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Powder filling of electrospun material in vials: A proof-of-concept study

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ABSTRACT

The present paper reports the powder filling of milled electrospun materials in vials, which contained voriconazole and sulfobutylether-β-cyclodextrin. High-speed electrospinning was used for the production of the fibrous sample, which was divided into 6 parts. Each portion was milled using different milling methods and sizes of sieves to investigate whether the milling influences the powder and filling properties. Bulk and tapped density tests, laser diffraction and angle of repose measurements were applied to characterize the milled powders, while a vibratory feeder was used for the feeding experiments. The correlation between the material property descriptors and the feeding responses was investigated by multivariate data analysis. Based on the results, three samples were chosen for the vial filling, which was accomplished with 3400 mg electrospun material containing 200 mg voriconazole, representative of the commercial product. The feed rate was set to fit the 240 g/h production rate of the electrospinning and the relative standard deviation of three repeated vial filling was determined to see the accuracy of the process. This research shows that by applying a suitable milling method it is possible to process electrospun fibers to a powder, which can be filled into vials and used as reconstitution dosage forms.

1. Introduction

The role of parenteral administration has always been prominent among the possible drug delivery routes since these dosage forms are well applicable in emergency cases, result in fast drug release and high bioavailability (i.e. low doses needed) and these formulations are needed for the majority of biopharmaceutics due to their large molecular size (Gulati and Gupta, 2011). The coronavirus pandemic situation revealed that parenteral medicines are still very important for handling quickly the worldwide rapidly spreading diseases. Liquid forms are mainly used for parenteral delivery, which are suitable for administration by injection. However, the liquid forms can become unstable thus the transport and storage of this type of medicines can be very costly and risky (Florence and Attwood, 2015). Reconstitution injections provide an excellent solution to avoid the stability problems since the solid dosage forms are more stable, can be dissolved quickly and used as parenteral administration (Jacobsen et al., 2000; Mitragotri et al., 2014). Besides the injection forms, powders for reconstitution also have an important role in the treatment of pediatric and geriatric patients since taking oral liquid medicines is much easier and more convenient for them in this way (Batchelor and Marriott, 2015; Boateng, 2017).

The most common technique for preparing reconstitution injection forms is lyophilization or freeze-drying and is generally considered the golden standard within the pharmaceutical industry for the stabilization of sensitive biopharmaceuticals and active pharmaceutical ingredients (APIs) (Cao et al., 2013; Luoma and Lim, 2020; Zimmermann et al., 2000). Although the low temperatures employed in freeze-drying facilitate the manufacture of stable formulation of suitable quality, the energy need and the time consumption of the process is relatively high (Patel and Pikal, 2011). Therefore, several research groups and companies are searching for alternative ways for drying and preparing reconstitution dosage forms. One of the novel developments is continuous freeze-drying, which is a well controllable process with reduced residence time and high productivity (Adali et al., 2020; Capozzi et al., 2019; De Meyer et al., 2015). While the continuous variant is generally considered to have a lower energy consumption per kg water removed, it

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is still believed to be higher than in the case of alternative drying technologies such as electrospinning and spray drying. Despite the high temperatures during spray drying, monoclonal antibodies were successfully formulated by this fast and well-known drying technique (Massant et al., 2020). In addition, spray drying was applied several times for preparing pediatric formulations as well (de Oliveira et al., 2018; Nese et al., 2020). To make reconstitution dosage forms, electroblowing and electrospinning are even gentler since the electrostatic forces facilitate the drying (Balogh et al., 2015). The techniques based on electrostatic forces enable the continuous preparation of reconstitution injection forms at room temperature while the energy consumption is lower compared to the above-mentioned methods (Vass et al., 2019a).

Besides the production, the packaging of the solid reconstitution dosage forms is also a crucial part of pharmaceutical product and process development. In the case of reconstitution injection forms, sterile vial filling needs to be accomplished to avoid contamination from the environment (Akers et al., 1996; Verjans and Reed, 2012). In addition, reconstitution dosage forms for pediatric and geriatric patients also require accurate dosing of powders into the vials to ensure the appropriate doses before usage (Boateng, 2017). Basically, two strategies can be applied for dry filling according to the properties of the powders (Bansal, 2002). If the API or the drug-loaded freeze-dried, spray-dried or similar formulation has good flow properties, it can be filled directly without adding excipients. Otherwise, another strategy needs to be applied such as blending with excipients, coating of the particles or compression of the powders into pellets, which are widely-used methods indicated by the several marketed products (Bansal, 2002). Despite using excipients and application of additional technological steps (e.g. dry coating, granulation, pelleting) are common and reliable strategies, several aspirations appeared in the literature for achieving direct filling. For instance, continuous manufacturing in freeze-drying including vial filling is a widely researched area (Pisano et al., 2019). Although the filling accuracy is high in the case of freeze-dried samples, the previously mentioned disadvantages of the technique (i.e. high energy need and time consumption) encourage researchers to develop alternative strategies (Dongare et al., 2015).

For vial filling, the powder properties such as flowability or electrostatic charging can be crucial since it has a huge impact on the product quality (Szabó et al., 2019). In the case of poor flowability, excipients are usually needed to achieve appropriate dosing. For instance, micronized APIs have a small particle size, typically below 10 μm, which generally leads to flow problems (Pingali et al., 2009). For handling poor flowability of the micronized APIs, silica is a suitable excipient for dry coating powders of poor flowability and thus for improving their flow properties (Escotet-Espinoza et al., 2021; Kunnath et al., 2018). Besides, different pharmaceutical technologies are suitable for improving the disadvantageous powder characteristics. In this way, the flow properties of electrospun fiber mats with low bulk densities and fiber diameters typically less than 10 μm can improve by milling. This formulation step reduces the length of the fibers, results in better flow properties and makes the technology more suitable for pharmaceutical applications (Vass et al., 2019b). In addition, spray-dried samples with spherical particles and typically larger than 20 μm particle size can also have flowability issues, which were successfully enhanced by roller compaction several times (Angi et al., 2019; Wagner et al., 2013). Furthermore, novel technologies for vial filling proved to be also suitable for accurate dosing of spray-dried particles (Seaward and Bailey, 2013; Wong et al., 2007). Another technological opportunity in this field is the optimization of the design of feeder equipment parts (e.g. feeder, screw type, screens), which might enable even the critical and challenging micro-dosing (Besenhard et al., 2016; Madarász et al., 2020). Furthermore, the selection of the appropriate feeding can be facilitated by investigating correlations between material property descriptors and feeding responses (Bekaert et al., 2021; Bostijn et al., 2019; Hsiao et al., 2020; Wang et al., 2017).

