by fitting the release data to Korsemeyers-Peppa’s equation. The diffusion exponent ‘n’ values of all formulations were found to be more than 0.5 indicating Non-Fickian diffusion in which the release continues regardless to the concentration difference between inside and outside the microspheres. Data of drug release was also fitted to Higuchi equation explaining the controlled diffusion mechanism of drug release from the insoluble matrix polymer of the microspheres. The highest entrapment efficiency and the slowest drug release rate were obtained with T3 formulation. These findings could be attributed to the highest polymer content in that formulation of trimetazidine HCl microspheres.

### Table 2: Kinetics of trimetazidine HCl release from different formulations of microspheres

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero</th>
<th>First</th>
<th>Higuchi</th>
<th>Peppa’s ($n$)</th>
<th>Overall Cumulative release % (0-12 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.962</td>
<td>0.915</td>
<td>0.986</td>
<td>0.985</td>
<td>81.3%</td>
</tr>
<tr>
<td>T2</td>
<td>0.966</td>
<td>0.896</td>
<td>0.980</td>
<td>0.991</td>
<td>79.4%</td>
</tr>
<tr>
<td>T3</td>
<td>0.976</td>
<td>0.862</td>
<td>0.979</td>
<td>0.965</td>
<td>71.8%</td>
</tr>
</tbody>
</table>

Similarity factor ($f_2$) T2 and T3: $f_2 = 80.85$ ; T2 and T3: $f_2 = 53.6$

3.4. Evaluation of Clopidogrel bisulphate layer-tablets

Formulation C2 (containing 4% of Crospovidone) as shown in Fig.4 showed the maximum cumulative overall drug release of 89.22% within 60 minutes in comparison to 87.44% and 86.22% obtained with formulations C1 and C3, respectively. On the other hand, the hardness, disintegration time, friability and weight variation of three layer formulations were within accepted limits as demonstrated in Table 3.
Table 3: Physical parameter (as mean ± SD) of Clopidogrel bisulphate layer-tablet.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Weight (mg) (n=20)</th>
<th>Hardness (Kg/cm²) (n=10)</th>
<th>Friability (%) (n=20)</th>
<th>Disintegration time (min.) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>199 ± 1.673</td>
<td>3.63 ± 0.27</td>
<td>0.231</td>
<td>0.551 ± 0.030</td>
</tr>
<tr>
<td>C2</td>
<td>200 ± 1.932</td>
<td>3.52 ± 0.21</td>
<td>0.432</td>
<td>0.547 ± 0.083</td>
</tr>
<tr>
<td>C3</td>
<td>199.5 ± 1.765</td>
<td>3.69 ± 0.25</td>
<td>0.342</td>
<td>0.5776 ± 0.040</td>
</tr>
</tbody>
</table>

CONCLUSION

The sustained release microspheres made of 1:2 trimetazidine HCl:ethyl cellulose and the immediate release clopidogrel bisulphate layer-tablet containing 4 % crospovidone was found to be promising for future work of preparation of bilayer tablets of the two drugs.

Acknowledgment

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REFERENCES