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Effects of the controlled temperature in the production of high-shear granulated protein-containing granules

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ABSTRACT

During wet granulation, the sample can be exposed to considerable mechanical effects, moisture content and elevated temperatures, and during high-shear granulation the impeller and chopper speeds can induce elevated temperatures and influence the parameters of the products. In our work, we therefore aimed to investigate the effect of cooling and process parameters on product parameters by factorial design in accordance with QbD guidelines. Our other goal was to study the effect of the type of granulation, therefore two series were used to prepare granules in a high-shear granulator, with water and binder solution as granulating fluid, at different chopper and impeller speeds with application of factorial design. The particle size was higher when cooling in the case of granules prepared with binder solution. The pepsin solution had a good granulating effect, the granules displayed a larger particle size, a higher breaking hardness and a favourable deformation process.

KEYWORDS

Protein, high-shear granulation, enzyme activity, factorial design, micro-CT

1. Introduction

Biologically active peptides and proteins are increasingly becoming a very important class of therapeutic agents because of their highly specific activity and very well tolerability by the human body [1, 2]. This may allow the direct application of these materials [3-5].

Pepsin, the major digestive enzyme of gastric juice, is responsible for the most digestive activity in the stomach [7]. It is a broad-specific endopeptidase that is commonly synthesized in gastric chief cells as a zymogen enzyme [8]. Hydrochloric acid produced by the gastric mucosa, is required to convert the inactive enzyme and maintain optimum acidity (pH=1-3) for pepsin function [9]. Typical therapeutic uses of pepsin include pathological condition associated with hypo- or acidity, such as Sjögren's syndrome. As an enzyme, pepsin is also a protein. Proteins are very sensitive materials: elevated temperature, mechanical effects, moisture content [10, 11], etc. can all decrease the enzymatic activity. The stability of enzymes is one of the most important parameter during formulation of dosage forms, in consequence of the great number of factors involved [12].

Pepsin is a stable enzyme, retaining its enzyme activity (A) level in solution for 1 year when stored at 4 °C, indicating that autohydrolysis is negligible [13], which means that it is still able to perform its enzymatic functions.

In the pharmaceutical industry fluid bed granulation [14], extrusion/spheronization [15] and high-shear wet granulation are frequently utilized in order to process fine cohesive powders into dense, round granules [16-19]. During high-shear granulation the dry powders are first mixed by the impeller, which rotates through the powder bed. In the second step, a liquid binder is added, the impeller ensuring spreading of the liquid and the chopper breaking down wet agglomerates [20-25]. This section can be divided into 3 further stages, wetting and nucleation, growth and consolidation, and breakage and attrition [26-28]. Finally, the granules

are rounded without the addition of liquid. On the basis of the impeller torque, Lin et al. identified 6 stages of the granulation process: wetting stage, nucleation, growing stage, granule shaping stage, granulation overwetting stage, binder overdosing stage and slurry stage [29]. Some of the advantages of this technology include increased bulk densities, improved flow properties, reduced powder segregation and better handling properties [30-33]. The other advantages of this granulation are short process time, efficient mixing of the powder blend and lower liquid amount required than with other granulation methods [34].

Reliable technology processes demand an understanding of the granulation processes, and identification and application of the critical factors that determine the granulation quality [35]. The ICH Q8, Q9, Q10 and Q12 guidelines emphasize the adoption of quality by design (QbD) [23, 36] in the development of pharmaceutical products; this is a systematic approach based on scientific principles [37-39].

During wet granulation, the sample can be exposed to considerable mechanical effects, moisture content and elevated temperatures, and during high-shear granulation the impeller and chopper speeds can induce elevated temperatures and influence the parameters of the products [40-43]. Several researchers have studied the effect of process parameters on product parameters during high-shear granulation [44-49], but the effect of temperature has not been studied. In our previous work, we therefore measured the change in temperature at different points in the chamber during granulation. We found that due to friction, a significant temperature rise should be expected with the use of high impeller speeds [41, 43]. In the present work, we therefore aimed to investigate the effect of critical product parameters (CPPs) (cooling, impeller and chopper speed) on critical quality attributes (CQAs) by factorial design in accordance with QbD guidelines. The CQAs were the *A* and the particle size.

This information can deepen the understanding of the effects of different technological processes, which is indispensable for determination of the critical control points in the

preparation of solid dosage forms containing proteins. Preservation of the A level of pepsin and other proteins should be taken into consideration during the formulation. This study emphasizes the importance of special aspects in the processing of solid dosage forms containing proteins. Its relevance is constantly increasing because of the spreading of biotechnology and protein-type active agents.

2. Materials and methods

2.1. Materials

In the course of the experimental work, purified water (distilled water, Ph. Eur. 10th Ed.) and microcrystalline cellulose (MCC; Vivapur 100, J. Rettenmaier & Söhne GmbH + Co., Rosenberg, Germany) (*D50* 65 μm ; bulk density 0.29 g/cm^3 ; data from producer) were used. The active pharmaceutical ingredient was porcine pepsin powder (Ph. Eur. 10th Ed., Meditop Ltd., Pilisborosjenő, Hungary) (*D50* 10 μm). Bovine hemoglobin (Sigma-Aldrich), Folin-Ciocalteu reagent (Sigma-Aldrich), trichloroacetic acid (Molar Chemicals Ltd.), hydrochloric acid (Ph. Eur. 10th Ed.) and sodium hydroxide (Ph. Eur. 10th Ed.) were used for pepsin activity measurement.