The main goal of this work was to accomplish the accurate dosing of electrospun fibers containing voriconazole (VOR) and sulfobuthylether-β-cyclodextrin (SBE-β-CD). The motivation of the research was that electrospinning can be an alternative to freeze-drying (Vass et al., 2019a), but vial filling of electrospun materials had not been investigated before. With this, a new way of reconstitution dosage forms might become industrially feasible, which applies low-temperature drying and potentially does not need high energy consumption and costs. Furthermore, the effect of the milling methods on the powder characteristics is also examined during this work. It can establish and speed up future development of electrospun reconstitution dosage forms since the results provide an overview about the importance of the selection of the ideal milling method and feeding circumstances. Overall, a process train including high-speed electrospinning, followed by milling and powder filling was tested during this research and the possibility of making it continuous was investigated.

2. Materials and methods

2.1. Materials

VOR with a molar weight of 349 g/mol and with low aqueous solubility (0.6 mg/mL at pH 7) was obtained from Sigma-Aldrich Ltd. (Budapest, Hungary). SBE-β-CD sodium (Dexolve™, average degree of substitution = 6.5) was purchased from Cyclolab Cyclodextrin Research and Development Laboratory Ltd. (Budapest, Hungary). The water used for the solution preparation was from a Millipore Milli-Q ultrapure water system.

2.2. High-speed electrospinning (HSES)

Scaled-up production of fibers containing VOR and SBE-β-CD was accomplished using an HSES setup coupled with a cyclone (Fig. 1) (Vass et al., 2019a). During the HSES experiment, a constant 120 m^3/h gas flow rate was set in the system using a fan, which helped the collection of the fibers into the collector bin at the bottom of the cyclone. Furthermore, some air knives can be found on the HSES chamber to further facilitate the collection via cutting the fibers stuck on the wall of the chamber. For the fiber formation, a rotating, round-shaped, stainless steel spinneret (d = 34 mm) with 36 orifices (d = 330 μm) was applied, which was connected to a high-speed motor. For the electrospinning experiments, a water-based solution containing 30 w/w% distilled water and 70 w/w% solid material was prepared and stirred with a magnetic

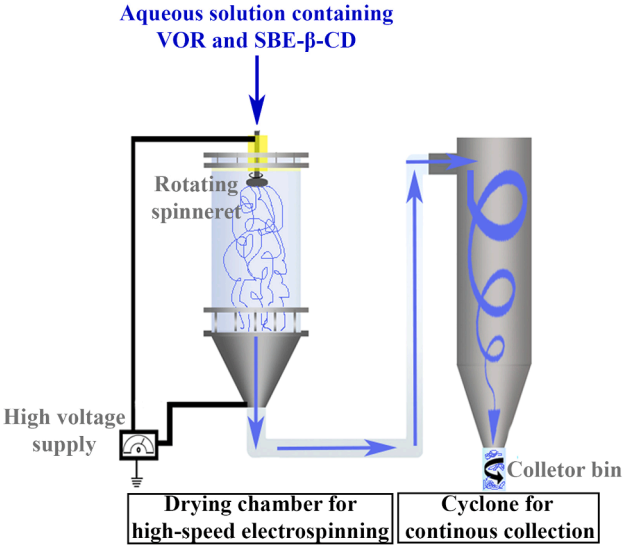


Fig. 1. Schematic design of the applied high-speed electrospinning apparatus coupled with a cyclone.

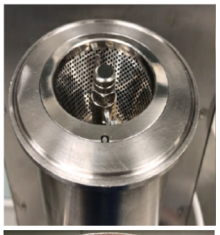
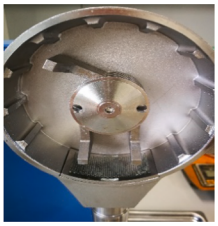
stirrer (600 rpm) at room temperature until complete dissolution. The solid material concentration of the solution was 5.9 w/w% VOR and 94.1 w/w% SBE-β-CD, which was equal to the 1:16 formulation of the commercial Vfend® composition (Pfizer, 2010). {Pfizer, 2010 #44} {Pfizer, 2010 #44}The prepared solution was fed towards the spinneret with a SEP-10 S Plus syringe pump with a flow rate of 300 mL/h. The rotational speed of the spinneret was adjusted at 40,000 rpm, while 40 kV voltage was applied during the experiments. The experiments were performed at ambient temperature (25 °C ± 1 °C) and 45% ± 5% relative humidity. The HSES took ~30 min to prepare the necessary quantity (~200 g) for further experiments. During this period, the electrospinning and the collection of the fibers was continuous. There was no need to stop the production since the capacity of the collector bin was enough to this amount.

2.3. Milling of the electrospun powders

To make the electrospun fibers suitable for powder filling, insertion of milling into the formulation steps is indispensable. Since the mode of the milling can influence the properties of the powders (e.q. particle size distribution, specific surface area etc.) three different types of milling were tested for preparing milled electrospun fibers (Table 1) (Nakach et al., 2004). In this way, the efficiency of the milling methods and their impact on the powder quality can be examined.

One of the applied devices was a QUICKmill Lab multifunctional milling apparatus (Quick2000 Ltd., Tiszavasvári, Hungary), which can be operated both in oscillating and conical milling mode depending on the applied milling head. During this research, both the oscillating and the conical milling modes were applied with different sizes of sieves (Table 1). The selected methods enabled us to investigate the effect of the size of the sieves and to compare the different types of milling since 1.5 mm and 2.0 mm screen sizes were applied both in the case of oscillating and conical milling. Furthermore, one of the aims of this work was to investigate whether the milling method influences the powder and feeding properties of the milled electrospun materials. Since it was never examined before, firstly, the limits of the milling in the case of the given electrospun material was tried to find. To achieve this, as much information as possible from the produced material was attempted to get thus the examinations were expanded to some extreme cases besides minimal material consumption. Therefore, besides the O1.5, O2.0, C1.5 and C2.0 settings the H1.0 and O0.8 were also included in the research. The holes of the sieves were round-shaped in all cases. As the research is a proof-of-concept work in the field of pharmaceutical application of electrospun materials, in which the implementation of vial filling of the given electrospun material and investigation of its continuous possibilities were the main goals, not all dependent variables were tested during the milling. For instance, the milling rate was a fixed parameter in the case of all different type milling methods to minimalize the number of the variables, and thus the number of experiments and the material consumption. Therefore, the applied milling rates were determined based on preliminary experiments and our previous researches (Szabó et al., 2021; Vass et al., 2019a). The main standpoint was to choose the maximal milling speed, which fit the production rate of HSES and does not result in the damage of the amorphous physical state of the fibers. In this way, connecting of the formulation step (HSES, fiber collection, milling, feeding) would be feasible. The milling rate was adjusted to 200 cycles/min in the case of oscillating milling, while 2000 rpm rotation speed was set during the conical milling, which were the highest value that could be set in both cases. The other applied equipment was a hammer mill (IKA MF10, IKA-Werke GmbH & Co. KG, Staufen, Germany) with a 1.0 mm sieve. The rotational speed of the hammers was fixed at 3000 rpm.

