**Regular Article**

**Tabletting Process of Pellets Containing Bioactive Fraction DLBS1033F Isolated from *Lumbricus rubellus*: Challenge and Strategy**

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DLBS1033 is a mixture of bioactive proteins fractionated from the earthworm *Lumbricus rubellus* which possesses antithrombotic and thrombolytic activities. DLBS1033 contains a potent fibrinolytic enzyme with excellent specificity for fibrin. A commercial oral enteric coated tablet for administration 3 times daily is available on the market from the DLBS manufacturer. This research aimed to develop a multiparticulate sustained-release DLBS1033 Forte (DLBS1033F) tablet (with more potent fibrinolytic activity than DLBS1033), which has targeted release in the small intestine and colon. The chemical and physical stability of the fibrinolytic enzyme was expected to be preserved in these bowel segments. The sustained-release system was designed to maintain the effective plasma concentration of the proteins and prolong the thrombolytic activity. This was done by incorporating the proteins in a multiparticulate solid that was subsequently compressed into the monolithic system of a sustained-release tablet. The challenge was to maintain pellet integrity after the compression process. A set of studies confirmed the preserved physical shape of the pellets. In addition, *in vitro* release studies of the multiparticulate tablet in reassembling media of the gastrointestinal tract indicated the successful formulation of DLBS1033F as an enteric as well as sustained-release solid dosage form.

**Key words** bioactive protein fraction; multiparticulate tablet; monolithic delayed release; thrombolysis; fibrinolysis

Thrombosis-related disease is currently becoming one of the most widely occurring disease. It can lead to other serious diseases such as cardiovascular disease. According to World Health Organization (WHO) report year 2012, number of deaths due to cardiovascular disease is 31% of all global deaths.¹)

Thrombosis-related disease which is characterized by the formation of thrombus or blood clot in circulatory system may be treated by administration of thrombolytic agents. However, the search for an ideal and effective thrombolytic agent is still a concern, due to the low activity and fibrin specificity, numbers of side effects, and also the cost needed.²)

*Lumbricus rubellus* is an earthworm originated from Indonesia and few other Asian countries. The bioactive fraction of this earthworm contains a group of serine protease enzymes with potent fibrinolytic activity, called the earthworm fibrinolytic enzymes (EFEs). Once the EFEs reach the gastrointestinal tract (GIT), they can be absorbed through intestinal epithelium and possess fibrinolytic and fibrinogenolytic properties. EFEs also have a good physical and chemical stabilities, which make them very potential to be developed into an oral thrombolytic drug.³)

DLBS1033F is a bioactive protein fraction isolated from *L. rubellus* which contains EFEs. DLBS1033 has characteristic similar to the general characteristic of lumbrokinase, i.e., the isoelectric pH is in the range of 3–5, water soluble, and logP of −3.4161. Lumbrokinase has strong proteolytic activity leading to its resistancy to GIT proteolytic enzymes and cellular enzymes. The stability pH and the optimal activity of DLBS1033 is in the range of 4–12. DLBS1033 consists of a various proteins with molecular weight is below 100 kDa. This group of protein is called Lumbricus low molecular-weight proteins (LLP), contributing in the thrombolytic activity of DLBS1033.³) DLBS1033F is the purified form of DLBS1033 which has 6-fold protease activity over DLBS1033.

The mechanism of absorption of DLBS1033 in the GIT is not explained clearly. However, our previous study demonstrated that EFEs are able to be transported through intestinal epithelium (Trisina et al.⁴). In addition, based on the study reported by Tjandrawinata et al.,⁵ the active components of DLBS1033 were transported through rat GIT in the intact forms.