2.2. Sample preparation and the factorial design

Two series of granules were produced. In Series 1 (granule prepared with binder solution), the granulating fluid was a 4% aqueous pepsin solution (100 ml), protein solutions exhibiting excellent adhesive properties [47]. The pepsin powder was dissolved in distilled water, and mixed with magnetic stirrer for 30 min at room temperature. The powder consisted of MCC (100 g).

In Series 2 (granule prepared with water), the granulating fluid was purified water (96 ml), and the powder mixture contained pepsin powder (4 g) and MCC (100 g). The powder was homogenized with a Turbula mixer (Willy A. Bachofen Maschinenfabrik, Basel, Switzerland) for 10 min.

The wet granulating process was carried out in a Pro-C-epT 4M8 high-shear granulator (ProCepTnv, Zelzate, Belgium). The diameter of the chamber is 18.5 cm and the height is 15.2 cm.

The impeller and chopper are both positioned vertically in the high-shear granulator, and their speeds were taken as the factors in the factorial design, chosen on the basis of the preformulation studies. As the temperature may increase during the process, the experiments were repeated with cooling. In this case, a jacketed vessel was used, with water at 20 °C flowing between the two layers of the vessel. The granules were dried at room temperature (25 ± 1 °C) for 24 h. The temperature was continuously monitored by the high-shear granulator's software. At high impeller speeds, the temperature increase was higher than at low impeller speeds [16]. Therefore, cooling was also used as a factor during factorial design.

The factorial design is a suitable method for modelling and predicting the effects of the technological parameters (the chopper and impeller speeds). The mixed 2-level and 3-level factorial design was applied (Table 1). The high levels were based on the technical parameters of the high shear granulator, and the low levels on the results of the preformulation studies. The variation interval was the range between the high and low levels. Cooling was -1 in the case of formulation without controlled temperature, and it was +1 in the case of controlled temperature. Impeller speed levels were 300, 900, and 1500 rpm, and for the chopper speed, 500, 2750, and 5000 rpm.

The other parameters were standard (Table 2).

The experiment was based on a mixed 2-level and 3-level factorial design, with the equation

$$y = b_0 + b_1(L)x_1 + b_1(Q)x_1^2 + b_2(L)x_2 + b_2(Q)x_2^2 + b_3(L)x_3 + b_3(Q)x_3^2 + b_1(L)_2(L)x_1x_2 + b_1(L)_2(Q)x_1x_2^2 +$$

$$b_1(Q)_2(L)x_1^2x_2+b_1(Q)_2(Q)x_1^2x_2^2+b_1(L)_3(L)x_1x_3+b_1(L)_3(Q)x_1x_3^2+b_1(Q)_3(L)x_1^2x_3+b_1(Q)_3(Q)x_1^2x_3^2+b_2(L)_3(L)x_2x_3+b_2(L)_3(Q)x_2x_3^2+b_2(Q)_3(L)x_2^2x_3+b_2(Q)_3(Q)x_2^2x_3^2 \text{ where}$$

y : the optimization parameter

b_0 : the average optimization parameter value

b_1 : a coefficient describing the effect of the chopper speed

b_2 : a coefficient describing the effect of the impeller speed

b_3 : a coefficient describing the effect of cooling

b_{12} : a coefficient describing the effect of the interaction of the chopper and impeller speeds

b_{13} : a coefficient describing the effect of the interaction of the chopper speed and cooling

b_{23} : a coefficient describing the effect of the interaction of the impeller speed and cooling

x_1 : the chopper speed

x_2 : the impeller speed

x_3 : cooling

Q: quadratic part

L: linear part

The optimization parameters were the average particle size (D50) and the enzyme activity (A) value.

2.3. Statistical evaluation

Tibco Statistica v13.4.0.14 (Statsoft, USA) software was used for the statistical evaluation of the results. This software can calculate the coefficients ($b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}$) and ignore the redundant effects. Additional coefficients were ignored manually until the

adjusted R^2 (Adj. R^2) reached its maximum value. The lack of overdetermination in the modified equations results in a better predictive power and promotes the determination of a robust design space [50, 51].

2.4. The average particle size (D_{50}) and the size distribution

The granules on the 2000 μm sieve were discarded. The samples were evaluated with an analytical sieve series ranging from 1400 μm to 125 μm (mesh sizes: 1400, 1250, 1120, 1000, 630, 500, 400, 315, 200 and 125 μm) (Retsch GmbH, Haan, Germany). Retsch EasySieve 2.0 software was used to calculate D_{50} and the distribution. The sieving analysis time was 10 min. Our preliminary results revealed no change in the distribution results over the 10 min. The sieve shaking was performed on an analytical sieve shaker. The amplitude was 1.5 mm. The D_{50} values were used for comparison.