Table 1
Summary of the applied milling methods.

Type of the milling method	Milling heads	Diameter of the holes by the applied sieve (mm)	Applied abbreviations
Oscillating milling		0.8 1.5 2.0	O0.8 O1.5 O2.0
Conical milling		1.5 2.0	C1.5 C2.0
Hammer milling		1.0	H1.0

2.4. Characterization of the milled electrospun product

The milled electrospun samples were examined with scanning electron microscopy (SEM) and with three basic powder characterization methods, namely with the bulk and tapped density tests, angle of repose measurements and laser diffraction measurements. Although the latter method is mainly ideal for spherical particles, it can also give an approximation to the particle size distribution of the milled electrospun samples. The reason for this is that the entangled fibers form different size agglomerates, which approach the spherical particle shape (Szabó et al., 2021). The used three basic characterization techniques, the measured and calculated material property descriptors and the abbreviations are summarized in Table 2.

Table 2
Summary of the used characterization techniques, the measured and calculated material property descriptors and the abbreviations.

Characterization method	Material property descriptor	Applied abbreviation
Bulk and tapped density	Bulk density	ρ_{bulk}
	Tapped density	ρ_{tapped}
	Hausner ratio	HR
	Carr index	CI
Angle of repose	Angle of repose	α
Laser diffraction	10% cumulative undersize of volumetric particle size distribution	$d_{0.1}$
	50% cumulative undersize of volumetric particle size distribution	$d_{0.5}$
	90% cumulative undersize of volumetric particle size distribution	$d_{0.9}$
	The span of volumetric particle size distribution	Span
	Specific surface area	SSA
	Surface weighted mean	D[3,2]
	Volume weighted mean	D[4,3]

2.4.1. Scanning electron microscopy (SEM)

A JEOL JSM 6380LA (JEOL, Tokyo, Japan) type scanning electron microscope was used for investigating the morphology and size of the milled electrospun samples. Before the measurements, the fibrous specimens were fixed with conductive double-sided carbon adhesive tape and sputtered with gold using an ion sputter (JEOL 1200, JEOL, Tokyo, Japan) to avoid electrostatic charging. The accelerating voltage was set to 10 kV while the working distance was between 10 and 16 mm. Each SEM examination was performed in a high vacuum.

2.4.2. Bulk and tapped density

Bulk and tapped densities of the milled electrospun samples were measured on an ERWEKA SVM12 (Heusenstamm, Germany) type tapped density tester. The bulk density was calculated as the quotient of the measured mass and the initial volume, which was determined using a graduated cylinder. The tapped density was calculated similarly but the volume applied for the calculation was measured after 1250 taps. Furthermore, the Hausner ratio and the Carr index were calculated as well (Carr, 1965; Hausner, 1967).

2.4.3. Angle of repose

The angle of repose was measured using a fixed funnel, where the diameter of the hole at the bottom of the funnel was 10 mm. The angle of repose was calculated according to Eq. (1).

$$tan\alpha = \frac{h}{0.5 \times d} \quad (1)$$

In the equation, h indicates the height of the cone of powder and d is the diameter of the base. Three repeated measurements were performed with each sample, where the base was the same and the height of the cone was measured with a digital height gauge.

2.4.4. Laser diffraction measurement

A Malvern Mastersizer 2000 type laser diffractometer (Malvern Instruments Ltd., Worchestershire, UK) was applied to examine the particle size distribution of the milled electrospun samples. A background recording was performed before each measurement. In the following step, a vibratory feeder added the powder into the diffractometer with 75% intensity of the vibrational amplitude. A single-lens system was applied for investigating the entire 0.02–2000 μm measurement range. During the 60-second long measurements, 1.5 bar pressure was used, which facilitated the disaggregation of the particles.

From the laser diffraction measurements, more measured and calculated values were used to describe the electrospun material as well as possible. The measured $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ values were described as the 10%, 50% and 90% cumulative undersize of the volumetric distribution, respectively. The 'Span' calculated from $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ was also determined to compare the width of the particle size distribution of the electrospun samples milled by different methods. Besides, surface and volume weighted means can give useful information about the particle size distribution thus these values were also used for the powder characterization (Scientific, 2012). In addition, specific surface area (SSA) was also determined since this property has an important role in the flowability of the powders. Important to note that the laser diffraction technique is an indirect analysis method for the estimation of specific surface area. The volumetric gas adsorption techniques probably give higher SSA values and result in a more accurate description of the samples. However, there can be a correlation between the two techniques thus laser diffraction can be suitable to distinguish the milled electrospun powders based on the SSA as well (Gómez-Tena et al., 2014). A further advantage is that the material needs can reduce in this way since more material property descriptors are determined only from one measurement.

2.5. Vibratory feeding

To the vial filling, a LABORETTE 24 vibratory feeder was utilized (Fritsch GmbH, Idar-Oberstein, Germany). During the feeding tests, a V-shaped channel was applied to direct the powder into the vials. The feeding rate could be controlled only by varying the vibration amplitude. An in-house developed software processed the mass data measured with a catch scale (Sartorius LC4800P, Göttingen, Germany). Furthermore, the software enabled the control of the feeder according to the data from the catch scale.

2.6. Multivariate data analysis

The multivariate data analyses were performed by using MATLAB 9.4.0. (MathWorks, USA) program with PLS Toolbox 8.6.1. (Eigenvector Research, USA).

First, principal component analysis (PCA) was applied to create an overview of the material property descriptors and the examined milling methods. Several pre-treatments were tested before the PCA model building. Finally, automatic scaling was selected during the evaluation of the measured data. 'Autoscaling' consists of a normalization, during which each column (variable) of the data is normalized to the standard deviation of the given column. Besides, mean centering is also a part of the 'autoscaling', which means the subtraction of the mean of the column.

The correlation between the material property descriptors and the feeding was investigated by partial least squares (PLS) model development. The relative standard deviation (RSD) values of the feed rates from three repeated measurements were applied as feeding responses (Y) during the PLS regression, while the material property descriptors of the milled electrospun samples were used as X values. Since the RSD values were determined at different vibration intensities, the applied intensities were also included in the X matrix. Prior to the PLS model building, the data was pre-treated with automatic scaling. During the cross-validation of the model, the 'leave one out' method was used since it is appropriate for small datasets with randomly distributed objects. In this case, one observation is used as the validation set while the remaining objects are applied as the training set (Sammut and Webb, 2010). Each observation in the data set is utilized as a test set during the 'leave one out' cross-validation.