Due to unstable at low pH and in gastric fluid as described above,⁶ in order to achieve successful oral delivery, manipulating the drug release in the safe place along the gastrointestinal tract with low enzymes content and neutral pH is absolutely necessary. By considering the complex physiological barrier in the GIT, a rigid solid form must be developed to prevent the leakage of the proteins in wrong place.⁷)

This report described the solid dosage form formulation of this potent protein. Pellet as a multiparticulate system was chosen to protect protein as well as to obtain a defined period of protein released in lower part of GIT, as an intermediate solid form. The final preparation was into tablet. The main challenge in pellet compression is maintaining its physical integrity and physicochemical stability. Weight and dose uniformity are also a concern after compression process.⁸ Many factors influencing the characteristic from multiparticulate system, such as pellet shape, size, porosity, density, excipients used, and the compression force were studied to obtain tablet with releasing the active DLBS1033 for 24h in the lower compartment of GIT.

**MATERIALS AND METHODS**

**Materials** DLBS1033F was prepared at pharma company

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Dexa Laboratories of Biomolecular Sciences, Cikarang, Indonesia, under Good Manufacturing Process (GMP) facility. Avicel® PH 101 (microcrystalline cellulose with particle size of around 50 μm), polyvinylpyrrolidone (PVP) K30, guar gum, calcium acetate, talcum, and magnesium stearate were purchased from PT Bratachem, Indonesia. Sodium alginate was purchased from Wako Pure Chemical Industries, Ltd., Japan. Bradford reagent and bovine serum albumine (BSA) were purchased from Bio-Rad Laboratories, Inc. All solvents and reagents used were analytical grade.

**Pellet Preparation** DLBS1033F pellets were prepared by extrusion–spheronization method. DLBS1033F, Avicel PH 101, PVP K-30, and release modifier matrices were mixed (Turbula mixer Tipe 2C), and subsequently granulated with deionized water, further on extruded with 1 mm pore size extruder (Erweka AR 401) and agitated at speed of 100 rpm. The extrudates were spheronized for 2 min with speed of 850 rpm (IKA® RW 20 Digital). Pellets were then dried at room temperature for 5 h. Detailed compositions of pellet formulations are presented in Table 1.

**Pellet Evaluation** Characterization of pellets consisted of size distribution analysis, morphology, flowability, bulk and tapped density, compressibility index, total protein content assay, and *in vitro* release study.

Size distribution of pellets was determined using sieving analysis method (Pharmatest, Germany). The particle size distribution was defined via the mass. Sieve analysis was used to divide the particulate material into size fractions and then determining the weight of these fractions. Furthermore, the percentage of weight of each fraction was plotted versus pellet size. In this way a relatively broad particle size spectrum can be analyzed quickly and reliably.

Flowability of pellet was measured using flow tester (Pharmatest). An empty glass beaker ($W_0$) was weighed and put under the apparatus. The funnel was filled with pellets. The flow tester was run, and the pellets were loaded in the empty glass beaker. Measurement was stopped before all the pellets were emptied from the funnel. The time required for the pellets to flow was noted. The loaded beaker glass ($W_1$) was weighed and the flow rate of pellets was calculated using following formula:

$$\text{Flowability} = \frac{W_1 - W_0}{\text{Time}}$$

Bulk density of the pellet was determined by weighing the pellet and slowly poured through the upper funnel until they overflow the receiving cup. The top of the receiving tube was leveled with a spatula such that it was completely full. The bulk density is the ratio of the mass of an untapped pellet sample and its volume including the contribution of the interparticulate void volume. The tapped density is an increased bulk density attained after mechanically tapping a container containing the pellet sample. After the bulk density test, 500 times of tapping was run. The resulting tap volume was noted and tapped density is the ratio of pellet mass and its volume after 500 times tapping. The compressibility index was further calculated:

$$C = 100\left(\frac{V_0 - V_t}{V_0}\right)$$

$V_0$=bulk volume; $V_t$=tapped volume

Total protein content was measured by using Bradford protein assay. Crushed pellet was dissolved in pH 6.8 phosphate buffer solution. An amount of 800 μL of this solution was mixed homogeneously with 200 μL of Bradford reagent and incubated at room temperature for 5 min. Total protein content was determined using UV-Vis spectrophotometer (Beckman DU 650i) at 595 nm, and a series of bovine serum albumin concentrations were used as a standard calibration curve.

