Effect of Particle Size and Some Formulation Components on the Mechanical Properties and \textit{in vitro} Release Profile of Metronidazole from Compressed Tablets

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Citation

Abstract
Properties of a tablet such as the mechanical properties, disintegration time and drug release characteristics have been found to be affected by particle size, the properties of the added excipient and the manufacturing process employed in formulation. The aim of the present work was to investigate the effect of particle size on the mechanical properties and \textit{in vitro} release profile of metronidazole from compressed tablets. Three tablet batches were prepared by the wet granulation method using different formulation components (binders, disintegrants and fillers). The granules were fractionated from three sieve fractions (1.0, 0.5 and 0.25 mm). Granule properties evaluated as a function of the particle size are hopper flow rate, angle of repose, bulk and tapped densities, Hausner’s quotient and Carr’s index. The effects of the particle size fractions on the mechanical properties of the tablets (crushing strength and friability) and on the \textit{in vitro} release profile of metronidazole was also investigated. Results obtained indicated that decrease in particle size improved the \textit{in vitro} release profile of metronidazole and caused a gradual decrease in the mechanical properties of the tablets. The projected \textit{in vivo} bioavailability of metronidazole using the dissolution efficiency parameter (DE) showed an increase in DE with a decrease in the particle size.

1. Introduction

Particle characterization of powder materials has become one of the most crucial aspects in the drug product development process and in the quality control of dosage forms. Studies have shown that particle size (PS) and particle size distribution (PSD) of pharmaceutical powders have a profound influence on almost every step involved in the formulation processes for solid dosage forms including pre-mixing / mixing, granulation, drying, milling, blending, coating, encapsulation and compression\textsuperscript{1-4}. The PSD of a drug substance has also been shown to have significant effect on the final drug product performance like dissolution, bioavailability, content uniformity, compactibility.
Furthermore, the PSD of both drug substances and other excipients used in formulation can also affect drug product formulation processes like flowability, blend uniformity, compactibility etc. and these can ultimately impact safety, efficacy and the quality of the drug product. Drug absorption from solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological condition and the permeability across the gastrointestinal tract, first pass metabolism, and susceptibility to efflux mechanisms. The incorporation of excipients like diluents, disintegrants, lubricants and surfactants in tablet formulations have also been found to cause significant effects on the dissolution rate of drugs especially those that are hydrophobic and poorly water soluble. Excipients are principally responsible for the properties of a tablet with respect to robustness, bioavailability, stability, safety and manufacturing feasibility. Pharmaceutical manufacturers therefore, have the option of using either a number of excipients or a single excipient with multifunctional properties, thereby reducing the number of excipients in inventory and as well as improved organoleptic properties. Excipient functionalities such as flowability, compaction, dilution, disintegration, and lubricating potentials are influenced by fundamental solid-state properties of the excipients such as morphology, particle size, shape, surface area, porosity, and density. Therefore, the choice of excipients to be incorporated in any formulation is often of critical importance in achieving the goal of the dosage form design. Metronidazole is an antiprotozoal and anti-parasitic agent that is very effective in the treatment of amoebiasis, trichomoniasis, gardiasis and many other parasitic diseases. Its poorly soluble in water and its oral absorption is dissolution rate limited which can lead to potential bioinequivalence problems. Numerous approaches have been reported in literature to enhance the solubility of poorly soluble drugs. The approach chosen is dependent on certain aspects such as properties of the drug under consideration, nature of excipients to be selected, site of drug absorption and nature of the intended dosage form. In the present study, the effect of various size fractions of granules on the mechanical and release profile of metronidazole from compressed tablets was investigated.

2. Experimental

2.1. Materials

Maize starch, gelatin, magnesium stearate (BDH Chemicals Ltd, England), Star Lac (85% lactose monohydrate and 15% maize starch) (Meggle group Wasserburg, W. Germany), talc, magnesium stearate, lactose, Acacia, Avicel PH101 (Qualichem, India), distilled water (Energy center, UNN, Nigeria). Metronidazole is a generous gift from SyCom Pharmaceutical, India. All other reagents and solvents used are of Analar grade and were used as supplied.