2.7. Reconstitution test

For the reconstitution tests, 19 mL of purified water was added to 3400 mg of milled electrospun sample (Pfizer, 2010). Then, the vials were shaken to dissolve the samples including powder stuck on the wall. The absorbance of the dissolved VOR was measured with an Agilent 8453 UV–VIS spectrophotometer (Hewlett-Packard, Palo Alto, USA) at the wavelength of 256 nm. The dissolution percentage was calculated from a preliminary built calibration. For the UV measurements, 1 mL of the solution was filtered using a regenerated cellulose filter with 0.45 μm pores.

3. Results and discussion

3.1. Characterization of the milled electrospun samples

The main challenge during the pharmaceutical application of electrospun fibers is the handling of the hardly flowable fibrous mats in the formulation steps. Therefore, developing a formulation resulting in electrospun powders with good flow properties is the key point of the development activities. During this research, oscillating, conical and hammer milling was used for grinding the VOR and SBE-β-CD containing electrospun fibers to achieve powder forms with better flowability. The characteristic of the milled electrospun materials was examined by bulk and tapped density test, angle of repose measurement and laser

diffraction, which results are summarized in Table 3. It can be seen that the highest differences were measured by laser diffraction. For instance, the $d_{0.5}$ values were the lowest in the case of the larger size of sieves, while smaller sizes of screens resulted in higher $d_{0.5}$ values. It assumes that in the case of smaller screen sizes the cohesion forces between the fibers, which formed the different size agglomerates, can be higher. Therefore, these agglomerates are closer to the spherical shape and can better retain this shape even under higher pressure used by the laser diffraction measurements. In contrast, the fibers can easier slip through the screens with higher diameters thus less cohesion force can be formed between the fibers. It leads that the agglomerates can fall apart to smaller units during the laser diffraction measurements, which results in smaller $d_{0.5}$ values. The results suggested that the laser diffraction measurements proved to be suitable to distinguish the samples milled by different methods, which can be the base of future work, relating to the in-depth investigations of the cohesion forces between the fibers.

Besides, the morphology of the milled fibers was investigated with scanning electron microscopy (Fig. 2). The SEM images confirm that the fibrous structure was retained after the milling in all cases while the fiber diameters were between 0.5 and 2 μm. However, agglomerates in the size range of 100–1000 μm were generated after the milling of the electrospun samples, which size was influenced by the screen size (Fig. 2). Furthermore, laser diffraction measurements also confirmed the appearance of the different size agglomerates since small differences between the repeated measurements were observed (Fig. 3). However, it proves the success of the milling process that the measured secondary particle size distribution can be found in a similar range in the case of the three repeated measurements. In addition, it is well visible that the particle sizes measured by laser diffraction were lower compared to the SEM images. The different sizes determined by the two analytical methods suggest that the cohesive forces between the fibers in the agglomerates are relatively weak and thus the dispersion pressure applied during laser diffraction measurements was able to reduce the size of the agglomerates. Nevertheless, these agglomerates may facilitate the downstream processing steps during the formulation since the round-shaped structures can have better flow properties than the fluffy fibers with low bulk density.

Although the appearance of the milled electrospun samples is quite similar, small differences are visible in the structure of the powders even based on camera images. Fig. 4 shows the photos of the non-milled fibers and the milled samples, which suggests that the milling method have an impact on the powder characteristic in the case of electrospun materials. It can be also stated that the electrospun sample before the grinding was fragmented, which is the result of the rotating movement of the material in the collector bin caused by the airflow in the cyclone. Although this collection method led to a low shear grinding, further milling processes proved to be more effective to achieve powder forms with round-shaped particles and better flow properties. However, the various particle size distribution due to the different size agglomerates and the distinct powder characteristics of the electrospun samples milled by the examined methods can cause difficulties in accurate feeding. For this reason,

Table 3
Results of the powder characterization.

Samples	C1.5	C2.0	O0.8	O1.5	O2.0	H1.0
ρ_{bulk} (g/cm^3)	0.18	0.12	0.31	0.28	0.22	0.32
ρ_{tapped} (g/cm^3)	0.22	0.15	0.38	0.35	0.27	0.37
HR (-)	1.21	1.28	1.23	1.23	1.22	1.16
CI (-)	17.56	21.97	18.47	18.67	17.78	13.65
α (°)	36.4	33.3	37.0	36.6	31.7	34.5
$d_{0.1}$ (μm)	26.7	8.0	28.0	43.0	2.6	38.5
$d_{0.5}$ (μm)	40.9	12.1	48.2	61.1	14.2	51.1
$d_{0.9}$ (μm)	55.2	17.6	60.1	88.2	48.9	75.1
Span (-)	0.8	1.3	1.2	0.8	2.4	0.7
D[3,2] (μm)	34.4	11.0	37.7	59.1	4.7	50.8
D[4,3] (μm)	40.4	12.6	49.1	64.2	20.8	54.4
SSA (m^2/g)	0.3	1.3	0.4	0.1	1.4	0.1

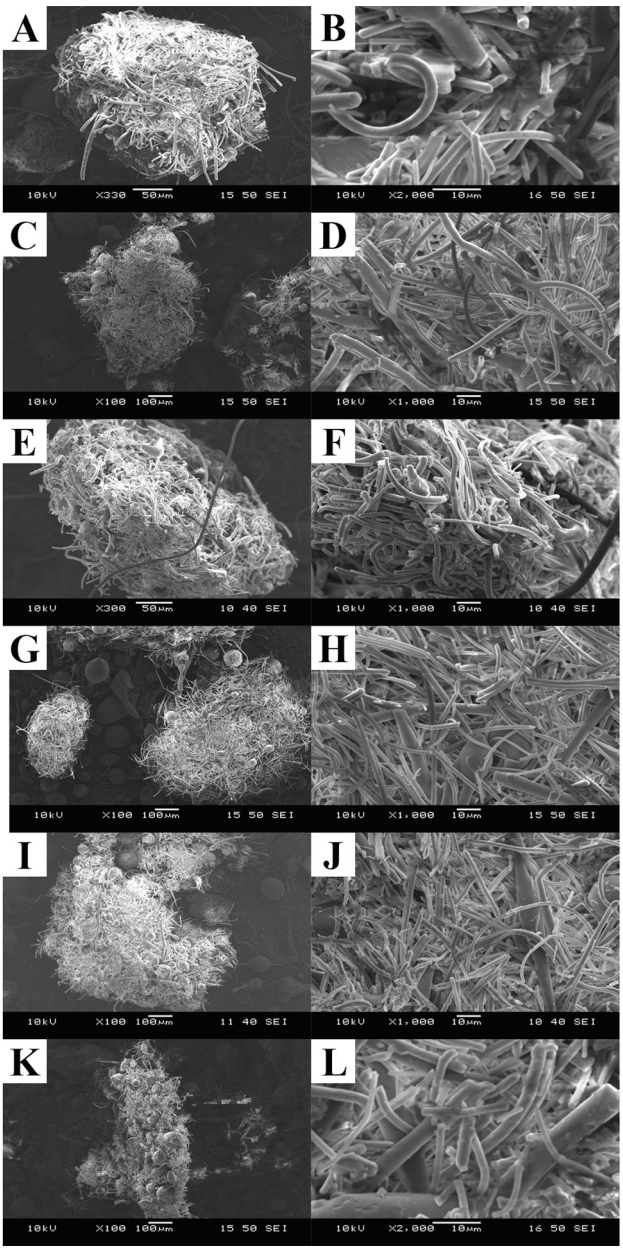


Fig. 2. Morphology of the fibers after milling (A-B: O0.8 sample; C-D: O1.5 sample; E-F: O2.0 sample G-H: C1.5 sample; I-J: C2.0 sample; K-L: H1.0).

an in-depth investigation of the main material property descriptors of the milled electrospun fibers is essential before technological developments.