The *in vitro* release study was carried out using dissolution apparatus 1 (basket, Hanson Research, U.S.A.) which was easily enabling continuous study with different media. The test was subsequently performed for 2 h in 250 mL pH 1.2 HCl medium and 6 h in 250 mL pH 6.8 phosphate buffer solution. A certain amount of pellets was put in the basket then the test was run in pH 1.2 HCl medium. After 2 h, the pellets remained in the basket, and the medium was changed to pH 6.8 phosphate buffer. In this medium, the test was run for 6 h observation. Both tests were performed at constant temperature of 37±0.5°C at 100 rpm. The total protein released at the sampling time were determined using Bradford protein assay.

**Scanning Electron Microscopic (SEM) Evaluation of DLBS1033F Pellets** In addition to visual observation, the pellets morphology as well as the surface appearance were also evaluated using SEM. SEM was performed on pellet formula F5 (formula of pellet with expected protein release profile) and tablet formula F8 (formula of tablet with expected protein release profile). The samples were fixed on a brass stub using double-sided tape and then gold coated in vacuum by a sputter coater. The pictures were taken at excitation voltage of 10 kV and at 10000–40000× magnification by using JSM-360LA Scanning Microscope (Jeol, Tokyo, Japan).

**Preparation of Tablet Containing Pellets of DLBS1033F** Detailed compositions of tablet formulations are presented in Table 2. Avicel granules were prepared by wet granulation method, consisted of 96% Avicel PH 101 and 4% PVP K-30. Combination of aqua deionized and 96% alcohol (1:1) was used as granulating agent. DLBS1033F pellet, Avicel granule, PVP K-30, and xanthan gum were mixed using Turbula Mixer for 10 min. Talcum was then added and mixed for 5 min. Lastly, magnesium stearate was added and mixed for 2 min. This mixture was then compressed using single punch tablet machine Erweka AR401, with round and flat punch.

### Table 1. Composition of DLBS1033F Pellet Formula

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
<th>F4 (%)</th>
<th>F5 (%)</th>
<th>F6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DLBS1033F</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Avicel PH 101</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>30</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>PVP K-30</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Sodium alginate</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Calcium acetate</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Guar gum</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Three batches of tablet were provided using 3 different compression forces resulted in tablet with hardness of 8, 10, and 12 kg/cm².

**Evaluation of Tablet** The multiparticulate tablet evaluations consisted of weight variation, thickness, hardness, friability, total protein, and *in vitro* release study. The weight variation and thickness of the tablets were determined using 20 samples taken randomly of each, respectively by weighing the individual tablet and measuring the individual thickness of the tablet with vernier calliper.

The tablet hardness was analysed using hardness tester (PharmaTest PTB 111, Germany). Twenty tablets were picked randomly and individually was tested. The average tablet hardness and its hardness variety based on its standard deviation (S.D.) were calculated.

The tablet friability was determined with friability tester (PharmaTest PTF). Twenty tablets was chosen randomly and placed into the device. One hundred rotations were run. All the twenty tablets were cleaned and weighed again. The percentage of weight loss during friability test was calculated as followed:

\[
\text{Percentage of weight loss} = \frac{\text{Weight loss}}{\text{Initial weight of 20 tablets}} \times 100\%
\]

The rigidity of the pellets after compression process was also observed under scanning electron microscope with the similar procedure as mentioned previously. The *in vitro* release study was carried out for 2h in 250mL pH 1.2 HCl medium and 22h in 250mL pH 6.8 phosphate buffer solution. This test was performed using United States Pharmacopoeia (USP) type I apparatus (basket), at constant temperature of 37°C for 10min. Trichloroacetic acid solution (100mM) was used to stop the degradation reaction. Centrifugation was then conducted to separate the soluble peptides. Further, the supernatant was mixed with Folin’s reagent under basic conditions, and the absorbance was measured at 660nm. A series of L-tyrosin solutions was used as a standard calibration curve.