2.5. Determination of enzyme activity (A)

The A values of the samples relative to the substrate bovine haemoglobin were measured according to the Ph. Eur. 10th Ed. The basis of the analysis was the measurement of the amount of protein which could not be precipitated with trichloroacetic acid. Haemoglobin was dissolved at 2% in 0.1 M hydrochloric acid solution, and the pH was adjusted to 1.6 ± 0.1 . The samples and the untreated pepsin powder were dissolved at 0.25% in 0.1 M hydrochloric acid, and the pH was adjusted to 1.6 ± 0.1 . Incubation was performed for 10 min at 25 °C. 4% trichloroacetic acid solution was used to precipitate the proteins. The samples were filtered twice through filter paper, leached with 5 ml of trichloroacetic acid and dried. After dilution, 1 ml of (5M) sodium hydroxide and 1 ml of Folin-Ciocalteu reagent were added as colour-producing reagent, and the solution was left to stand for 15 min at room temperature. The relative A was determined; the A value of the untreated pepsin was taken as 100%. The

amount of non-precipitating protein was determined at 540 nm with a UV spectrophotometer (Unicam Helios Alpha, Spectronic Unicam, Cambridge, UK).

2.6. Mechanical property

The breaking hardness was tested for granules measuring between 630 and 1120 μm . The self-developed device contains a special specimen holder and a stamp, and is connected to a computer via an interface; thus, not only can the ultimate deformation force be measured, but the process (force–time and force–displacement curves) can also be followed. If the measured plot (force–time) is parallel to the x-axis the deformation is viscoelastic; if the plot rises linearly, the deformation is elastic. The specimen is located horizontally and the stamp moves vertically (Figure 1). Twenty parallel measurements were performed. The measuring range was 0–200 N, the speed of the stamp was 20 mm/min, the output was 0–5 V, and the sensitivity was $\pm 0.5\%$ ± 0.1 digit. The sensor was UNICELL force measuring equipment, calibrated with the C9B 20 kN cell (Hofinger Brüel & Kjaer GmbH, Darmstadt, Germany).

2.7. Scanning electron microscopy (SEM)

A Hitachi S2400 (Hitachi Scientific Instruments Ltd, Tokyo, Japan) scanning electron microscope was used to determine the shape and the surface of the particles. A sputter coating apparatus, Polaron E5100 (Polaron Equipment Ltd, Hertfordshire, England), was applied to induce electric conductivity on the surface of the sample. The air pressure was 1.3–13 mPa.

2.8. Micro computed tomography (micro-CT) measurements

The micro-CT (computed tomography) measurements for further morphological and structural characterization of the samples were carried out using a Bruker Skyscan 2211 X-ray nanotomograph. The two different types of granules were packed into quartz capillary tubes

(1 and 2 mm in diameter) (Hilgenberg GmbH, Germany) and were scanned using an open type pumped X-ray source operating at 70 kV tube voltage and 450 μ A emission current. Each sample was measured with 3 μ m pixel resolution using an 11 Mp cooled CCD camera with an exposure time of 350 ms. A total of 1293 projection images were obtained by a 180° rotation of the sample with 0.15° rotation step in 120 min scan time. After reconstruction of the images with NRecon (Skyscan Bruker, Belgium) software, the volume rendered 3D CT images were visualized using the CT-Vox (Skyscan Bruker, Belgium) software.

3. Results and discussion

3.1. Particle size of granules

In Series 1 (granules prepared with binder solution), the particle size (D_{50}) for the various samples ranged between 319 and 1410 μ m (Table 3), with an average D_{50} for the complete series (b_0) of 972.25 μ m (Eq.1). In Series 2 (with pepsin in the powder mixture), D_{50} was less than in Series 1 (the average D_{50} (b_0) was 768.83 μ m (Eq.2)). The reason is that the pepsin powder started to dissolve during the operation, and the growth of the particles then began. In Series 1, pepsin was in the granulating liquid, and the growth of the particles therefore began directly. In Series 2, the dissolution of pepsin occurred during the process time, and the time of growth of the particles was therefore less than in Series 1. The tendency was the same both with and without cooling and in both series (Figure 2).

The highest D_{50} was observed in the case of the minimum-minimum impeller-chopper combination because the particles are subject to lower levels of breakage and friction at low impeller speed. The other reason for this is that in this case the lower impeller speed resulted in a lower temperature, which also resulted in lower evaporation. Thus when the temperature was controlled (20°C), the effect of the impeller speed was lower.

It can be concluded that the application of cooling has a significant effect on the D50 value for both series. In Series 1 the b_3 coefficient was 216.5, while in Series 2 it was 454, which is almost twice as high (Eq. 1-2). This may be due to the fact that at Series 1, pepsin is present in the granulating liquid, which is continuously added to the chamber in the form of a room temperature solution. The binder thus absorbs the internal temperature of the chamber as soon as it enters the chamber, which can be very high (48-50° C) in the case of granulation without cooling at high impeller speeds. In Series 2, the coefficient b_3 is much higher, (454), which can be explained by the fact that in this case the pepsin powder, which will act as a binder, is present in full in the chamber from the beginning of the granulation process. Therefore, in this case, the total amount of pepsin will be the same as the temperature of the chamber, so it can be seen that cooling plays a more significant role in this case. Thus, it can be concluded that using cooling, the particle size shows a clear increase (Figure 2). This is explained by the fact that at higher temperatures water evaporates faster, while with cooling the rate of evaporation decreases, which helps to maintain the proper moisture content of the particles and thus their growth for a longer period of time.