An overview of the material property descriptors and the milling methods was accomplished by using PCA modeling (Fig. 5). A model including two PC was selected, which explained 90% of the variation in the material property database. The small scale of the database and the similarity of the powders are the main reasons for the relatively high explained variance.

The score plot including the scores on PC1 and PC2 shows that the samples milled by oscillating milling with 0.8 and 1.5 mm screen, and the sample milled by conical milling with 1.5 mm screen were located in the same region, referring that these milling settings resulted in powders with similar material properties (Fig. 5a). In contrast, the samples milled by oscillating milling with 2.0 mm screen is anti-correlated since it can be found on the opposite side of the scores plot. Furthermore, it can be stated that the C2.0 sample is located in the same part of the score plot as

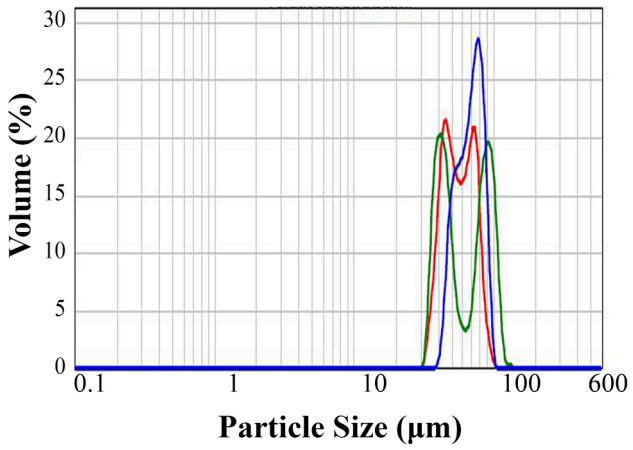


Fig. 3. Particle size distribution of O1.5 samples. (Different colors indicate three repeated measurements. The x-axis is depicted in logarithmic scale.)

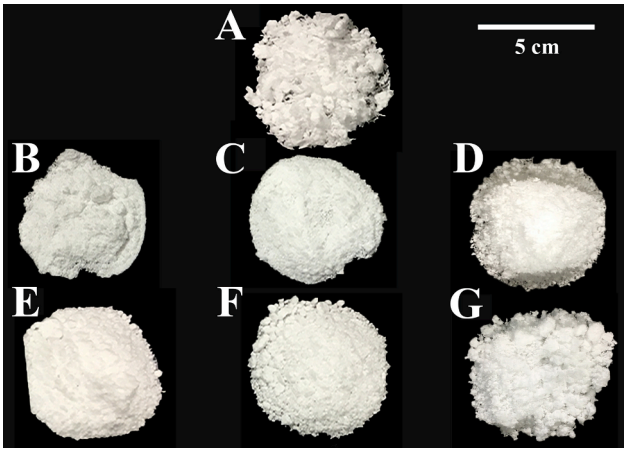


Fig. 4. Photos to compare non-milled and milled voriconazole-loaded fibers (A: before grinding; B: O0.8 sample; C: O1.5 sample; D: O2.0 sample; E: H1.0 sample; F: C1.5 sample; G: C2.sample).

HI and CI in the loading plot. It means that C2.0 can be characterized with higher HI and CI values, which indicates worse flow property. This part of the PCA modeling gives a quick overview of the correlation between different milling methods based on the descriptors of the milled electrospun samples. The results may suggest that the samples with similar properties will behave similarly during the feeding or other formulation steps.

The loading plot of PC1 and PC2 reveals that the first PC is mainly influenced by the particle size descriptors because the mean values from the laser diffraction measurements (D[3,2], D[4,3]) and the median values of the particle size distribution ($d_{0.5}$) are located on the right side of the PC1 and PC2 loading scatter plot (Fig. 5b). In addition, the flowability descriptors (HR, CI) have the highest impact on the second PC. The scores and loading scatter plots are completed by each other, which means that samples with a given location at the scores plot have high values for the given variables at a similar location on the loading plot. Looking at the two plots together, it can be stated that smaller screen sizes resulted in samples with higher D[3,2], D[4,3], and $d_{0.5}$ values. It suggests that the cohesion forces were higher between the fibers in those samples (C1.5, O1.5, O0.8, H1.0) and the formation of more stable agglomerates was achieved. This means that these agglomerates disaggregated less during the laser diffraction due to the pressure. Overall, the whole presented PCA modeling can provide useful information from the further formulation steps' point of view. For instance, if the particle size would have a high impact on the feeding the samples with the C1.5, O1.5 and O0.8 abbreviations will show similarities during the feeding.

3.2. Preliminary feeding experiments

Feeding of the electrospun materials can be very challenging due to their low bulk densities, even after the milling. Besides, electrospun materials usually stick to the surface of the sample holders or to the wall of the devices, which indicate their high electrostatic chargeability. Since the electrostatic properties can cause difficulties during the feeding, it would be also interesting to quantify them in future research. To handle the above-mentioned problems, pre-blending the electrospun samples with well flowable excipients enables the application of the most used twin-screw gravimetric feeders because appropriate feedability and accurate feeding can be achieved in that way (Szabó et al., 2018). However, this type of feeder is a wrong choice in the case of the pure milled electrospun materials since the screw elements may cause further milling of the powder, which will behave during the feeding as a micronized API (Beretta et al., 2020; Worku et al., 2017). During this work, the electrospun material was intended to be fed without any excipients thus the use of a vibratory feeder, widely used for feeding poorly flowable materials, was investigated (Besenhard et al., 2016; Coperion).