2.2. Method

2.2.1. Preparation of Metronidazole Tablets

<table>
<thead>
<tr>
<th>INGREDIENTS (mg)</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
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<td>20</td>
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<tr>
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<td>Talc</td>
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<td>Lactose q.s to 400 mg</td>
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<td>Avicel q.s to 400 mg</td>
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<td>-</td>
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<td>132</td>
<td>-</td>
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</tr>
<tr>
<td>Star lack® q.s to 400 mg</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>132</td>
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</table>

*Particle size fractions of granules (mm)

Three batches of metronidazole tablets (A – C) were prepared by wet granulation method. Batch A contained gelatin (binder), corn starch (disintegrant) and lactose (diluent). Batch B one the other hand, contained acacia (binder), corn starch (disintegrant) and microcrystalline cellulose, MCC (diluent) while Batch C contained maize starch (binder), corn starch (disintegrant) and StarLac® (diluent). Metronidazolopowder, gelatin, corn starch and lactose were weighed out using an analytical balance (Adventurer AR3130, China) according to the composition presented in Table 1. The weighed samples were mixed and triturated in a mortar to form a homogenous mix. Weighed quantity of gelatin was dispersed in 7 ml distilled water with continual stirring to form a mucilage. The mucilage was incorporated into the powder mixture in a mortar and triturated to a homogenous wet mass. The wet mass was screened through a 1.70 mm stainless steel sieve and dried in the oven at 50 °C for 1h. The granules were further fractionated into three sieves.
fractions in order of descending particle sizes (1.0, 0.5 and 0.25 mm). The fractions were labelled A1, A2 and A3 from the largest to the least particle size. They were stored in well closed amber coloured bottles for further experiments. Two other granule/tablet batches were similarly prepared following the same procedure and their fractions were labelled B1, B2, B3 and C1, C2, C3 in descending order of particle size for each batch.

2.2.2. Micromeritic Properties of the Granules

(i). Bulk and Tapped Densities

A 50 g quantity of the granules was placed in a 100 mL measuring cylinder and the volume occupied by the sample was noted as the bulk volume. The bulk density was obtained by dividing the mass of the sample by the bulk volume, as shown in Equation 1:

\[ \text{Bulk Density} = \frac{\text{Mass of Powder (M)}}{\text{Bulk volume of powder (V_B)}} \]  

(ii). Flow Rate and Angle of Repose

A funnel was properly clamped on to a retort stand. The funnel orifice diameter, base diameter and efflux tube length were appropriately measured. A 25 g sample was transferred into an open funnel orifice had been closed with a shutter. The time taken for the entire sample in the funnel to flow through the orifice was weighed out and transferred into a funnel that the funnel was then recorded as the tapped volume. The tapped density was calculated using the formula:

\[ \text{Tapped Density} = \frac{\text{Mass of Powder (M)}}{\text{Tapped volume of powder (V_T)}} \] 

(iii). Carr’s Index and Hausner’s Quotient

The Carr’s compressibility index (%) of the granules were obtained using the formula:

\[ \text{Carr’s index} = \frac{\text{Tapped density (T_p)} - \text{Bulk density (B_d)}}{\text{Tapped density (T_p)}} \times 100 \]  

While Hausner’s quotient was determined by finding the ratio of the tapped density to bulk density using Equation 5:

\[ \text{Hausner’s quotient} = \frac{\text{Tapped density (T_p)}}{\text{Bulk density (B_d)}} \]  

2.2.3. Compression of Tablets

Prior to compression, all the granule batches were lubricated with 1% magnesium stearate and 1% Talc. Lubricated granules were compressed into tablets using an F-3 Manesty single punch tabletting machine fitted with 9.5 mm flat faced punches. Compression pressure was maintained at a constant pressure unit of the tabletting machine. Seventy tablets were produced for each batch. Tablet weights ranged from 360 to 396 mg. Prepared tablets were evaluated for hardness, friability, disintegration time, assay of active ingredients and dissolution profile studies using standard methods.