D50 was found to be described by the following equations:

In Series 1:

$$y = 972.25^* - 212.75x_1^* + 250.125x_1^{2*} - 518.25x_2^* + 216.5x_3^* + 295.25x_1x_2^* - 55.875x_1^2x_3^* + 306.75x_2x_3^*$$

(Eq.1)

R^2 : 0.99577; Adj. R^2 : 0.98096; MS Residual: 2611.62

In Series 2:

$$y = 768.833^* - 46.5x_1 + 432.25x_1^{2*} - 327.5x_2^* + 454x_3^* + 137.5x_1x_2 - 282x_1^2x_3 + 188x_2x_3 + 21x_1x_3$$

(Eq.2)

R²: 0.99923; Adj. R²: 0.99307; MS Residual: 882

* Statistically significant

where

y: *D50*

3.2. Activity of enzyme (A)

The results showed that the effect of cooling (b_5), on the *A* for Series 1 (56.99) was 65% higher than for Series 2 (36.61) (Figure 3, Eq. 3-4). The reason is that the pepsin solution is less stable than the solid form. In Series 1, high extremities were observed in the *A* values. The minimum *A* in Series 1 was 6.48% and the maximum *A* was 101.1%, as compared with a minimum of 3.48% and a maximum of 90.91% in Series 2. This can be explained by the fact that in Series 2 the total quantity of pepsin was exposed to the effect of the high impeller speed throughout the operation time. In Series 2, however, the mechanical effects influenced *A* throughout the process. In Series 1 pepsin was added to the powder mixture continuously, and in this case, therefore, it was exposed to mechanical effects for less time than the total process time. This phenomenon can also be explained by the better stability of the pepsin powder than that of the solution. Our earlier results revealed that the pepsin solution was more sensitive to temperature than the pepsin powder [15]. In Series 1, average *A* was 45.96% (b_0), while in Series 2 it was 47.85% (b_0). Pepsin in powder form is more stable, and the increase in this case is therefore lower (Eq. 3-4).

When the impeller and chopper speeds were high (1500 and 5000 rpm), A was extremely low (6.48% and 3.48%) (Table 3) without controlled temperature, but significantly higher (58.23 % and 46.40%) at controlled temperature.

Higher speed leads to greater thermal and mechanical effects, which result in lower A (Eq.3-4), therefore, significantly high b_2 (impeller speed) coefficient values were obtained in both cases. The effect of the impeller speed (b_2) was higher than the chopper speed (b_1) in both cases. The reason is the shape of the impeller and its higher mechanical effect.

A could be described with the following equations:

In Series 1:

$$y = 45.958^* - 8.3375x_1^* - 25.25x_2^* + 56.99x_3^* + 6.58x_1x_2 - 2.19x_1x_3 - 7.09x_2x_3 \quad (\text{Eq.3})$$

$$R^2: 0.99717; \text{Adj. } R^2: 0.99152; \text{MS Residual: } 9.19$$

In Series 2:

$$y = 47.85^* - 15.49x_1 - 50.25x_2 + 36.61x_3^* + 17.59x_1x_2 + 9.485x_1x_3 - 11.24x_1^2x_3 + 11.71x_2x_3 \quad (\text{Eq.4})$$

$$R^2: 0.9793; \text{Adj. } R^2: 0.96685; \text{MS Residual: } 101.77$$

where

y : A

3.3. Mechanical property

The mechanical properties of the granules were better for Series 1 than for the compositions containing the pepsin powder in the powder mixture. In the central point (900/2750) there are two or three breaking point. The highest values were found for the

samples in the central point, independently of the technological process. The best mechanical properties were those of the sample preparing with pepsin solution granulation fluid (Series 1). The number of bridges formed may be lower in the case of Series 2, because pepsin is in the powder mixture therefore the first step is pepsin solving and it can be only particular. The other reason is the bridges formed may be stronger because bridge forming started immediately, and accordingly hardness may be higher. The texture of this sample was therefore the most compacted.

Not only the breaking hardness, but also the deformation process can provide information on the processability. The breaking curve of Series 1 (Figure 4) was very similar to those of the compacted pellets or granules (41). There were three phases: a short elastic part was followed by a viscoelastic phase, and finally an elastic section up to the breaking point ($3.80 \text{ N} \pm 0.79 \text{ N}$). In this case, the first breaking point was followed by an elastic section up to the second breaking point ($30.66 \text{ N} \pm 2.84 \text{ N}$). There were no meaningful irregularities in the curves, which revealed only small deformations caused by the slightly inhomogeneous structure. Series 2, prepared with pepsin powder mixture, did not exhibit first an elastic curve, only a viscoelastic section (Figure 4). However, this section was not exactly parallel with the X axis. In this case there were also two breaking points and both were lower than for Series 1 ($3.14 \text{ N} \pm 0.77 \text{ N}$ and $16.26 \text{ N} \pm 1.75 \text{ N}$). In this case 50% of the samples had a third breaking point near the second breaking point. It is well known that air exhibits elastic properties, and in this case the amount of entrapped air was higher because of the loose structure and irregular shape (Figure 4). The curve of Series 1 was better than that of Series 2, but there were more irregularities than for Series 1, and the separation of the different phases was also less marked. The structure of Series 2 was loose, contained more coherent pores than Series 1, which can be seen in the micro-CT results (Figure 6). The shape of these particles was very

similar to that of Series 1 particles. These results correlate well with our previous results, the protein (human serum albumin) solution has very good granulating effect [47].