The applied vibratory feeder could be operated only in volumetric mode therefore the relationship between the adjustable intensity of the vibratory amplitude and the feed rate was investigated at first (Fig. 6). For this purpose, a catch scale was placed under the end of the V-shaped

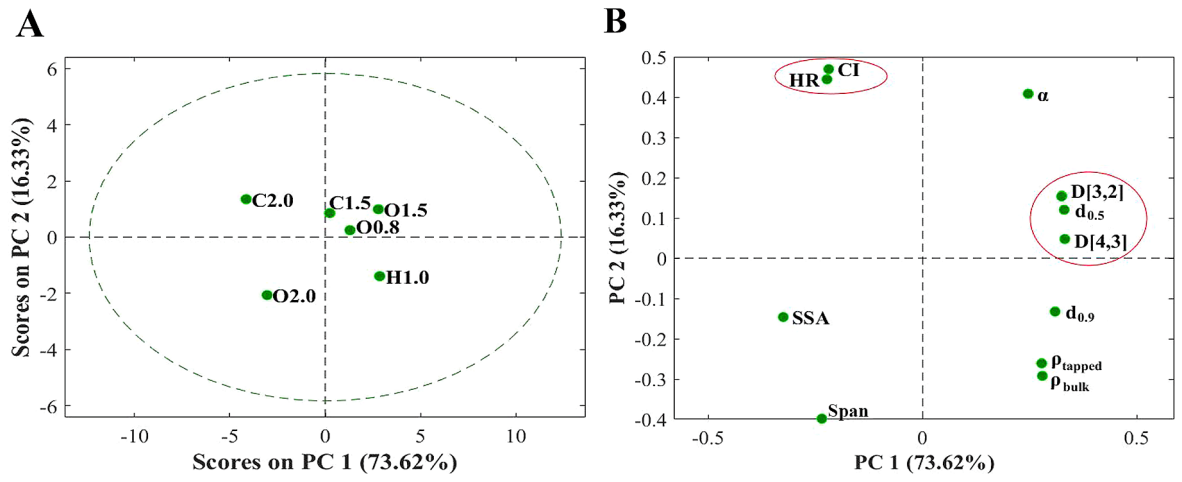


Fig. 5. PCA model of the milled electrospun samples: score plot (a) and loading plot (b).

channel and a computer connected to the scale recorded continuously the current weight. To find the correlation between the intensity and the feed rate, three repeated feeding experiments were fulfilled by 20, 40, 60, 80 and 100 % intensity with each milled sample for a specified period. The feed rates were calculated as the quotient of the total weight of the fed electrospun samples and the experiment time. It is well visible that the feed rates at the investigated intensities were changed by sample to sample, which indicate that the milling methods have an impact on the powder characteristic, and thus the feedability of the milled electrospun samples as well. Since the productivity rate of the electrospinning was ~240 g/h, the feed rate fitting to this value was searched during the preliminary feeding experiments. Based on the results, intensities between 60% and 80% can be appropriate for vial filling in the case of a continuous manufacturing line is planned to contain the electrospinning, milling and feeding (Fig. 6b).

In addition, the laser diffraction results and the SEM images highlighted that the milled electrospun samples formed different size agglomerates and is highly influenced by the milling method. For this reason, the accurate feeding of the milled electrospun samples can be challenging. However, it is considered to be satisfying if the mass variance described with the RSD of the repeated measurements is low because it indicates that uniform powder feeding is feasible. Consequently, the RSD values were also calculated from the results of three repeated measurements (Fig. 7) and used as the main output of the feeding experiments. The RSDs were higher at lower vibratory intensities since too low vibratory amplitude proved to be not enough to start the movement of the powders (Fig. 7a). In this way, the different size agglomerates did not properly distribute in the V-shaped channel thus in some cases bigger particles fall in the sample holder while in other cases the powders did not start to move and no particles fall down. It is also well visible that larger RSD values at 60% and 80% intensities were observed in the case of C2.0, O2.0 and H1.0 samples, which indicates that less reproducible feeding can be achieved with the powders after the application of these milling settings. In contrast, the C1.5 sample, which was characterized with higher $d_{0.5}$, D[3,2] and D[4,3] values compared to the C2.0 and O 2.0 samples, seemed to be very promising according to the preliminary experiments, since the RSD values were under 5%. This result assumes that the C1.5 samples could be well fit into a continuous manufacturing line because reproducible feeding can be performed with the applied vibratory feeder. Furthermore, it can be seen that O0.8 and O1.5 samples also showed lower RSD values but their feeding results were different from the feeding properties of C1.5 sample. These results highlighted that the milling method and the screen size have an impact on the feeding of the electrospun fibers, which was never investigated before according to the best knowledge of the authors. However, better understanding the correlations in the case of the feeding of milled electrospun materials, further investigations are needed.

3.3. Correlation between the material property descriptors and the feeding

The previous two sections gave a quick overview and highlighted that the material property descriptors of the electrospun materials milled by different methods are different and the RSD values of the feeding experiments also changed from sample to sample. Building on these results, the next step of the work was to determine the correlation between the material property descriptors and the feeding in the selected vibration intensity regions. It was examined by PLS regression included the RSD values from the 60% and 80% intensity feeding. The selection of the vibration intensities was based on the calculated feed rates since fitting the feeding step to the productivity of the electrospinning was investigated as well. Among several tested models, a four-component PLS method was selected, which explained 91% and 57% of the variation in the X and Y dataset, respectively. The lower percentage corresponding to the Y dataset suggests the greater differences between the RSD values.

Scores and loadings 'biplot' was utilized for better understanding the correlations and the reasons for the explained percentage values (Fig. 8). Since the first and the second latent variables (LV) have the highest explained percentage values, the evaluation of the model focused on these two components. In the LV 1 vs 2 'biplot', the RSD can be found in the right corner of the plot while the defining material property descriptors were observed on the opposite side. According to these locations, it can be concluded that the main material properties are anti-correlated with the RSD. It means that powders for instance with low bulk and tapped densities and low volume and surface weighted mean have higher RSD during the feeding thus the reproducibility of the vial filling will be worse. Consequently, the feeding properties of the milled materials can be estimated based on these material property descriptors. The powder characterization showed that C2.0 and O2.0 samples were described with lower density values, and lower volume and surface weighted means, while the characteristic of C1.5, O0.8 and O1.5 samples were closed to each other. The preliminary results correlate well with the scores and loadings 'biplot' since C2.0 and O2.0 samples were located on the same side as the RSD, which indicate that higher RSD values were observed during the feeding experiments of these powders. Besides, the vibration intensity was located close to the origin referring that there is no significant correlation with the RSD. Consequently, the reliability and the performance of feeding is more predictable based on the material property descriptors. The results of the PCA and PLS modelling made it illustrative that the milling methods influence the powder characteristic, which affects the efficiency of the reliable feeding