2.2.4. Dissolution Profile Studies

The Erweka DT dissolution apparatus fitted with a paddle that was operated at 50 rpm was used. The dissolution medium consisted of freshly prepared 0.1 N HCl maintained at 37±1°C. A tablet from each batch was placed in a basket (mesh size 325 mm) immersed half way into the dissolution medium. Five milliliter (5mL) volumes were withdrawn at predetermined time intervals and replaced with an equal volume of the dissolution medium maintained at the same temperature. Each withdrawn sample was analyzed spectrophotometrically at 278 nm using a Pye Cam SP8-1000 UV spectrophotometer. From the Beer’s calibration plot, the concentration of metronidazole released over time was calculated. The percentage amount of metronidazole released for each batch was plotted against time. The dissolution efficiency was calculated from Equation 6 and the DE at 60 minutes was expressed as a percentage of the ratio of the area under the dissolution profile curve (AUC) and of the rectangle described by 100% dissolution at the same time:

\[ \text{DE} = \frac{\text{AUC at Time, X}}{\text{AUC over the entire course of release}} \]  

Where DE = Dissolution efficiency of brand X, and AUC = area under the dissolution time curve.

The time taken for 50% of metronidazole to be released from each batch was also estimated from the dissolution profile curve. The dissolution efficiencies were obtained at 60 min.

3. Results and Discussion

3.1. Some Micromeritic Properties of the Metronidazole Granules

As earlier stated PS, PSD and the other formulation excipients have profound influence on almost every step involved in the formulation processes for solid dosage forms as well as affect the final drug product behavior, bioavailability/ efficacy and the overall quality of the product. In the drug product development process and quality control of dosage forms therefore, the need for adequate particle characterization of powder materials cannot be overemphasized. The particle size fractions of the batches...
as well as the type of binder and bulking agent used in the formulation had variable effects on the granule properties of the batches (Table 2) and on the mechanical properties (hardness, friability and hardness-friability ratio, (HFR) of the tablets (Table 3). The bulk and tapped densities of powders and granules provides an insight on their packing and densification behavior on compaction. From the results in Table 2, there was an increase from bulk to tapped densities as the particle size of the granules increased. This increase in densities might have resulted from densification produced by the tapping process. The results for the hopper flow rate of the granules ranged from 0.85 to 19.20 g/s and were generally observed to increase as the particle sizes of the granules increased within the batches. The Hausner’s quotient, Carr’s index and angle of repose are considered as indirect measurements of powder flowability and are also essential in determining the fluidity of the granules during tableting. They also give indication of the densification behavior on compaction which will eventually affect the weight and content uniformity of the final drug product. The flow indices based on angle of repose (Table 2), indicates that all the batches except A2, A3, B3 and C3 had fair to passable flow properties within the acceptable standard limit of 40 to 450.15 The results for Hausner’s quotient (Table 2) indicate that all the batches with the exception of A3, B3 and C3 were within the acceptable limit of < 1.25 indicating good flow properties. The HQ values of A3, B3 and C3 were within the limit of 1.26 – 1.59 indicating granules that are fluid and cohesive. The results for Carr’s index indicate excellent flowability for A1, B1 and C1, a fairly passable flow for A2, B2 and C2 and poor flow for A3, B3 and C3. The results from these indices of flow (AR, HQ and CI) indicate fairly good flow properties with increasing particle size. This should be expected as relatively small particles develop static charges, aggregate together and hinder free flow of granulations.

Table 2. Some micromeritic properties of the metronidazole granules.