3.4. Scanning electron microscopy (SEM)

Figure 5 demonstrates that the surface of the granules from Series 1 was not smooth, free particles of MCC can be seen in some places on the surface. In this case pepsin was in the granulation liquid, therefore it was in the solution. It can cause the formation of a pepsin film in the deeper layers, not only on the surface, and resulted in higher mechanical stability as demonstrated in Figure 5. The deformation process of granules initially followed the mild viscoelastic state characteristic of the pellets. The first cracking of the particle occurred later in time, since in this case it can be seen in the micro-CT results (Figure 6) that granules like pellets were formed. The complete deformation of the granules occurred under the effect of a larger force after a longer elastic section.

The surface of the granules from Series 2 was smoother because in this case pepsin was in the powder mixture and partially soluble. The particle size of pepsin was very small, approximately 10 μm . Pepsin with a small particle size dissolved partially and undissolved, therefore it was able to fill the surface irregularities between the particles of MCC. Original MCC particles were rarely seen. The less bound particles on the surface can crack faster under the influence of force (about 7-8 s), and then the deformation continues due to the elastic property of the pepsin film. The uneven distribution of the bonds is shown by the fact that the deformation process takes place in two steps. This was supported by the high porosity seen inside the particle in the micro-CT image.

3.5. Micro-CT

The internal structure of the granules can also be observed in the micro-CT images. It can be seen that in the case of Series 1, pellet-like, compacted granules were formed, which is also supported by the deformation curves, since in this case a curve characteristic of typical pellets with one breaking point was obtained. In this case, only few pores of small size are observed inside the granules, which are scattered (Figure 6).

In the second case, typical crust granules were formed, since pepsin was in the powder mixture, but only partially dissolved during the process. As a result, a well-observed crust was formed on the granules in the micro-CT images, and an enrichment of pores was observed inside the granules to the extent that they formed a coherent cavity, while the outer layer of the granules formed a coherent crust.

4. Conclusion

It has been found that the temperature rise due to friction during high-shear granulation has a significant effect on the properties of pepsin-containing granules. The evaluation of the results indicated that the effect of the impeller speed was definitely greater than that of the chopper speed. The effect of the impeller on $D50$ was exactly 243% in Series 1 and 704% in Series 2. It can be concluded that the application of cooling has a statistically significant effect on the $D50$ value for both series (b_3 : 216.5 and 454). Both reduced both $D50$ and A . The interaction of the factors (b_{23}) was taken into consideration during the evaluation. In the case of a high impeller speed, the temperature was elevated, and the application of cooling is therefore recommended. Additional developments are planned with a sample that demonstrates good A (100%) with a practically useful $D50$ (1410 μm). This granule was produced in Series 1, with low impeller and chopper speeds (300 and 500 rpm). As compared the two technological processes, the granules formed with pepsin solution displayed higher breaking hardness and favourable deformation process. With pepsin solution as granulation

liquid, the particles were larger, and from the aspect of A, this technological process is recommended under well controlled conditions. Based on the SEM and micro-CT images, we were able to detect the surface of the particles and some of their structural differences. We found that in the case of Series 2 (granules prepared with water), the surface of the particles is more uniform and contains larger and more cohesive pores inside than in Series 1 (granules prepared with binder solution). During high shear granulation, as a technological process, we have found that cooling plays an important role in the formulation of a protein-type drug because the thermal effects of the operation irreversibly denature the proteins.

CRedit author statement

Katalin Kristó: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, **Elvira Csík:** Investigation, **Dániel Sebők:** Investigation, **Ákos Kukovecz:** Investigation, Funding acquisition, **Tamás Sovány:** Investigation, **Géza Regdon:** Writing - Review & Editing, **Ildikó Csóka:** Funding acquisition, **Botond Penke:** Funding acquisition, **Klára Pintye-Hódi:** Supervision, Writing - Review & Editing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure list

Figure 1. Sample holder of breaking hardness tester

Figure 2. The response surfaces of D50 (a) Series 1; (b) Series 2

Figure 3. The response surfaces of A (a) Series 1; (b) Series 2

Figure 4. The breaking hardness curves of central point (2750/900) of Series 1 and Series 2

Figure 5. SEM images of the central points of granules (2750/900) of Series 1 (A and C) and Series 2 (B and D)

Figure 6. The results of the micro-CT measurements: the volume rendered 3D CT images of the central points of granules (2750/900) of Series 1 (A) and Series 2 (B) packed in quartz capillary tubes; the volume rendered 3D images of selected granules from each type (C and D) and the grayscale visualization of one vertical slice from images C and D (E and F)

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Table 1. Design matrix for the factorial design

Factors	Low level (rpm)	Zero level	High level (rpm)
Chopper speed (x_2)	-1	0	+1
Impeller speed (x_1)	-1	0	+1
Cooling	-1	-	+1

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Table 2. Standard parameters

Standard parameters	First series	Second series
Amount of granulation liquid (ml)	100	96
Amount of pepsin (g)	4	4
Amount of MCC (g)	100	100
Amount of distilled water (ml)	96	96
Dosing speed of granulation liquid (ml/min)	5	5
Process time (s)	1200	1152