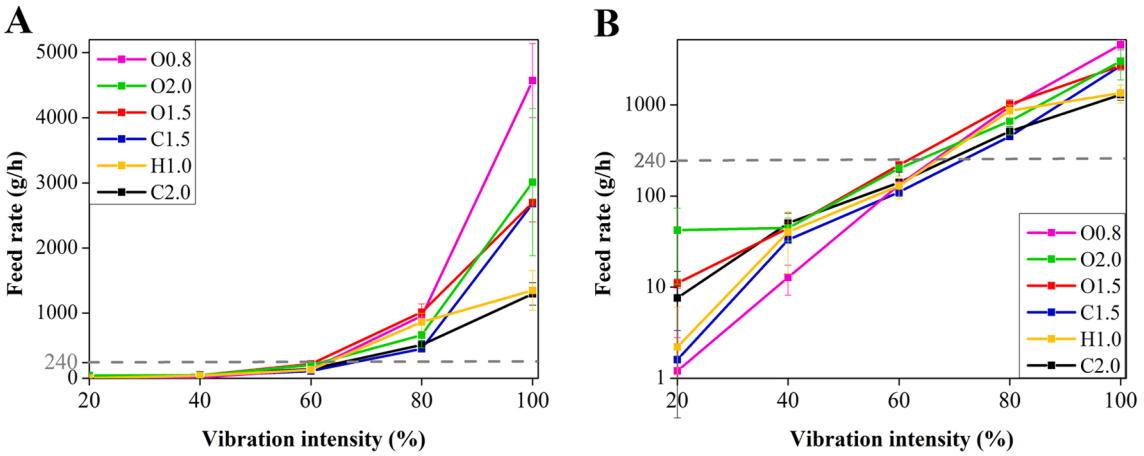


Fig. 6. Correlation between the feed rate and the intensity of vibratory amplitude represented on the original scale (a) and logarithmic scale (b).

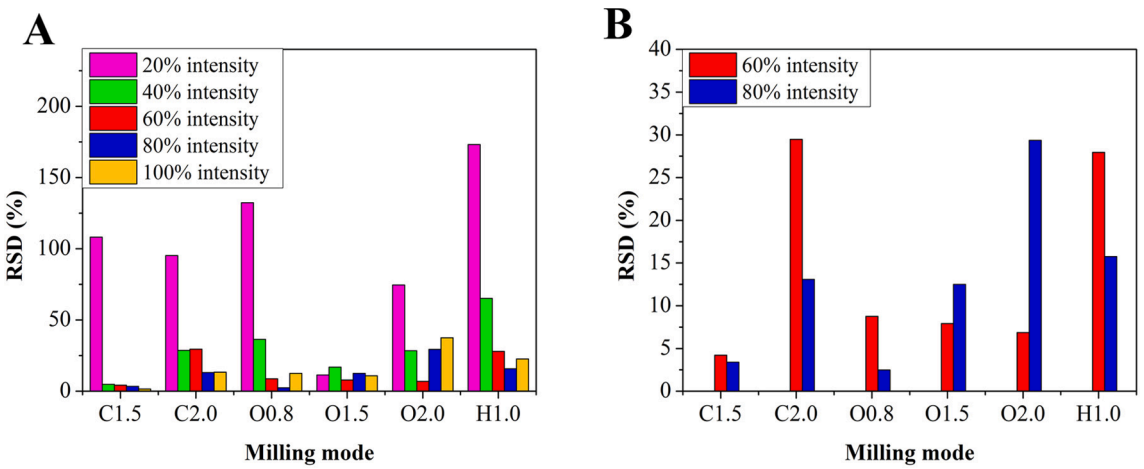


Fig. 7. RSD values at different vibratory intensities.

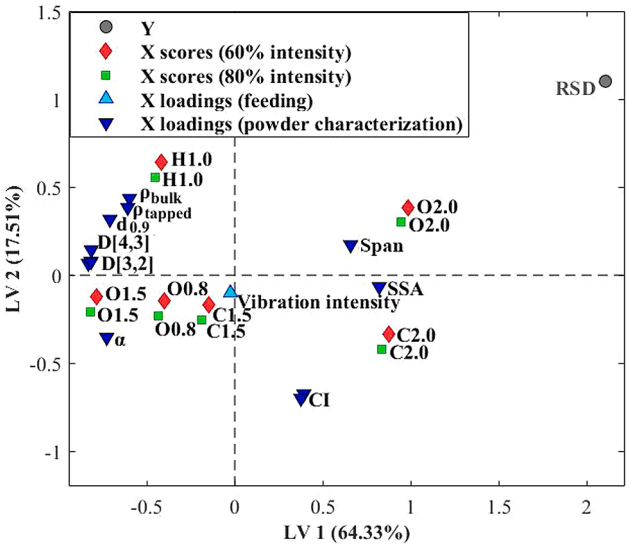


Fig. 8. Scores and loadings 'biplot" of the built PLS model.

and thus it could have a high impact on the appropriate and precise vial filling. Furthermore, the results of the modeling suggest that extending the model with more material property descriptors or different electrospun samples (different API-excipient) compositions can further increase the performance of the model. In that way, the method could be applicable during the formulation of electrospun samples for the selection of the appropriate milling method in the case of a new composition.

3.4. *Vial filling with the selected powders and feeding adjustments*

The targeted weight during vial filling was 3.40 g since the dosage of the VOR and SBE-β-CD-loaded marketed product was intended to be achieved (Vass et al., 2019a). For accurate dosing, two different methods were tested (Fig. 9), which both could be operated in continuous mode.

First, tared sample holders were put on a moving conveyor belt, while the filling was accomplished by the vibratory feeder (Fig. 9a). To fit the productivity of the vial filling to the electrospinning experiments, a 240 g/h rate was used. It meant that filling a vial with 3.40 g electrospun samples required a time of 51 s. Therefore, the computer software stopped the feeder after 51 s feeding and restart it when the next sample holder was under the feeder. The time between the filling of two vials was aligned with the moving rate of the conveyor belt, which was determined during preliminary experiments. The shortest possible idle time between the filling of two vials was attempted to achieve, which means that the feeding was restarted immediately when the mouth of the next vial was under the feeder. The vibratory feeder was calibrated with each investigated milled sample before the continuous experiments to adjust the required feed rate with the setting of the vibration intensity. The vial filling tests were performed only with the O0.8, O1.5 and C1.5 samples because these materials showed anti-correlation with RSD according to the previously presented PLS model. The anti-correlation meant that the RSD values during the feeding experiments

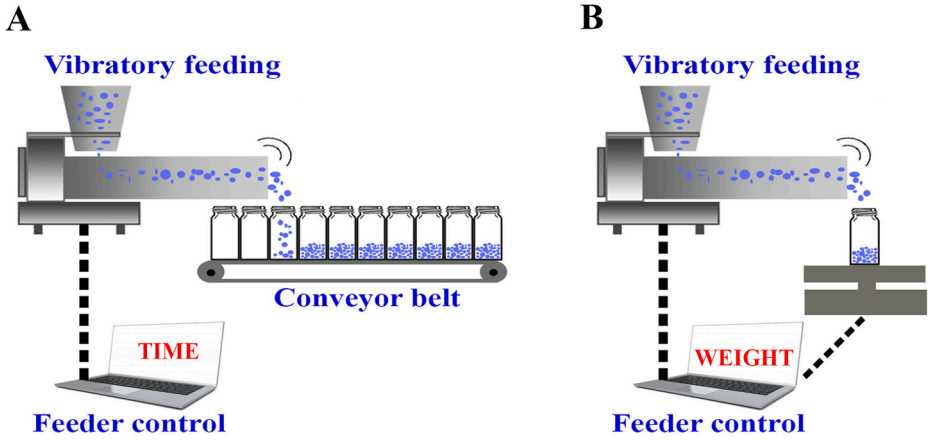


Fig. 9. Vial filling based on time-control (a) and weight-control (b).