<table>
<thead>
<tr>
<th>Granule properties</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD (g/ml)</td>
<td>0.62±0.06</td>
<td>0.61±0.06</td>
<td>0.58±0.04</td>
</tr>
<tr>
<td>TD (g/ml)</td>
<td>0.67±0.12</td>
<td>0.77±0.20</td>
<td>0.83±0.14</td>
</tr>
<tr>
<td>FR (g/s)</td>
<td>19.20±0.18</td>
<td>2.40±0.12</td>
<td>2.40±0.01</td>
</tr>
<tr>
<td>AR (°)</td>
<td>41.50±0.02</td>
<td>47.90±0.22</td>
<td>52.30±0.54</td>
</tr>
<tr>
<td>HQ</td>
<td>1.98±0.12</td>
<td>1.26±0.04</td>
<td>1.43±0.54</td>
</tr>
<tr>
<td>CI</td>
<td>7.5±0.18</td>
<td>2.70±0.12</td>
<td>30.12±0.25</td>
</tr>
</tbody>
</table>

Where BD is the bulk density ±standard deviation (SD), TD is tapped density, FR is flow rate, AR is angle of repose, HQ is Hausner’s quotient and CI is Carr’s index. The values represent the means ±Standard deviations(SD) for n=3 sampling time per batch. Statistically significant differences between the batches were measured using the Student T-test with SPSS version 16.

3.2. Mechanical Properties and In vitro Release Profile of the Metronidazole Tablets

The presence of different particle size fractions and excipients (binders and fillers) used in the formulation was also observed to have variable effects on the hardness, friability, hardness-friability ratio (HFR), disintegration time and invariably, the in vitro release profile of metronidazole from the tablets (Table 3 and Fig. 1, 2 and 3). The percentage weight deviations of the tablet batches from standard limits were in the range of 1.5 to 4.93%. According to BP 2009, not more than two of the individual tablets should deviate by 5% of the average weight for tablets >250mg. Based on this, the results (Table 3) indicate that all batches passed the weight uniformity test. According to some previously published work, uncoated tablets with hardness of ≥ 4 kgf are considered adequate for handling and transportation. From the results (Table 3), all the batches except A2 and A3 (containing gelatin as binder) were considered adequate in terms of their hardness. Despite the higher gelling property of gelatin when compared to acacia and starch, it was observed that all the batches in A with the exception of A1 (1.00 mm particle size fraction) had significantly lower hardness values than the other batches. A result consistent with that of its flow indices based on the AR, HQ and CI.

The lower hardness values observed in A could be due to the presence of lactose in the formulation since the other batches B and C both contained a more compressible direct compression excipient (MCC and StarLac®) as diluent. On the other hand, Batch B containing acacia as binder had a relatively higher hardness and friability value than all the other batches and this might be due to the binding mechanism involved with acacia. The results for friability, indicate that with the exceptions of A1, A3 and B2 all the batches were within the acceptable limit of ≤ 1%17,18. The values of hardness and friability provide measures of tablet strength and weakness, respectively. The parameter, hardness-friability ratio (HFR) have been employed as an index to measure the mechanical properties of ibuprofen tablets. The higher the HFR, the stronger the tablet19,20. The assay results of the batches ranged from 66.0±98.5% (Table 2). The different particle size fractions of the granules prior to compression and the different formulation excipients (binders and fillers) used in the batches was also found to have enhanced the disintegration time of the formulation batches with a resultant increase in its dissolution rate. This was especially observed in batch C containing maize starch as binder, corn starch as disintegrants and
StarLac® (85% α-lactose and 15% maize starch) as fillers. The presence of StarLac® as fillers in formulations has been found to improve tablet hardness, faster disintegration with a resultant increase in dissolution rate and these improvements are usually independent of the level of lubricant and the compaction pressure used. In the least, all the batches with the exception of A2, B1 and B2 released up to 50% of metronidazole within 30 min. This is likely due to the larger particle size fractions that make up these batches (A2, B1 and B2) when compared to the other batches. Improved dissolution and release rates of drug particles have been associated with smaller particle size resulting in enhancement of drug solubility. A graphical representation of the dissolution profile of metronidazole released over 2 h from the different batches is shown in Fig. 1, 2 and 3.

Table 3. Some mechanical and release properties of the metronidazole tablets.