Table 3. Average particle sizes (D_{50}) and the activity (A) of the enzyme (Series 1: granules prepared with binder solution; Series 2: granules prepared with water)

Series	Cooling	Chopper speed (rpm)	Impeller speed (rpm)	D_{50} (μm)	A (%)
Series 1	-1	5000	1500	380	6.48
		500	300	1394	30.79
		5000	300	955	21.46
		500	1500	319	9.44
		2750	900	1068	19.14
	1	5000	1500	962	58.23
		500	300	1410	101.10
		5000	300	833	80.59
		500	1500	858	58.78
		2750	900	1210	73.57
Series 2	-1	5000	1500	244	3.97
		500	300	934	90.91
		5000	300	601	40.62
		500	1500	260	3.48

		2750	900	660	16.42
	1	5000	1500	805	46.40
		500	300	863	90.95
		5000	300	828	75.23
		500	1500	607	42.70
		2750	900	1490	68.02

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CRedit author statement

Katalin Kristó: Conceptualization, Methodology, Formal analysis, Writing - Original Draft,

Elvira Csík: Investigation, **Dániel Sebők:** Investigation, **Ákos Kukovecz:** Investigation,

Funding acquisition, **Tamás Sovány:** Investigation, **Géza Regdon:** Writing - Review &

Editing, **Ildikó Csóka:** Funding acquisition, **Botond Penke:** Funding acquisition, **Klára**

Pintye-Hódi: Supervision, Writing - Review & Editing

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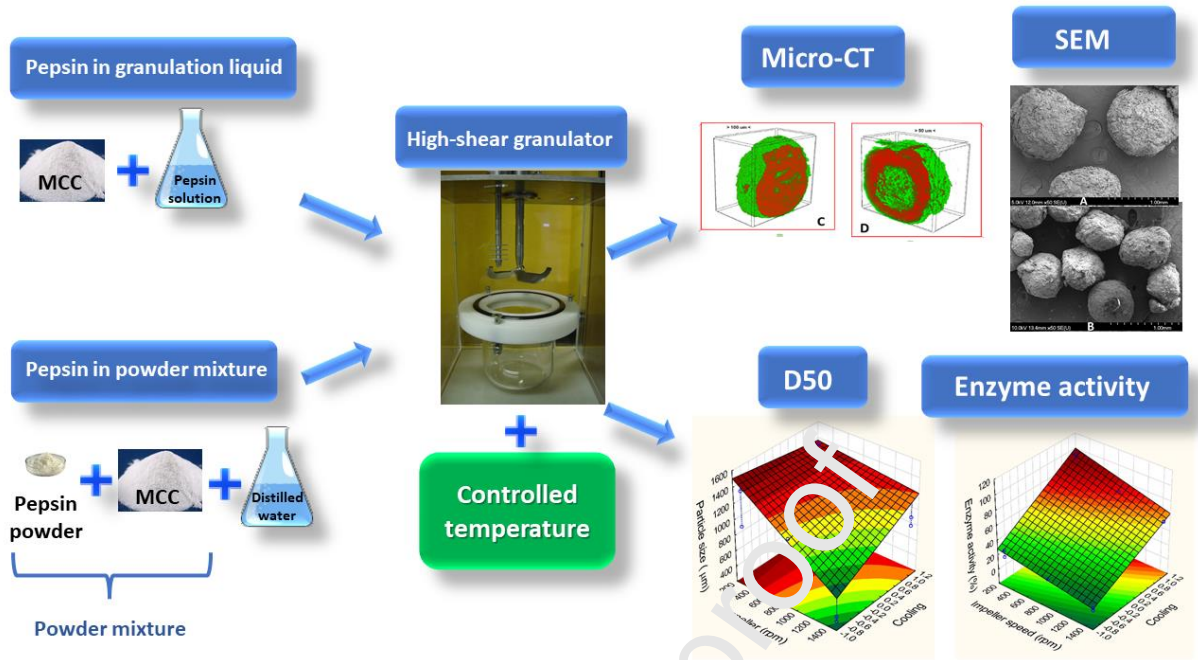
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Graphical Abstract



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Highlights

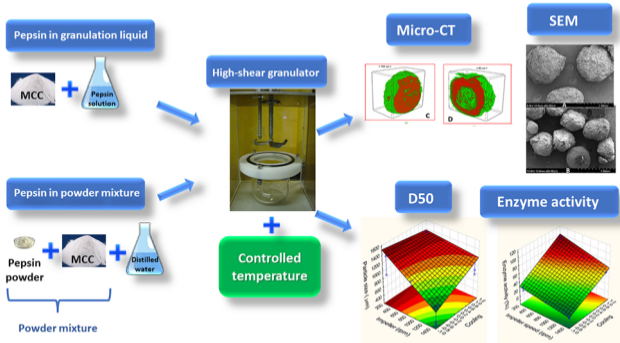
The effect of cooling on the activity of pepsin.

The application of cooling has a significant effect on the D50 value.

The granules prepared with binder solution has favourable deformation process.

The production of granules prepared with binder solution with cooling is recommended.