**Protein Profile Characterization** Protein profile from both pellet and multiparticulate tablet were evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method using 12% polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Low molecular weight proteins were used as a protein marker.

**Protease Assay of DLBS1033** Protease assay of DLBS1033 in both pellet and multiparticulate tablet were performed as previously described by Trisina et al.²³ Crushed sample of pellet or tablet was dispersed in pH 6.8 phosphate buffer solution. Centrifugation was carried out to precipitate component in the formulas which were not dissolved in the medium. The supernatant containing DLBS1033 was collected and then mixed with casein solution (0.65%) and incubated at 37°C for 10min. Trichloroacetic acid solution (100mM) was used to stop the degradation reaction. Centrifugation was then conducted to separate the soluble peptides. Further, the supernatant was mixed with Folin’s reagent under basic conditions, and the absorbance was measured at 660nm. All data were analyzed with SPSS program. Statistical differences were calculated by unpaired Student’s *t*-test and considered significant at *p*<0.05.

**RESULTS**

**Pellet Evaluation** A set of parameters was evaluated to ensure the physical characteristic of pellet formulations. All measurements were done in triplicate (Table 3).

As presented in Table 3, all pellet formulations exhibited a good spherical shape with narrow size distribution between 500–841µm, good flowability and compressibility indicating good physical properties.

**In Vitro Total Protein Released from Pellet** This evaluation was performed to measure the total protein release profile

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**Table 2. Composition of Tablet Formula Containing DLBS1033F Pellet**

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>F7 (%)</th>
<th>F8 (%)</th>
<th>F9 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DLBS1033F pellet</td>
<td>33.33</td>
<td>33.33</td>
<td>33.33</td>
</tr>
<tr>
<td>2</td>
<td>Avicel granule</td>
<td>49.67</td>
<td>39.67</td>
<td>29.67</td>
</tr>
<tr>
<td>3</td>
<td>PVP K-30</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Xanthan gum</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Talcum</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium stearate</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3. The Parameters of Pellet Containing DLBS1033**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size distribution (%)</td>
<td>80.27</td>
<td>81.23</td>
<td>78.30</td>
<td>82.94</td>
<td>86.32</td>
<td>75.43</td>
</tr>
<tr>
<td>Flowability (g/s)</td>
<td>9.34±0.73</td>
<td>8.99±0.21</td>
<td>9.42±0.68</td>
<td>11.10±0.35</td>
<td>10.38±0.15</td>
<td>9.66±0.27</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>0.699±0.016</td>
<td>0.649±0.023</td>
<td>0.643±0.040</td>
<td>0.715±0.014</td>
<td>0.724±0.025</td>
<td>0.736±0.011</td>
</tr>
<tr>
<td>Tapped density (g/cm³)</td>
<td>0.740±0.054</td>
<td>0.720±0.037</td>
<td>0.731±0.018</td>
<td>0.772±0.007</td>
<td>0.755±0.013</td>
<td>0.786±0.023</td>
</tr>
<tr>
<td>Compressibility index (%)</td>
<td>9.59±0.10</td>
<td>9.88±0.58</td>
<td>10.02±0.31</td>
<td>7.41±0.49</td>
<td>6.42±0.64</td>
<td>6.15±0.22</td>
</tr>
<tr>
<td>Protein content per mg of pellet (µg)</td>
<td>7.16±0.27</td>
<td>7.23±0.64</td>
<td>7.18±0.41</td>
<td>7.25±0.94</td>
<td>7.24±0.33</td>
<td>7.14±0.78</td>
</tr>
</tbody>
</table>

* The data of size distribution shown in the table represents % of pellets in the range of diameter 500–841µm.
from pellet formulations. Figure 1 shows the comparison of protein release profile for pellet formulated using guar gum as a release modifier matrix. Those 3 release profiles were statistically analyzed using one way ANOVA with confidence level approximately 95% and showed significant difference at p value of 0.0336 ($p < 0.05$). All formulations released more than 20% of total protein after first 2 h in HCl medium pH 1.2. Formula F2 shows expected profile with 88.24% of total protein released at the end of 8 h observation.