were the lowest in the case of O0.8, O1.5 and C1.5 materials. Therefore, these samples seemed to be suitable for accurate vial filling. During the powder filling, three vials were filled and the fed weights were subsequently measured to check the reproducibility and accuracy of the continuous vial filling (Table 4). Investigation of filling of three vials allowed to calculate standard deviation and RSD values while the material usage was minimal, which can be advantageous for instance during early developments of expensive APIs. The low RSD values revealed that the continuous dosing of milled electrospun materials is possible with a vibratory feeder. Moreover, the preliminary calibration can be omitted if a gravimetric vibratory feeder is used. In that way, the needs of the pharmaceutical industry can be met even better and the vial filling with electrospun materials might become more accurate.

As for the other option, the application of a catch scale enables the determination of the current weight precisely (Fig. 9b). During these experiments, the computer software stopped the feeder based on the weight measured by the catch scale thus the feedback control of the feeder was performed. Only the O1.5 sample was used for the weight-controlled tests, where a 3.40 g limit of weight was set in the software to stop the feeder. The results of three repeated measurements are summarized in Table 5. The measured weights showed that there is a short idle time during the feedback-control of the feeder since the vials contained more than 3.40 g electrospun sample in all cases. Although the continuous operation of the time-controlled version coupled with a conveyor belt can be accomplished more easily, the application of automatic sample robots might allow continuous dosing based on weight control as well. Besides, calculation of the idle time and adjusting the limit of weight according to that can lead to more accurate feeding results.

Nonetheless, the application of vibratory feeding seemed to be a suitable choice in the case of high target weight since the deviations from the target values were low. Consequently, the presented powder filling methods could be used for other reconstitution powders with high target values such as for the FDA-approved Virazole®, which contains 6 g of lyophilized powder per vial (Drugs.com, 2019). In addition, vibratory feeding would be probably applicable for dosing smaller amounts since the intensity of the vibratory amplitude and the time of the feeding periods can be varied. Consequently, further research and optimization can make the vibratory feeding suitable for preparing formulations with lower fill weights (e.q. below 0.5 g) as well.

The reconstitution tests of the O1.5 samples dosed by the weight-control method were performed by adding 19 mL of water to each filled vial (Pfizer, 2010). The vials were shaken to dissolve the samples stuck on the wall. After 30 s, the 3.40 g of electrospun samples were fully dissolved and showed ~ 100% dissolution. The reconstitution tests confirmed that the advantages of the fibers with large surface area remained even after the milling and the feeding. Consequently, the electrospinning followed by oscillating milling and vibratory feeding proved to be suitable for the production of reconstitution dosage forms and it has the potential with further development to evolve to a continuous manufacturing line.

4. Conclusions

Different milling methods were tested to make suitable VOR and SBE-β-CD-loaded electrospun materials for vial filling. The SEM images showed that the fibrous structure remained after all types of milling. However, the powder characteristic of the milled samples was different according to the results of three basic characterization methods and the PCA model building. The reason for the variations could be the formation of different size agglomerates after the milling, which were confirmed with the SEM images and with the results of the laser diffraction measurements.

After the milling, the flow properties of the electrospun material became better and each sample proved to be appropriate for vial filling with a vibratory feeder. The feed rate was controlled by adjusting the vibration intensity, which influenced the distribution of the powders in the channel too. The tested intensity values resulted in different feed rates in the case of the examined milled samples while the standard deviations were also differed based on three repeated measurements at each setting. The powder characterization and the preliminary feeding experiments assumed that the flow properties of the samples milled by different methods affect the accuracy and efficiency of the feeding. The correlation between the material property descriptors and the feeding was investigated by PLS regression, which presented that the RSD values was the lowest in the case of the samples with higher bulk and tapped densities, and higher volume and surface weighted means. The results of the built PLS model showed that the samples milled by oscillating milling with 0.8 and 1.5 mm of sieve diameter and by conical milling with 1.5 mm of sieve diameter are the most promising from the accurate and reproducible vial filling point of view.

Two methods were tested for vial filling of milled electrospun materials, which both can be operated in the continuous mode. The reconstitution tests of the O1.5 samples showed quick and complete dissolution, which proved the applicability of milled electrospun fibers for reconstitution dosage forms. The presented work revealed that milling is essential during the formulation of electrospun sample-loaded drug products since the flow properties of the fibrous materials can be increased in this way. Furthermore, vibratory feeding proved to be suitable for feeding the electrospun products without any further excipients, which can make easier the developments of the final dosage forms. Besides, the applied multivariate data analysis methods enabled us to get a clear overview of the effect of the milling methods and the material property descriptors on the feeding. Increasing the dataset with more sample and characterization methods, and further development of the models may lead to improving predictive performance of the models; therefore the time and material consumption of the developments could be decreased.

Table 4
Data of vial filling after time-control.

Sample code	Adjusted intensity (%)	Average weight (g)	Deviation from the target value (%)	Standard deviation (g)	RSD (%)
O0.8	68	3.43	0.9	0.18	5.1
O1.5	64	3.36	2.7	0.10	2.9
C1.5	64	3.31	1.2	0.16	5.0

Table 5
Results of vial filling besides weight-control.

Repetition	Measured weight (g)
1	3.60
2	3.52
3	3.47
Average (g)	3.53
Standard deviation (g)	0.07
RSD (%)	1.87

CRediT authorship contribution statement

Edina Szabó: Investigation, Writing – original draft, Writing – review & editing. **Petra Záhonyi:** Investigation, Writing – original draft. **Dorián L. Galata:** Investigation, Visualization, Writing – original draft. **Lajos Madarász:** Investigation, Writing – original draft. **Panna Vass:** Visualization, Writing – review & editing. **Attila Farkas:** Resources, Writing – review & editing. **Jens Dhondt:** Supervision, Writing – review & editing. **Sune K. Andersen:** Supervision, Writing – review & editing. **Tamás Vígh:** Resources, Writing – review & editing. **Geert Verreck:** Resources, Writing – review & editing. **István Csontos:** Supervision, Writing – original draft, Writing – review & editing. **György Marosi:**

Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Zsombor K. Nagy:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, 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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