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Graphics Abstract

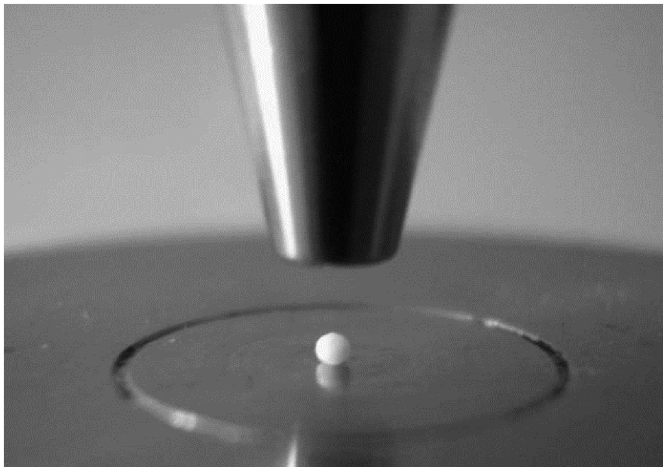


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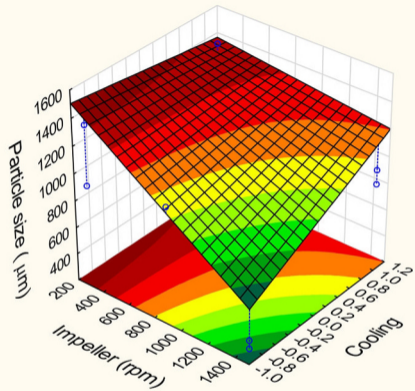
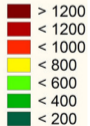
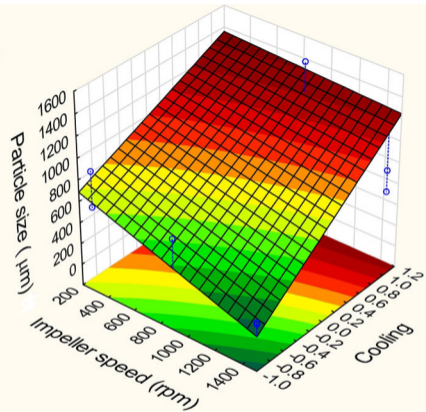
a**b**

Figure 2

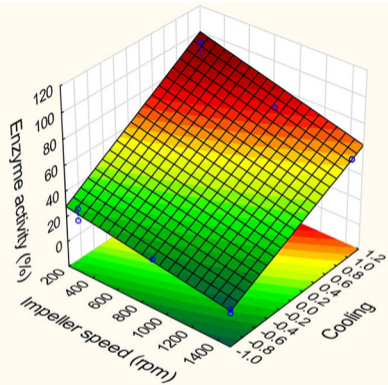
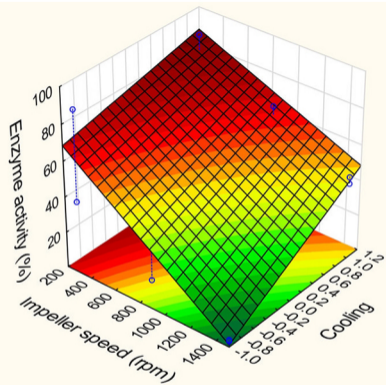
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Figure 3

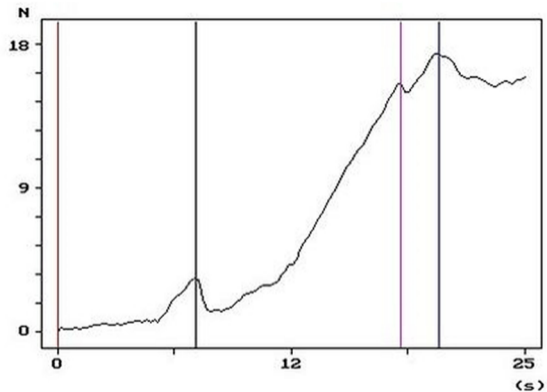
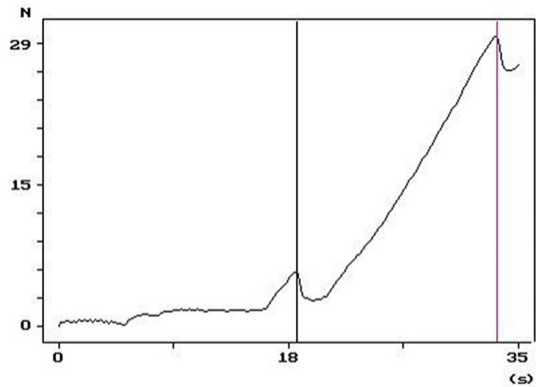