Figure 2 shows the profile of in vitro protein released from pellet with combination of sodium alginate and calcium acetate as release modifier matrices. Formula F4–F6 exhibited a better profile as compared to formula F1–F3 presented in Fig. 1. This indicated a lower initial protein release at the first 2 h. Formula F5 shows the best protein release profile and was used for further compression process. Figure 3 shows the visual (a) and microscopic (b) morphology of pellet on formula F5. The release profiles from Formula F4–F6 were statistically analyzed using one way ANOVA with confidence level approximately 95% and showed significant difference at $p$ value of 0.0421 ($p < 0.05$).

**Optimization of Tablet Compression Force** Figure 4 shows the pellets morphology (SEM analysis) embedded in the tablet compressed using three different compression forces resulting in tablet hardness of 8, 10, and 12 kg/cm². Figure 4a shows spherical intact pellets dispersed in the tablet and Fig. 4b confirms that the pellet morphology was preserved after

![Fig. 2. In Vitro Protein Release Profile of Pellet on Formulas F4, F5, and F6](image)

A significant difference analyzed by one way ANOVA; 95% of confidential level was shown with $p = 0.0421$.

![Fig. 3. The Morphological Observation of Pellet on Formula F5: Visual Observation (a) and Scanning Electron Microscopic Observation (b) Magnification 30× and 100×](image)

![Fig. 4. SEM Presentation of Tablet on Formula F8 with Hardness of 8 kg/cm² (a) 30× Magnification, (b) 100× Magnification; 10 kg/cm² (c) 30× Magnification, (d) 100× Magnification; and 12 kg/cm² (e) 30× Magnification, (f) 100× Magnification](image)
compression with the lowest force. Using higher compression forces resulting hardness of 10 and 12 kg/cm² did not cause physical damage of the pellets in the tablet (Figs. 4c–f).

**Evaluation of Tablet Containing Pellet of DLBS1033**

Figure 5 shows the visual observation of all multiparticulate tablet formulations. As presented, all formulas resulted in a round and flat tablet with yellowish spots indicating the pellets. A set of parameters as presented in Table 4 were evaluated to confirm the successful compaction process of the tablet formulas in the presence of sufficient amount of pellets.

As shown in the table, the presence of sufficient amount of DLBS1033 pellets did not influence the physical performance of the tablets. Importantly, the protein DLBS1033 was not deteriorated after compression procedure, indicated by high protein content was remained. This conclusion was confirmed by data in Figs. 6 and 7 below.

**In Vitro Protein Release from Tablet** The profile of protein release in vitro was depicted in Fig. 6. Tablet formula F8 shows the best release profile i.e., lower release of 5.11% at first 2 h in pH 1.2 HCl medium, and followed by released protein in pH 6.8 phosphate buffer medium until end of experiment (24h). The release profiles of tablet formula F7–F9 were statistically analyzed using one way ANOVA with confidence level approximately 95% and showed significant difference at p value of 0.0154 (p<0.05).

**Protein Characterization** This study was performed to confirm the stability of protein during formulation processes. The stability of protein in the pellet as well as after compression procedure was shown in Fig. 7 below.

**Table 4. Parameter of Tablet in the Presence of DLBS1033 Pellet**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of sample</th>
<th>Formula F7</th>
<th>Formula F8</th>
<th>Formula F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width (cm)</td>
<td>20</td>
<td>0.505±0.009</td>
<td>0.505±0.005</td>
<td>0.500±0.005</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td>20</td>
<td>1.01±0.005</td>
<td>1.01±0.005</td>
<td>1.02±0.008</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td>10</td>
<td>9.9±0.2</td>
<td>9.8±0.2</td>
<td>9.8±0.3</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>20</td>
<td>750.6±4.4</td>
<td>750.2±3.0</td>
<td>750.5±2.5</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>10</td>
<td>1.37</td>
<td>0.91</td>
<td>1.31</td>
</tr>
<tr>
<td>Protein content per mg of pellet (µg)</td>
<td>10</td>
<td>2.27±0.29</td>
<td>2.31±0.18</td>
<td>2.36±0.53</td>
</tr>
</tbody>
</table>