<table>
<thead>
<tr>
<th>Granule properties</th>
<th>BATCHES/PARTICLE SIZE FRACTIONS(mm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>0.50</td>
<td>0.25</td>
<td>1.00</td>
</tr>
<tr>
<td>WD (%)</td>
<td>1.55</td>
<td>1.35</td>
<td>4.93</td>
<td>2.48</td>
</tr>
<tr>
<td>CS (KgF)± SD</td>
<td>4.19 (0.74)</td>
<td>2.96 (0.38)</td>
<td>2.98 (0.77)</td>
<td>9.52 (1.89)</td>
</tr>
<tr>
<td>F (%)</td>
<td>1.25</td>
<td>0.97</td>
<td>1.78</td>
<td>0.78</td>
</tr>
<tr>
<td>HFR</td>
<td>3.35</td>
<td>3.05</td>
<td>1.67</td>
<td>12.21</td>
</tr>
<tr>
<td>DT (min)</td>
<td>0.917</td>
<td>13.66</td>
<td>4.82</td>
<td>8.33</td>
</tr>
<tr>
<td>Assay (%)</td>
<td>98.50</td>
<td>96</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>DE</td>
<td>80.39</td>
<td>80.61</td>
<td>98.42</td>
<td>51.61</td>
</tr>
<tr>
<td>T_{50} (min)</td>
<td>18.10</td>
<td>33.06</td>
<td>7.02</td>
<td>44.01</td>
</tr>
</tbody>
</table>

where WD is percentage weight deviation, CS is crushing strength ± standard deviation, SD, F is friability, HFR is the hardness friability ratio, DT is the disintegration time, DE is dissolution efficiency and T_{50} is the time taken for 50% metronidazole release from the tablets. The values represent the means ± Standard deviations(SD) for 10 tablets per batch. Statistically significant differences between the batches were measured using the Student T-test with SPSS version 16.

(Fig. 1)
Generally, batches (C1 - C3) containing maize starch as binder and disintegrant and StarLac® as filler showed a higher release rate than the other batches. This was as a result of a higher concentration of starch present in the formulation than the other batches. In Fig. 1-3, it was observed that there was a significant (p < 0.05) increase in the rate of release of metronidazole from the tablets as the particle size decreased. The batches (A3, B3 and C3) with a particle size fraction of 0.25 mm exerted a more significant (p < 0.05) improved rate of drug release than the other batches with larger particle size fractions. Further analysis of in vitro release of metronidazole from the batches using the dissolution efficiency (DE) parameter indicated a similar effect in the rate of release. In an earlier study, we had established that the mechanism of improved dissolution by reduction in particle size is usually through the enhancement of drug solubility and this is consistent with the Nernst-Brunner theory\textsuperscript{20, 22}. According to the theory, the dissolution rate of a drug is directly proportional to the surface area available for drug dissolution; since the surface area increases with decreasing particle sizes, higher dissolution rates may be achieved through reduction of particle size\textsuperscript{[22]}.

Similar findings were observed in an earlier study that demonstrated an increased absorption rate for griseofulvin after reduction in particle size (micronization)\textsuperscript{22,23}. The expected in vivo bioavailability of metronidazole on the basis of the dissolution efficiency (DE\textsubscript{60})
parameter from the batches with 0.25 mm particle size fraction is in the order A3 (98.42%) > C3 (96.39%) > B3 (94.20%) while those with 0.5 mm particle sizes is in the order of C2 (81.36%) > A2 (80.61%) > B2 (53.47%) and those with 1.0 mm particle sizes is in the order of C1 (84.27%) > A1 (80.39%) > B1 (51.61%). There was an increase in the DE with decrease in particle size. Overall, batch C that contained the highest proportion of starch exhibited the best release profiles. The overall results further demonstrate that particle size and type of excipients used in formulation play vital role in the micromeritic properties of granules and on the performance of the final dosage form.

4. Conclusion

The results of this study, show the effects of different particle sizes and the presence of different formulation excipients on the mechanical strength and release characteristics of metronidazole from compressed tablets. The presence of the different excipients and particle size fractions influenced the rate and extent of dissolution of metronidazole from the prepared tablets. The results show that the maize starch – StarLac® combinations used in A and the gelatin-maize starch-lactose combinations used in C could be useful as good formulation additives that enhance the solubility and rate of release of metronidazole from tablet formulations and are therefore good candidates for the manufacture of metronidazole tablets.

References