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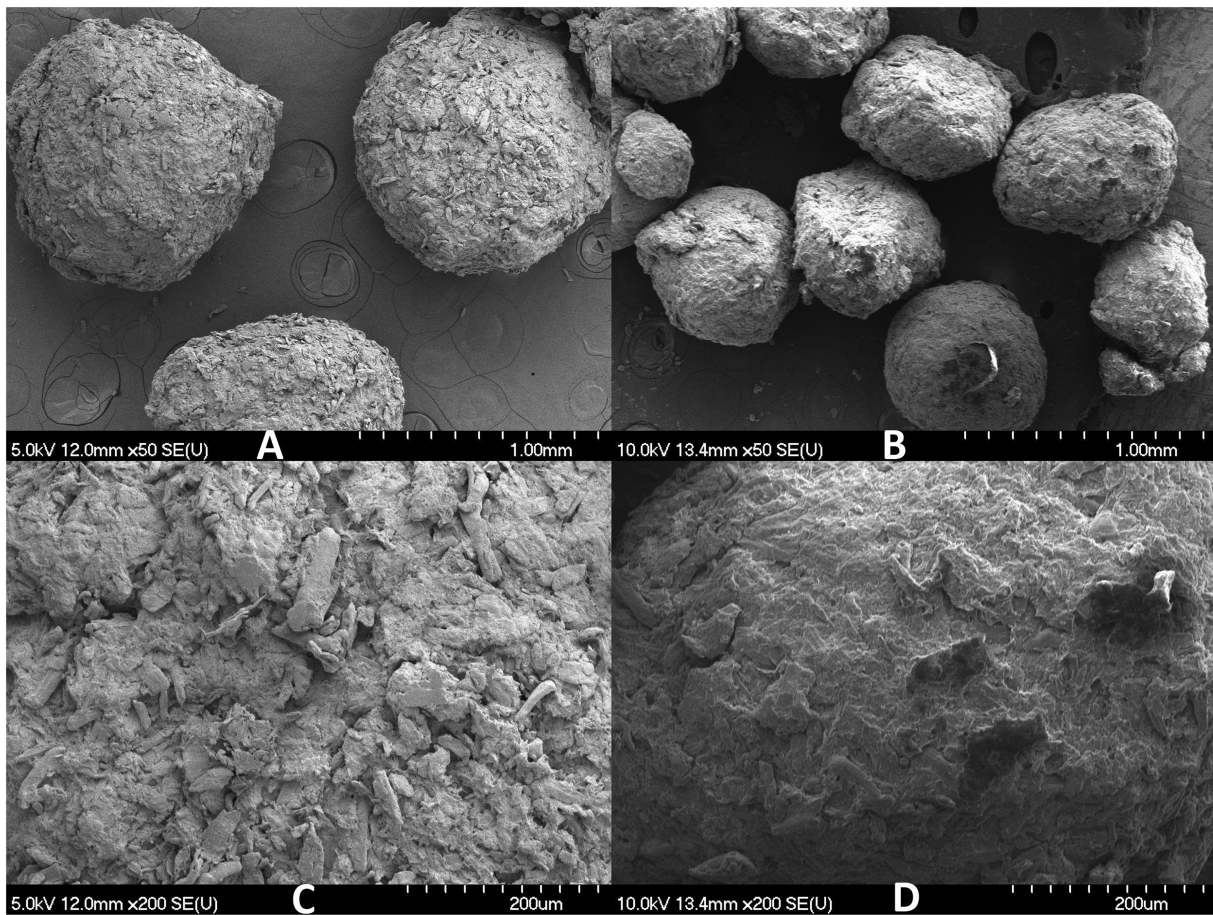


Figure 5

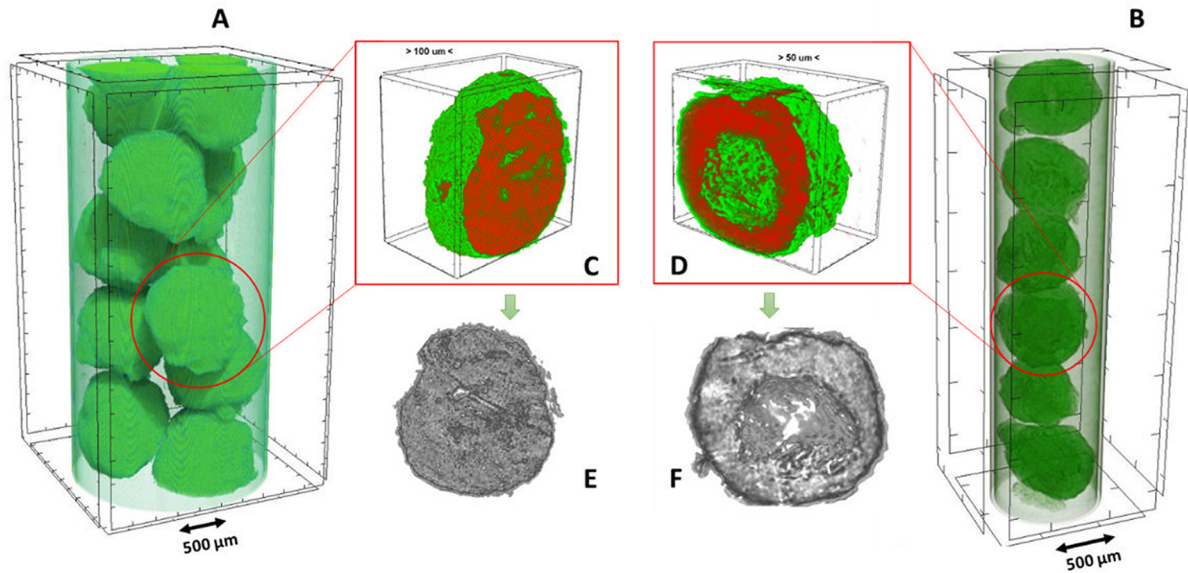


Figure 6