Fig. 5. Visual Appearance of Tablet in the Presence of Sufficient Amount of DLBS1033 Pellet Formula F7 (a), Formula F8 (b), and Formula F9 (c)

Fig. 6. In Vitro Release Profile of Pellet Tablet on Formulas F7 (10% Xantan Gum, XG), F8 (20% Xantan Gum, XG), and F9 (30% Xantan Gum, XG)

A significant difference analyzed by one way ANOVA; 95% of confidential level was shown with p=0.0154.

Fig. 7. Electrophoregram of Protein on SDS-PAGE Gel

pression was studied using SDS-PAGE. As shown in Fig. 7, protein in the pellet F5 and in the tablet F8 revealed similar profile with pure DLBS1033F as a standard.

Protease Activity Assay  Protease activity assay of DLBS1033 was done to ensure the stability of the protein after a serie of formulation processes. In addition, this step was also to confirm the presence of protein in the formula detected by SDS-PAGE (Fig. 6). Table 5 shows the protease activity assay result for pellet F5 and multiparticulate tablet F8.

**DISCUSSION**

The multiparticulate system is a beneficial approach to control the release of active substance from the solid dosage forms. In this report, we developed multiparticulate system to control the release of bioactive fraction DLBS1033F aiming for 24 h release in the intestinal compartment. Due to partition of the active compound in small unit form pellet, our target to prevent the immediate leakage of the protein in the gastric compartment is enabling to obtain. Compression of pellet into tablet has to guarantee that no significant physical alterations occur during and after the process. Therefore a careful consideration must be taken into account on each material used and each step of process.  

In this study, pellet was prepared using extrusion-spheronization method i.e., a technique that incorporates multiple processes involving extrusion followed by spherization to produce uniform size and free flowing spherical particles. Avice1 PH 101 was used as a pellet matrix due to its ability to produce spherical pellets with good flow properties, especially when combined with PVP as a binder.  

The addition of release modifier matrix in the formulation was aimed to sustain the protein release from the pellet formulation, further to prolong the drug diffusion from the dosage form over a specific amount of time. Therefore, the drug can be absorbed continuously into the bloodstream maintaining the action. In this study, the ability of guar gum to sustain the drug release was compared with combination of sodium alginate and calcium acetate.  

In *in vitro* release data exhibited that combination of sodium alginate and calcium acetate was able to sustain the protein release better than guar gum alone. This is due to the natural behavior of sodium alginate, which is insoluble in acidic pH and only soluble in neutral to basic pH. The insoluble sodium alginate functioned as a drug diffusion barrier in the acidic pH. At higher pH, sodium alginate started to dissolve and cross-linked with calcium acetate, hence creating a stable 3 dimensions structure. This structure was able to sustain the diffusion of drug dissolved in the aqueous media inside the pellet. Pellet formula F5 showed the best protein release profile with less than 20% of protein detected in HCl medium and about 90% in phosphate buffer medium.  

Compression of multiparticulate system into tablet absolutely needs the addition of suitable excipient to prevent the rupture and damage of the pellet. In this formulation, Avicel granule was used as a cushioning agent that helped to stabilize the pellet during the compression process. This excipient also helped to maintain the uniformity amount of pellet in each tablet compressed. Many studies have been carried out on the ability of Avicel in compression process. It shows plastic deformation during compression and therefore offers better protection for pellet shape and reduce the tendency of alteration in release characteristics.  

Significant changes in pellet porosity, sphericity, and morphology after compression are able to alter the physical properties and drug release profile of the tablet. Therefore a careful optimization of compression force during tableting process was in the range of hardness of 8–12 kg/cm². As seen, pellets with hardness of 8 and 10 kg/cm² remained in good physical integrity guaranteeing no leakage of protein and subsequently controlled its release in the right compartment. Pellets with hardness of 12 kg/cm² showed physical deformation. Therefore, the compression force resulting tablets with hardness of 10 kg/cm² was selected and used for further studies.  

While, application of xanthan gum in our study was aimed to control the protein release in subsequent media mimicking the fate of formulation in GIT. Xanthan gum is a natural polysaccharide consisted of 2 glucose residues, 2 mannose residues, and 1 glucoronic acid residue per monomer. Xanthan gum was chosen due to its stability in the aqueous solution and its ability to act as a matrix that sustains the drug release from a system and not dependent on the environment pH value. Xanthan gum mediates the drug release by hydration and swelling process that followed by drug diffusion. It will hydrate and swell continuously in the aqueous environment that allows the drug to diffuse out of the system.  

All tablet formulas show protein release in HCl medium less than 5% *i.e.*, the maximum release percentage requirement for enteric dosage forms. This gave a benefit of preventing the degradation of the protein both by low pH as well as by proteolytic enzymes presence in the gastric. As targeted, protein DLBS1033 was released completely in phosphate buffer pH 6.8 with time as expected *i.e.*, 24 h, depicted by tablet formulas F8 and F9. Between these two formulas F8 and F9, only F8 was showing perfectly desired characteristic

<table>
<thead>
<tr>
<th>Samples</th>
<th>DLBS1033 content in the sample (%)</th>
<th>Protease activity (unit/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBS1033F powder</td>
<td>100</td>
<td>66.44±1.78</td>
</tr>
<tr>
<td>Pellet F5</td>
<td>59.1</td>
<td>38.19±2.05</td>
</tr>
<tr>
<td>Multiparticulate tablet F8</td>
<td>18.8</td>
<td>11.77±0.65</td>
</tr>
</tbody>
</table>

Table 5. Enzymatic Activity of DLBS1033 in Different Samples

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order equation</th>
<th>First order equation</th>
<th>Higuchi equation</th>
<th>Korsmeyer–Peppas equation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$R^2$</td>
<td>$R^2$</td>
<td>$n$</td>
</tr>
<tr>
<td>F7</td>
<td>0.789</td>
<td>0.804</td>
<td>0.923</td>
<td>0.893</td>
</tr>
<tr>
<td>F8</td>
<td>0.931</td>
<td>0.971</td>
<td>0.976</td>
<td>0.980</td>
</tr>
<tr>
<td>F9</td>
<td>0.962</td>
<td>0.952</td>
<td>0.950</td>
<td>0.954</td>
</tr>
</tbody>
</table>

Table 6. The Kinetic Release Model of Tablet Formulas
with slow release profile and >80% of the protein released after 24 h of observation. In addition, when the release pattern of tablet formula F8 was analyzed for kinetic models such as zero order, first order, Higuchi, and Korsmeyer–Peppas, the Korsmeyer–Peppas model seems to be the best fit model with n value >1 (Table 6). This means that the release of protein from the dosage form follows the super case-II transport model. The drug release was suggested via diffusion mechanism and also the relaxation of polymer chain.24)

DLBS1033 was a protein with in general prone to degradation under various conditions including high stress of mechanical force like spheronomization process during pelleting and compression during tableting process. The question about conformation stability influencing the enzymatic activity therefore must be confirmed by a series of studies. As we reported, the preserved protein profile on SDS-PAGE gel clearly indicated that there was no degradation signs of protein observed. Further confirmation described the stability of protein during formulation is the protease activity. In line with the data on SDS-PAGE gel, there is insignificant reduction in the protease activity of DLBS1033 in both pellet and tablet.

CONCLUSION

Multiparticulate tablet containing 33.33% pellet and 20% xanthan gum slowed the release of DLBS1033 in the lower part of gastrointestinal tract up to 24 h. The protein remained stable and the protease activity was preserved after a series of formulations involving pelleting as well as tableting process. The parameter process and the component used in this study were able to maintain the rigidity of pellet in the tablet avoiding protein leakage from the pellet. Our strategy therefore is promising to develop a dosage form containing bioactive molecule for oral sustained release target.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES