European Journal of Pharmaceutics and Biopharmaceutics 137 (2019) 77–85

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Optimisation of an in-line Raman spectroscopic method for continuous API quantification during twin-screw wet granulation and its application for process characterisation

Julia Harting, Peter Kleinebudde*

Institute of Pharmaceutics and Biopharmaceutics, Heinrich Heine University, Universitaetstrasse 1, 40225 Duesseldorf, Germany

ARTICLE INFO

Keywords:
Twin-screw granulation
In-line Raman spectroscopy
Mixing
Blend uniformity
Process Analytical Technology
Multivariate analysis

ABSTRACT

In a previous publication, the development of an in-line Raman spectroscopic method for continuous API quantification during twin-screw wet granulation was presented. An in-line method was developed successfully and the developed method showed an acceptable prediction error. A disadvantage of the developed method was that a measurement was only possible in the dark since light influenced the Raman spectra and made a data interpretation impossible. Therefore, the measurement setup for the implementation of the Raman probe was optimised in the present study to allow a measurement in interior light and to further improve the predictive performance. With the optimised setup, two different calibration models were developed and compared. For the first calibration model, spectra were collected in the dark as before and for the second in interior light. The dark calibration model was able to predict the API content with an RMSEP of 0.31% and the light model with an RMSEP of 0.29%. Thus, both PLS models showed prediction errors in the same order. Consequently, it was possible to evaluate Raman spectra which were collected in interior light. Further, the previous prediction error of 0.60% could be clearly decreased.

The optimised Raman method was applicable to evaluate the mixing efficiency of the twin-screw granulator during a split feeding process. The quality of the mixture was monitored behind different barrel sections by Raman spectroscopy and the corresponding API concentrations were predicted by the developed calibration model. For a screw length of 40 D and a screw configuration with two kneading blocks a good mixing ability was observed. For a screw length of 20 D and one kneading block the mixing efficiency was largely acceptable whereas a broad scattering of the API content was observed when no kneading blocks were used.

In a second part, an experimental design was performed for each screw configuration to evaluate the influence of the barrel-fill level and screw speed on the mixing efficiency. The quality of the mixture using the entire barrel length was minimally influenced by the fill-level. For the other two positions, the screw speed influenced the quality of the mixture slightly. Thus, for an appropriate mixing, a certain barrel length and a screw configuration with two kneading blocks were necessary.

1. Introduction

Twin-screw granulation is gaining more interest in the pharmaceutical industry since it is a promising continuous wet granulation technique for solid dosage manufacturing [1]. The twin-screw granulation process stands out by its high design flexibility and also by a short residence time [2,3], which is beneficial for a continuous process. Nevertheless, until today the produced granules are mostly characterised by often destructive and further time consuming off-line analysis methods which degrade the former mentioned advantages [4]. Thus, there is still a high demand for new non-destructive and fast in-line analysis methods. This need is also supported by the Food and Drug Administration, which released a Process Analytical Technology initiative already in 2004 [5].

During the twin-screw wet granulation process, critical quality attributes as moisture content, particle size, solid state of the API and the content uniformity of the produced granules should be taken into consideration and monitored with appropriate Process Analytical Technologies (PAT).

A possible analytical instrument to monitor the Active Pharmaceutical Ingredient (API) content of the continuously produced granules and thus, to control the content uniformity, is Raman

---

* Corresponding author.
E-mail address: Kleinebudde@hhu.de (P. Kleinebudde).

https://doi.org/10.1016/j.ejpb.2019.02.015
Received 7 November 2018; Received in revised form 18 February 2019; Accepted 18 February 2019

spectroscopy. Raman spectroscopy is a molecular vibrational spectroscopic technique which does not require any sample pre-treatment [6]. Furthermore, Raman spectroscopy allows a fast and non-destructive measurement and is further just minimal sensitive towards interference by water [7], and therefore, suitable to monitor a wet granulation process. Besides the mentioned advantages, a considerable disadvantage of Raman spectroscopy is its sensitivity towards light and background signals [7]. Background signals like fluorescence and ambient light caused e.g. by illumination of displays and fluorescent tubes can overlay the Raman spectra of the particular sample and make a data interpretation impossible or at least exceedingly difficult. Thus, so far Raman measurements were only possible in the dark or darkened rooms.

Nevertheless, due to the before mentioned advantages, Raman spectroscopy was evaluated as a PAT tool for continuous API quantification during twin-screw wet granulation in a previous study [8]. An in-line Raman spectroscopic method was developed successfully, which showed a good predictive performance over the applied concentration range. Nevertheless, it was stated that the developed method has to be optimised regarding its predictive performance since the observed prediction error was still too high for a content uniformity analysis. Furthermore, as mentioned above, a measurement was only possible in a darkened room. This compromised the applicability and also the user convenience.

Thus, in the first part of the present study, the developed method for an in-line API quantification was optimised regarding its predictive precision. Further, the measurement setup was enhanced to allow a measurement in interior light. Two different PLS calibration models were built to predict the API concentration and compared with the previous results.

In a second part of the present study, the optimised method for an in-line API quantification was used to evaluate the mixing behaviour of the twin-screw granulator. The quantitative Raman method allows a direct measurement of the blend quality since a direct prediction of the API content is possible. Thus, not just surrogate or process parameters are used to control [9,10]. Furthermore, Raman spectra contain many multivariate information. Therefore, Raman spectroscopy allows the collection of data rich profiles of the mixing quality [11]. First results regarding the mixing ability of the twin-screw granulator were also published in the previous study [8]. There, split feeding experiments were done since split feeding has only been investigated to a limited extent besides some applications feeding just a small amount of API [12]. An appropriate mixing efficiency of the twin-screw granulator for the chosen settings and a simple binary mixture was observed. Also, Van Melkebeke et al. showed that the twin-screw granulator had a good mixing efficiency independent of screw configuration, granulation time and granule size [12]. Since these results are very promising so far, the investigations should be extended in the present study.

It is well known today that the kneading elements are necessary for a good liquid distribution in the powder bed [13], but to our best knowledge no investigations have been made so far regarding the powder mixing during a split feeding process. For a twin-screw granulation process in a continuous manner or to shorten production lines, it would be desirable to avoid a batch-wise pre-blending step or an additional continuous mixer. Thus, more knowledge about the mixing ability of the twin-screw granulator has to be required to figure out how many powder feeders can be placed along the granulator barrel. Furthermore, it is well known today that the barrel fill-level influences the granulation process and the granule attributes [14–16]. Kumar et al. found out that a higher fill degree caused a restriction of the material flow [17]. Thus, it can be assumed that the fill-level has also an influence on the mixing ability. Therefore, the quality of a binary blend was evaluated behind different barrel sections and further, the influence of the process parameters fill-level and screw speed on the blend quality were examined since to our best knowledge no studies exist which combine fill-level and split feeding experiments. To keep it as simple as possible, the specific feed load (SFL) [18] was used as a surrogate parameter for the barrel fill-level.

2. Materials and methods

2.1. Materials

Diclofenac sodium (UNIQUE Chemicals, Mumbai, India) was chosen as a model API in concentrations from 5 to 25%. Lactose monohydrate (Granulac 200, Meggle, Wasserburg, Germany) was used as a filler. Since the flow properties of the powder mixture were not sufficient, fumed silica (Aerosil® 200 VV Pharma, Evonik Industries AG, Hanau, Germany) was required as a flow-regulating agent.

2.2. Methods

2.2.1. Optimisation of the experimental setup

The in-house created construction for the implementation of the Raman probe, which has been described in a former publication [8], was optimised regarding the stability and light transmission, to improve the predictive performance and to allow a measurement in interior light (light of the cold cathode fluorescent tubes in the lab). To decrease the amount of scattered light and to improve the signal to noise ratio, the installation angle of the probe head was changed from 90° to 60°. Further, the construction was reworked to enhance the implementation of the Raman probe and ensure that the probe head can be fixed always in exactly the same position. Moreover, the construction was closed besides a small opening for the granules to fall out in order to decrease the amount of light in the measuring chamber (Fig. 1). To reduce light reflexion, the walls of the measuring chamber were painted matt black.

2.2.2. Development of a PLS calibration model

New calibration models were developed using the SIMCA software (Version 13.0.3, Sartorius Stedim Biotech, Sweden) as described in the previous publication. For a detailed method description, the reader is referred to our former publication [8].

2.2.2.1. Blending. Powder batches of 1.5 kg containing 5 to 25% [w/w] diclofenac sodium, lactose monohydrate and 0.2% fumed silica (Table 1) were blended for 20 min at 30 rpm in a lab scale blender (LM 40, L.B. Bohle, Ennigerloh, Germany).

2.2.2.2. Twin-screw wet granulation. After pre-blending the powder batches were granulated using a Pharma 16 twin-screw granulator (Thermo Fisher Scientific, Karlsruhe, Germany) with a screw diameter of 16 mm and a total screw-length of 40 × D. For the development of the PLS calibration model, a standard screw configuration with two kneading blocks was applied in the following setting: 8 D conveying – 5 × kneading discs 60° (1 ¼ D) – 10 D conveying – 6 × kneading discs 60° (1 ½ D) – 1 D distributive flow element – 18 D conveying (Fig. 2a). The twin-screw granulator was equipped with a loss-in-weight powder feeder (K-CL-24-KT 20, K-Tron, Niederlenz, Switzerland) and a micro annular gear pump (MZP 7205, HNP-Mikrosysteme, Schwerin, Germany) for the liquid feeding. The twin-screw granulator was run with a screw speed of 150 rpm, a throughput of 40 g/min and a liquid to solid (L/S) ratio of 0.115. The barrel temperature was set to 30 °C to keep the temperature constant during all experiments.

2.2.2.3. Raman spectroscopy applied for the model development. The Raman RXN2™ Hybrid Analyzer (Kaiser Optical Systems, Ann Arbor, USA) and the iC Raman™ 4.1 software package (Kaiser Optical Systems, Ann Arbor, USA) were used to collect in-line Raman spectra. The excitation laser had a wavelength of 785 nm and 400 mW power. Raman spectra were collected in front of the granulator barrel (Fig. 2a) using the PhAT (Pharmaceutical Area Testing) probe head, which was equipped with an immersion optic and fixed in an angle of

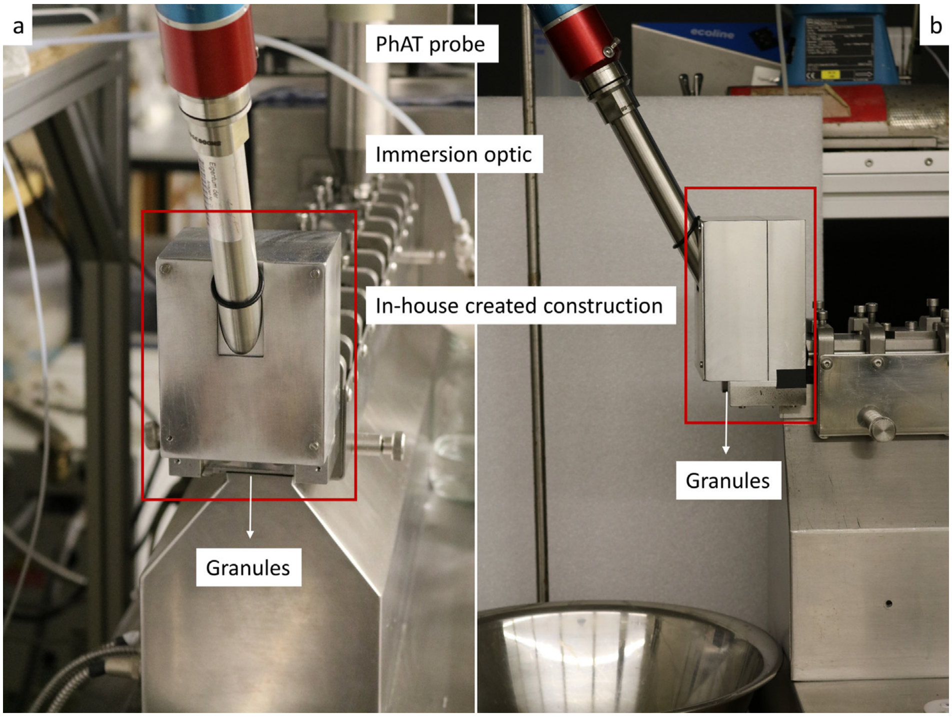

Fig. 1. In-house created construction for the implementation of the Raman probe a. frontal view b. side view.

Table 1
Composition of the calibration and test samples.

	Diclofenac sodium [%, w/w]	Lactose monohydrate [%, w/w]	Fumed silica [%, w/w]	L/S - ratio
Calibration samples				
1	5	94.8	0.2	0.115
2	10	89.8	0.2	0.115
3	15	84.8	0.2	0.115
4	20	79.8	0.2	0.115
5	25	74.8	0.2	0.115
Test samples				
1	7.5	92.3	0.2	0.115
2	12.5	87.3	0.2	0.115
3	17.5	82.3	0.2	0.115
4	22.5	77.3	0.2	0.115

60° in the in-house created and optimised construction (Fig. 1). Thus, Raman spectra were collected with a working distance of about 25 cm from the granule surface, with a spot size diameter of 6 mm and an exposure time of 2 s. One spectrum was collected every 20 s and the first spectrum was collected after a start-up period of ten minutes. Raman spectra were collected in the dark and in interior light (cold cathode fluorescent tube). For both setups respectively 25 Raman spectra were collected per calibration and test mixture. The spectra were collected in the spectral range from 150 to 1890 cm^{-1} with a Raman shift interval of 1 cm^{-1}. Further, a cosmic ray filtering and dark subtraction were performed for each measurement by the software.

2.2.2.4. Development and validation of a PLS model. With the optimised setup, two separate PLS models were developed and compared. For the first calibration model, spectra were collected in the dark and for the second in interior light. Hereinafter referred to as dark model and light model.

For the data evaluation, preprocessing and model building the SIMCA software (Version 13.0.3, Sartorius Stedim Biotech, Sweden) was used. Before the model building, the spectral data were preprocessed by Standard Normal Variate (SNV) transformation and mean-centering. After preprocessing, a PLS calibration model was built by regressing the 125 in-line collected Raman spectra of the calibration samples (5 concentration levels × 25 spectra per concentration) against the known API concentrations calculated by weight. The spectral range from 300 to 620 cm^{-1} and 1000 to 1650 cm^{-1} was used for the modelling.

In-line Raman spectra of new binary mixtures (test samples) containing different API concentrations were collected on a different day to evaluate the predictive performance of the developed PLS calibration models. To estimate the quality of the calibration and the predictive performance, the root mean square error of calibration (RMSEC), the standard error of calibration (SEC), the coefficient of determination (R^2) and the root mean square error of prediction (RMSEP) were calculated according to the European Pharmacopoeia monograph 5.21. [19]. To verify the predicted API concentrations, the API concentration was calculated by weight and further, UV-analysis was used as a reference method (See Section 2.2.3.4).

2.2.3. Evaluation of the mixing efficiency

2.2.3.1. Split feeding process. To evaluate the mixing efficiency, the Pharma 16 twin-screw granulator was equipped with two loss-in-weight feeders which were placed in different positions along the barrel (Fig. 2b-d). The first feeder added lactose monohydrate and the second diclofenac sodium resulting in a total powder feed rate of 40 g/min. An API concentration of 15% [w/w] was adjusted by the feed rates of both feeders for all experiments.

The feeders were placed at different ports to vary the effective screw length for wet granulation in order to investigate different barrel sections. Thus, the quality of the mixture could be examined for barrel section 4 and 5 (before and behind the first kneading zone (Fig. 2c-d) and behind the entire barrel length (Fig. 2b). All other process parameters were the same as described for the previous experiments.

2.2.3.2. Design of experiments. For each barrel length/screw configuration a 3-level full factorial design (Modde 12.0, Sartorius Stedim Biotech, Sweden) with three replicates of the design center point was performed to evaluate the influence of the barrel fill-level

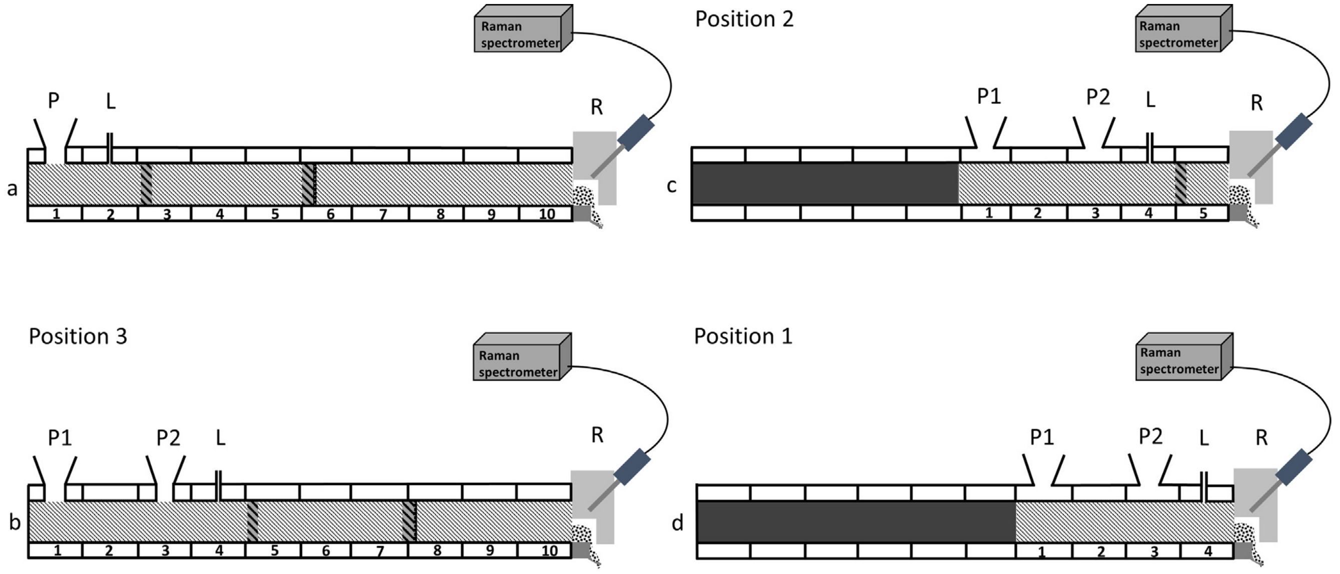

Fig. 2. Experimental setup a. for the development of the PLS calibration models b-d. for the split feeding experiments ▨ conveying elements ▧ 60° kneading elements ■ distributive flow element P: loss-in-weight powder feeder L: liquid feed R: Raman probe.

Table 2
Raw data of the DoE – factors and response variables (n = 60 (Raman); n = 6 (UV)).

Experiment	Specific feed load [g]	Screw speed [1/min]	Resulting powder feed rate for lactose [g/min]	Resulting powder feed rate for diclofenac [g/min]	Resulting liquid feed rate [g/min]	Absolute standard deviation [%] P3	Absolute standard deviation [%] P2	Absolute standard deviation [%] P1	UV Absolute standard deviation [%] P3	UV Absolute standard deviation [%] P2	UV Absolute standard deviation [%] P1
N 1	0.134	150	17	3	2.30	0.51	1.35	2.19	0.48	1.32	1.57
N 2	0.20	150	25.5	4.5	3.45	0.44	1.01	2.08	0.13	0.99	1.49
N 3	0.267	150	34	6	4.60	0.19	0.80	1.47	0.55	1.28	0.83
N 4	0.134	300	34	6	4.60	0.44	0.67	1.94	0.22	1.65	2.55
N 5	0.20	300	51	9	6.90	0.29	0.62	1.64	0.34	1.26	1.44
N 6	0.267	300	68	12	9.20	0.33	0.51	0.83	0.18	0.96	0.62
N 7	0.134	450	51	9	6.90	0.33	0.64	0.84	0.34	0.97	0.70
N 8	0.20	450	76.5	13.5	10.35	0.41	0.61	1.24	0.27	0.53	0.81
N 9	0.267	450	102	18	13.80	0.17	0.32	0.57	0.32	1.08	1.07
N 10	0.20	300	51	9	6.90	0.38	1.09	0.93	0.39	1.00	0.48
N 11	0.20	300	51	9	6.90	0.28	0.98	1.06	0.58	0.38	1.19
N 12	0.20	300	51	9	6.90	0.35	0.46	0.78	0.14	0.57	0.53

(ratio of throughput to screw speed) and screw speed on the mixing efficiency (Table 2). The screw speed was varied from 150 rpm to 450 rpm and the fill-level from 0.134 g to 0.267 g. The influence was quantified by calculating the absolute standard deviation of the API content as a response parameter for the quality of the mixture leaving the granulator. For the calculation the values recorded during the steady state were used. Thus, a standard deviation of the API concentration over ten minutes corresponding to 60 spectra was calculated. The model for the absolute standard deviation was optimised via backwards regression after fitting to the experimental data in order to improve the coefficient of prediction (Q^2).

Furthermore, a relative bias was calculated by Eq. (1) referred to a reference value y_i calculated by UV-analysis to proof the correctness of the predicted values and to show that the predictive performance of the developed model was independent of the process parameters.

$$Rel.\ bias = \frac{(\hat{y}_i - y_i)}{y_i} * 100\% \tag{1}$$

2.2.3.3. Raman spectroscopy applied during the split feeding process. In-line Raman spectra were collected in front of the granulator barrel as described before. After a start-up period of ten minutes, spectra were collected every ten seconds for 30 min for the first split feeding application and for ten minutes for every setting of the DoE. The chosen interval of ten seconds was below the measured mean residence times for all examined granulator settings (data not shown) and thus appropriate to investigate the mixing ability. The corresponding API concentrations were predicted by the developed PLS calibration model. All measurements took place in interior light.

2.2.3.4. UV-analysis. To verify the API concentrations predicted by the developed PLS models, UV–VIS spectroscopy was used as a reference method. Therefore, samples were collected every 100 s during the granulation of the test samples, every 180 s during the monitoring behind different barrel sections and every 120 s during the DoE. Afterwards the samples were dried in an oven at 40 °C for 48 h. A quantity of 40 mg of dried samples was dissolved in 100 mL demineralised water and measured at a wavelength of 250 nm using a UV-1800 SHIMADZU UV spectrophotometer (Shimadzu, Duisburg, Germany).

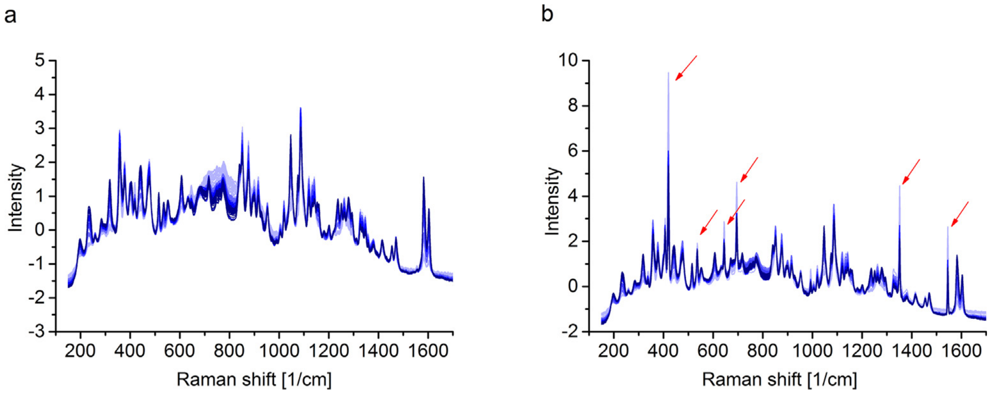

Fig. 3. a. SNV corrected, in-line collected Raman spectra of the calibration mixtures during granulation in the dark. b. SNV corrected, in-line collected Raman spectra of the calibration mixtures during granulation in interior light. The arrows indicate the peaks caused by the interior light. Diclofenac sodium concentrations increase from light blue to dark blue. (For interpretation of the references to colour in this figure the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Results for the optimised setup

3.1.1. Development of a PLS calibration model – Dark model

In the present study, the concentration range from 5 to 25% diclofenac sodium was examined and the developed calibration models were compared with the second developed calibration model for diclofenac sodium described in our previous publication [8]. The concentration range was not extended to 50% as in the former study since for a higher diclofenac sodium concentration a higher L/S ratio was needed for an adequate granulation and additional experiments proved that the L/S ratio had a considerable influence on the Raman spectra and the predicted values (data not shown). Thus, the developed in-line method was just applicable for one L/S ratio, which restricted the concentration range.

The in-line collected and preprocessed Raman spectra of the five calibration mixtures are shown in Fig. 3a with increasing diclofenac concentrations from light blue to dark blue. The intensity of the characteristic diclofenac sodium peaks at 1046 cm^{-1} and from 1578 to 1605 cm^{-1} increased linear to the concentration level. Thus, a first requirement for a successful calibration was met. For the model building, not the entire recorded spectral range was used. Instead, spectral regions which contained important information for the model building were extracted. Thus, the spectral range from 300 to 620 cm^{-1} and 1000 to 1650 cm^{-1} was chosen since these regions could be related to variations in the concentration level. The spectral range from 620 to 1000 cm^{-1} was left out since in this wavenumber range great variations in the spectra were visible (Fig. 3a) which could not be related to changes in the API concentrations. So far no root cause was found for this variations. Further, a peak at 420 cm^{-1} was removed before the model building since this peak was caused by the background. All raw data were preprocessed by SNV and mean-centering before the model building. To develop a PLS calibration model, all in-line collected and preprocessed Raman spectra were regressed against the known diclofenac concentrations calculated by weight since it was supposed that the blended mixtures were homogeneous and did not segregate during the process time.

The PLS model was established with two latent-variables, since the RMSEP was the lowest in this case (Table 3). These two latent-variables captured 97.5% of the spectral variation. The observed versus predicted plot of the calibration samples (Fig. 4a) showed a good correlation between the estimated and predicted diclofenac sodium concentrations. The developed PLS calibration model had an SEC of 0.34% and an R^2 of 0.998. Thus, the calculated model performance indicators were comparable to the ones of the first published PLS calibration model for diclofenac sodium [8]. There, an R^2 of 0.997 and a SEC of 0.36% were observed. Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were calculated regarding the ICH validation guideline [20] and were found to be 1.1% and 3.4%. Thus, also these detection limits were in the same order compared to the first calibration model where the LOD was 1.2% and the LOQ 3.6%.

To evaluate the predictive performance of the developed PLS calibration model, in-line Raman spectra of new binary mixtures with different concentration levels (Test samples, Table 1) were collected on a different day and projected onto the developed PLS calibration model to predict the corresponding API concentrations. The observed versus predicted plot for the test samples (Fig. 4b) indicated a good correlation ($R^2 = 0.998$) between the known and predicted diclofenac concentrations. Furthermore, a small y-axis intercept of 0.305% and a slope near 1 were observed. In comparison, the first published calibration model had a y-axis intercept of 0.817%. This already indicated that the predictive precision could be improved. Consequently, the RMSEP of the new calibration model was lower and the model was able to predict the API content with an RMSEP of 0.31%. Thus, the previous prediction error of 0.60% could be clearly decreased.

To verify the predicted diclofenac sodium concentrations, UV-analysis was used as a reference method. The received diclofenac sodium concentrations of the two different analysis methods were plotted against the process time to compare the results (Fig. 5a). This comparison showed that the predicted diclofenac concentrations were highly correlated with the concentrations calculated by the UV measurement which further underlined the good predictive performance of the new calibration model.

3.1.2. Development of a PLS calibration model – Interior light

To improve the applicability of the developed method for an in-line API quantification and to improve the user convenience, a further PLS calibration model was developed with Raman spectra, which were collected in interior light and compared to the dark calibration model. Further, Raman spectra were collected in day light but due to negative peaks in the entire spectral range the spectra were not evaluable. Some additional peaks became obvious in the spectra collected in interior light as well (Fig. 3b). In order to identify these peaks, a background

Table 3
Model performance indicators for the PLS dark model.

Component	R^2X (cum)	R^2Y (cum)	Q^2 (cum)	RMSEC [%]	SEC [%]	RMSEP [%]
1	0.890	0.997	0.997	0.37	0.38	0.35
2	0.975	0.998	0.998	0.34	0.34	0.31
3	0.983	0.998	0.998	0.31	0.31	0.42

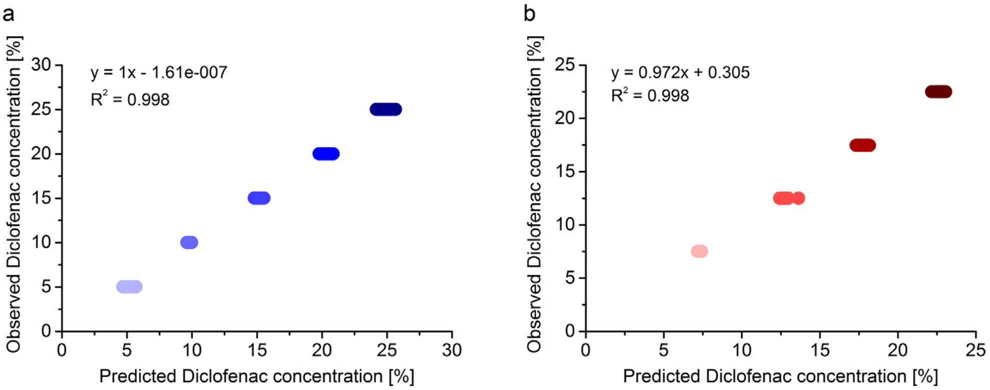

Fig. 4. a. Observed versus predicted diclofenac concentrations for the calibration samples of the dark model. b. Observed versus predicted diclofenac concentrations of the test samples of the dark model.

spectrum of the interior light was manually collected and compared with the strong lines of different materials [21]. Thus, the additional peaks (highlighted with arrows) could be related to argon and krypton, which are used in the cold cathode fluorescent tubes. Consequently, a total exclusion of light could not be achieved with the optimised construction. However, it was not possible to further shade the construction since the granules had to get out of the granulator in one place. Therefore, before the model building, the peaks caused by the light of the fluorescent tubes as well as the spectral range from 620 to 1000 cm^{-1} had to be excluded.

After preprocessing, all in-line collected Raman spectra were regressed against the known diclofenac sodium concentrations calculated by weight as described before. For this model one latent-variable was chosen, which described 92.3% of the spectral variation. The observed versus predicted plot for the calibration samples (Fig. 6a) showed a good correlation ($R^2 = 0.998$) between the estimated and predicted diclofenac sodium concentrations as well. The model performance indicators (Table 4) were in the same order as for the before described dark calibration model, in particular an SEC of 0.31% and an R^2 of 0.998 were observed. The LOD and LOQ were found to be 1.0% and 3.0%. Overall, both models were comparable regarding the model performance indicators. Consequently, it was possible to evaluate Raman spectra which were collected in interior light after a careful preprocessing. The evaluation of the Raman spectra without removal of the peaks caused by the light of the fluorescent tubes resulted in a PLS model with a SEC of 0.49% for three latent-variables.

Also for this model the predictive performance was determined by collecting in-line Raman spectra of new binary mixtures on a different day. Again, the observed versus predicted plot (Fig. 6b) indicated a good correlation ($R^2 = 0.998$) between the known and predicted diclofenac concentrations. The observed y-axis intercept of 0.120% was even smaller as for the dark model and the slope with 0.987 closer to 1. The model was able to predict the diclofenac concentration with a comparable low prediction error of 0.29%. Thus, it was possible to evaluate Raman spectra, which were collected in interior light, without a loss in the predictive performance. The UV-analysis (Fig. 5b) verified the good predictive performance.

In the first part of this study, the predictive performance of the calibration model for an in-line API quantification during twin-screw wet granulation could be clearly improved due to an optimised setup. Further, the applicability and the user convenience could be improved by enabling a measurement in interior light.

3.2. Application of the in-line Raman spectroscopic method during a split feeding process

3.2.1. Mixing in twin-screw granulation

The optimised in-line Raman spectroscopic method was applied to monitor the API content during different split feeding processes to acquire more knowledge about the mixing behaviour in the twin-screw granulator. In a former study, it was shown that the mixing behaviour of the twin-screw granulator was appropriate for the chosen screw configuration and the adjusted process settings. Furthermore, a high mixing ability of the twin-screw granulator was observed [8]. Therefore, in this study the quality of the mixture should be investigated behind different barrel sections to gain more knowledge about the mixing behaviour inside the granulator barrel. In the previous study the entire length of the barrel was exploited. Since pharmaceutical formulations consist in most cases of more than two ingredients, usually more than two powder feeders are required and some excipients or APIs have to be added in the last barrel sections. Thus, just a short distance is available for the mixing and granulation to take place and it should be investigated if this short distance is sufficient. It is commonly known that especially the kneading elements ensure a uniform distribution of the granulation liquid [22,23]. Verstraeten et al. even showed that for some formulations one kneading block is not sufficient [13]. Thus, it is

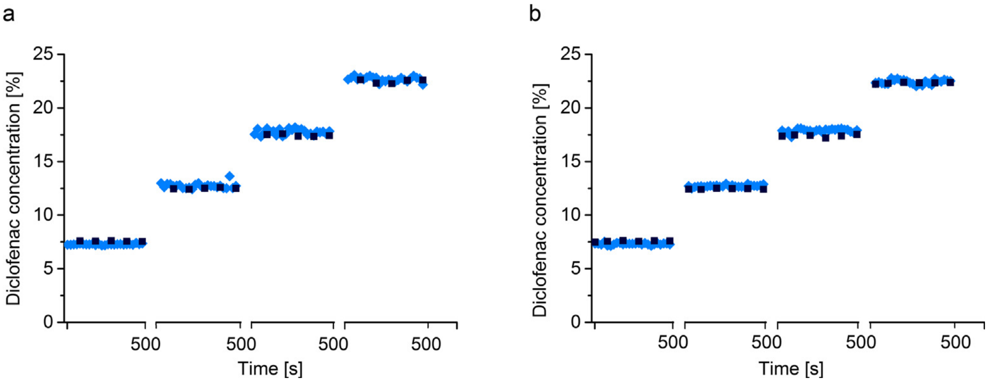

Fig. 5. a. Comparison of the diclofenac concentrations predicted by the developed dark model (diamonds, light blue) and calculated by the UV-analysis (squares, dark blue) for the four different test levels. n = 25 (Raman); n = 5 (UV) b. Comparison of the diclofenac concentrations predicted by the developed light model (diamonds, light blue) and calculated by the UV-analysis (squares, dark blue) for the four different test levels. n = 25 (Raman); n = 6 (UV).

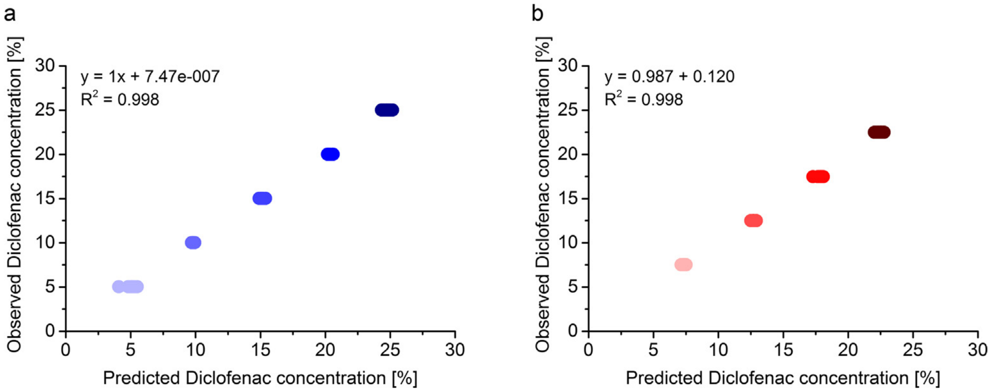

Fig. 6. a. Observed versus predicted diclofenac concentrations for the calibration samples of the light model. b. Observed versus predicted diclofenac concentrations of the test samples of the light model.

obvious that the quality of the powder mixture depends on the number of kneading blocks as well.

Since a direct collection of in-line Raman spectra in the different barrel sections was not possible, the loss-in-weight feeders were placed in different positions along the barrel and the screw configuration was adapted to investigate the different barrel sections. Thus, the influence of the kneading blocks on the mixing quality could be investigated. This research work focused on the mixing efficiency and thus, granule properties like particle size and moisture content were not taken into consideration.

3.2.2. Evaluation of the mixing behaviour behind different barrel sections

For all following results, Raman spectra were collected in interior light and corresponding API concentrations were predicted by the light PLS calibration model.

In this part of the study, the API concentration of the granules was monitored in-line behind three different investigated barrel sections. Therefore, diclofenac sodium and lactose monohydrate containing respectively 0.2% fumed silica were metered by two independently controllable loss-in-weight feeders which were placed at different positions along the barrel (Fig. 2b-d). Thus, Raman spectra were collected behind the entire granulator length (Fig. 2b; Position 3), behind the first kneading block (Fig. 2c; Position 2) and before the first kneading block (Fig. 2d, Position 1).

As supposed before, the kneading elements had a great impact on the content uniformity of the produced granules leaving the granulator. The predicted diclofenac concentrations and the concentrations calculated by the UV measurement were plotted against the process time to visualise the results (Fig. 7). Furthermore, the feeder values were recorded by an in-house written Labview application and a concentration was calculated by this recorded feeder values. For reasons of clarity a moving average over ten seconds was built. The fluctuation of the concentration increased with a decreasing barrel length and a decreasing number of kneading blocks. For a screw length of 40 D and a screw configuration with two kneading blocks a good mixing ability was observed. For a screw length of 20 D and one kneading block the mixing efficiency was largely acceptable whereas a broad scattering of the API concentrations was observed when no kneading blocks were used. To further compare and evaluate the results, the absolute standard deviation of the API content was calculated as a parameter for the quality of the mixture. Therefore, the concentrations measured during the steady state were used. At the beginning of the experiments two und three (Fig. 7, Position P2 and P1) greater variations of the predicted concentration were visible. These fluctuations were caused by an inaccurate powder feeding of the loss-in-weight feeder and were therefore not included in the calculation of the absolute standard deviation of the API content. The calculated standard deviations (Table 5) confirmed the decreasing scattering of the measurement values in dependency of the number of kneading blocks. The absolute standard deviation decreased from 0.62% for no kneading block to 0.40% for one kneading block and 0.25% for two kneading blocks. These findings were also confirmed by the UV-analysis. Nevertheless, the observed variation was acceptable for all examined positions so far since the standard deviation was below 0.94%. Thus, the content uniformity requirements of the European Pharmacopeia for a final dosage form should be fulfilled.

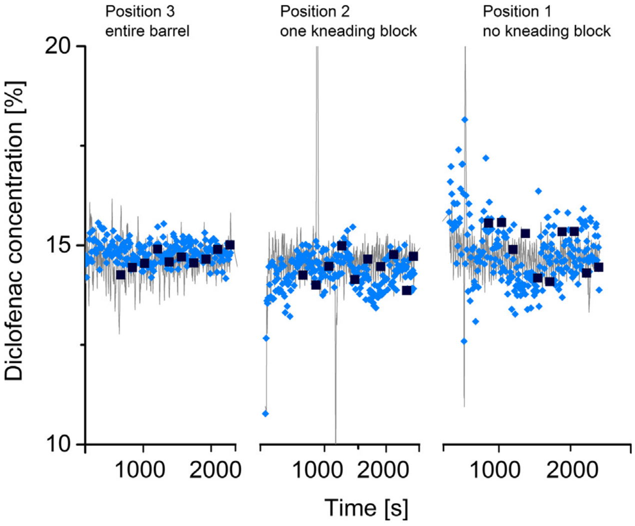

Fig. 7. Diclofenac concentration calculated by the different analysis methods during the evaluation of the mixing behaviour behind different barrel sections. Light blue diamonds: Raman measurement; dark blue squares: UV-analysis; grey line: feeder data. (For interpretation of the references to colour in this figure the reader is referred to the web version of this article.)

Based on these results, it could be assumed that the kneading blocks and a certain barrel length are not just important for an adequate liquid

Table 4
Model performance indicators for the PLS light model.

Component	R^2X (cum)	R^2Y (cum)	Q^2 (cum)	RMSEC [%]	SEC [%]	RMSEP [%]
1	0.923	0.998	0.998	0.30	0.31	0.29
2	0.957	0.999	0.998	0.27	0.27	0.35

Table 5
Mean values and standard deviations of the concentrations predicted by the PLS model and calculated by the UV-analysis for the three different examined positions. n = 162 (Raman); n = 10 (UV).

	Position 3 (entire barrel length)		Position 2 (one kneading block)		Position 3 (no kneading block)	
	Raman	UV	Raman	UV	Raman	UV
Mean value	14.80	14.66	14.36	14.43	14.64	14.90
Standard deviation	0.25	0.23	0.40	0.36	0.62	0.60

distribution but also for an adequate powder mixing during a split feeding application. This knowledge might be helpful when more than two components, metered in different ports along the barrel, should be mixed and granulated in one step since it might be problematic to add excipients or APIs at the end of the granulator.

3.2.3. Evaluation of the mixing behaviour in dependency of different process parameters

To further investigate the obtained findings, for each simulated barrel section a 3-level full factorial design with three replicates of the design center point was performed. The effects of variations in the barrel fill-level and screw speed on the quality of the mixture were determined (Table 2). It is well known today that the barrel fill-level affects the granule properties [14–16] and thus, it is conceivable that the barrel fill-level is essential for the powder blending as well. To keep it as simple as possible, it was decided to use the specific feed load (SFL) by Kolter et al. [18] as a surrogate parameter for the barrel fill-level. The SFL [g] is the ratio of the gravimetric throughput [g/min] and the screw speed [1/min] and correlates with the volumetric fill-level [16].

As a response for the quality of the mixture, the absolute standard deviation of the API content was calculated. The values recorded during the steady state were used for the calculation. Further, a relative bias was calculated referred to a reference value calculated by UV-analysis for every process setting to proof the correctness of the predicted values and to show that the predictive performance of the developed model was independent of the process parameters. The relative bias was below 10% for all performed granulation processes (data not shown), consequently the predicted API concentrations could be assumed to be correct.

For each position, the predicted concentrations and the concentrations calculated by the UV measurement were plotted against the process time (Fig. 8a-c). The arrows indicate new process settings (Table 2). The great variations visible at the beginning in Fig. 8c were again caused by an inaccurate feeding of the used loss-in-weight feeder and thus, these values should be considered with caution. The comparison of the three figures showed clearly what was stated before: the fluctuation of the measured concentration increased with the decreasing number of kneading blocks and a decreasing barrel length. This observation was confirmed by the calculated absolute standard deviations which decreased from position 1 to position 3 for all performed experiments besides for the experiment N 10. Nevertheless, the evaluation of the mixing behaviour for each barrel section resulted in a poor design model since the relationship between the factors and the responses was weak. Hence, it can be concluded that the influence of the process parameters fill-level and screw speed on the quality of the mixture was not as high as the influence of the barrel length and the number of kneading blocks. The highest scattering was observed at the lowest fill-level and the lowest screw speed, less scattering at higher fill-levels and screw speeds. Nevertheless, the quality of the mixture using the entire barrel length (position 3) was only minimally influenced by the fill-level. For the other two positions the screw speed influenced the

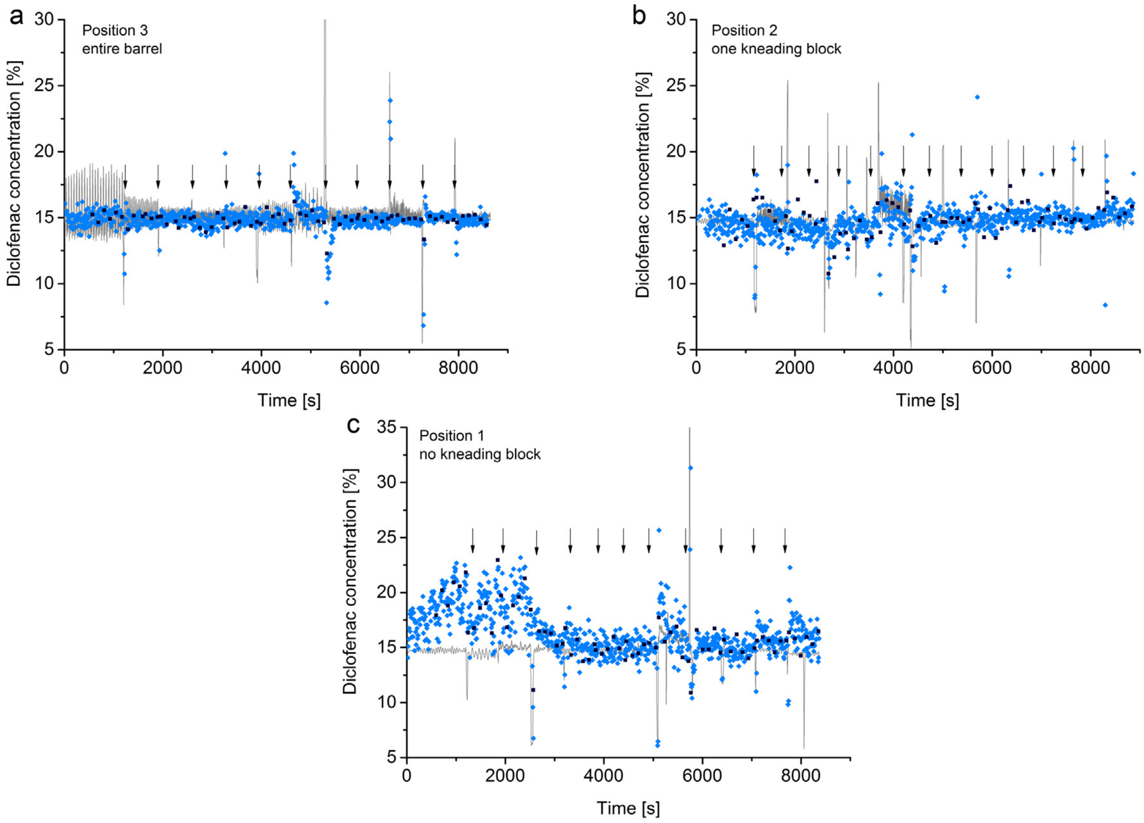

Fig. 8. Diclofenac concentration calculated by the different analysis methods during the evaluation of the mixing behaviour in dependency of different process parameters. Light blue diamonds: Raman measurement; dark blue squares: UV-analysis; grey line: feeder data. The arrows indicate new process settings. (For interpretation of the references to colour in this figure the reader is referred to the web version of this article.)

quality of the mixture slightly. These observations were not confirmed by the reference values calculated by UV-analysis. The absolute standard deviation calculated by the UV values was not influenced significantly by the fill-level or screw speed. However, for the UV measurement only six samples were collected for every experiment during the granulation to verify the concentrations predicted by the developed PLS calibration model. Thus, the sample size was low compared to the Raman measurement with 60 spectra per setting. Furthermore, it was difficult to differentiate if the lower standard deviation was really caused by an increased blending or just because the sample volume was higher. Further studies showed that the sample size, hence the granule mass measured during an exposure time of two seconds, also increased with a higher fill-level and screw speed. With an increased fill-level and screw speed the granules left the granulator with a higher velocity, and therefore, more granules passed the Raman probe during an exposure time of two seconds.

Nevertheless, it was proven that the quality of the mixture was different behind the investigated barrel sections for all adjusted process settings and it can be assumed that not all produced granules would pass the content uniformity requirements of the European Pharmacopeia (2.9.40 Uniformity of dosage units). Certainly, in this case no final dosage unit exists and therefore, no precise limits are defined. Nevertheless, some calculated standard deviations (≥ 0.94) led assume that the dosage unit would not pass the requirements when the mean content conforms the desired value. In this case a variation of the API content of 6.25% in the final unit dosage form is acceptable. An overall appropriate quality of the mixture could only be reached when using the entire barrel length and a screw configuration with two kneading blocks. Thus, kneading blocks are not only required for an adequate liquid distribution but also for a good powder mixing. This should be taken into consideration when no pre-blending step is used before the granulation process.

4. Conclusion

The predictive performance of an in-line Raman spectroscopic method for continuous API quantification during twin-screw wet granulation could be clearly improved by revising and optimising the measurement setup for the implementation of the Raman probe. The previous prediction error could be reduced by half. Furthermore, for the first time it was possible to evaluate Raman spectra, which were collected in interior light without a loss in the predictive performance. Thus, the applicability and the user convenience of the in-line analysis method were improved.

With the improved model the mixing efficiency of the twin-screw granulator was examined further. The results indicated that two kneading blocks are required to ensure a content uniformity of the produced granules. Thus, it might be problematic to add APIs and excipients at the end of the granulator barrel.

Acknowledgments

Dr. Klaus Knop and the fine mechanics of the faculty of Mathematics and Natural Sciences of the Heinrich Heine University of Duesseldorf are kindly acknowledged for the support in the planning of the optimisation and the manufacturing of the construction for the Raman probe. Dr. Björn Fischer is kindly acknowledged for his help in identifying the Raman peaks of the fluorescent tubes.

References

[1] T.C. Seem, N.A. Rowson, A. Ingram, Z. Huang, S. Yu, M. de Matas, I. Gabbott, G.K. Reynolds, Twin screw granulation — A literature review, Powder Technol. 276 (2015) 89–102.

[2] J. Vercruysse, D. Cordoba Diaz, E. Peeters, M. Fonteyne, U. Delaet, I. Van Assche, T. De Beer, J.P. Remon, C. Vervaet, Continuous twin screw granulation: influence of process variables on granule and tablet quality, Eur. J. Pharm. Biopharm. 82 (2012) 205–211.

[3] J. Vercruysse, M. Toiviainen, M. Fonteyne, N. Helkimo, J. Ketolainen, M. Juuti, U. Delaet, I. Van Assche, J.P. Remon, C. Vervaet, T. De Beer, Visualization and understanding of the granulation liquid mixing and distribution during continuous twin screw granulation using NIR chemical imaging, Eur. J. Pharm. Biopharm. 86 (2014) 383–392.

[4] S. Laske, A. Paudel, O. Scheibelhofer, S. Sacher, T. Hoermann, J. Khinast, A. Kelly, J. Rantanen, O. Korhonen, F. Stauffer, F. De Leersnyder, T. De Beer, J. Mantanus, P.-F. Chavez, B. Thoorens, P. Ghiotti, M. Schubert, P. Tajarobi, G. Haeffler, S. Lakio, M. Fransson, A. Sparen, S. Abrahmsen-Alami, S. Folestad, A. Funke, I. Backx, B. Kavsek, F. Kjell, M. Michaelis, T. Page, J. Palmer, A. Schaepman, S. Sekulic, S. Hammond, B. Braun, B. Colegrove, A Review of PAT strategies in secondary solid oral dosage manufacturing of small molecules, J. Pharm. Sci. 106 (2017) 667–712.

[5] Food and Drug Administration, Guidance for Industry, PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance, 2004.

[6] T. De Beer, A. Burggraeve, M. Fonteyne, L. Saerens, J.P. Remon, C. Vervaet, Near infrared and Raman spectroscopy for the in-process monitoring of pharmaceutical production processes, Int. J. Pharm. 417 (2011) 32–47.

[7] N.L. Jestel, Raman Spectroscopy, in: K.A. Bakeev (Ed.) Process Analytical Technology - Spectroscopic Tools and Implementation Strategies for the Chemical and Pharmaceutical Industries, 2010, 198-200.

[8] J. Harting, P. Kleinebudde, Development of an in-line Raman spectroscopic method for continuous API quantification during twin-screw wet granulation, Eur. J. Pharm. Biopharm. 125 (2018) 169–181.

[9] B. Nagy, A. Farkas, M. Gyurkes, S. Komaromy-Hiller, B. Demuth, B. Szabo, D. Nusser, E. Borbas, G. Marosi, Z.K. Nagy, In-line Raman spectroscopic monitoring and feedback control of a continuous twin-screw pharmaceutical powder blending and tableting process, Int. J. Pharm. 530 (2017) 21–29.

[10] M. Fonteyne, J. Vercruysse, F. De Leersnyder, R. Besseling, A. Gerich, W. Oostra, J.P. Remon, C. Vervaet, T. De Beer, Blend uniformity evaluation during continuous mixing in a twin screw granulator by in-line NIR using a moving F-test, Anal. Chim. Acta 935 (2016) 213–223.

[11] D. Riolo, A. Piazza, C. Cottini, M. Serafini, E. Lutero, E. Cuoghi, L. Gasparini, D. Botturi, I.G. Marino, I. Aliatis, D. Bersani, P.P. Lottici, Raman spectroscopy as a PAT for pharmaceutical blending: advantages and disadvantages, J. Pharm. Biomed. Anal. 149 (2018) 329–334.

[12] B. Van Melkebeke, C. Vervaet, J.P. Remon, Validation of a continuous granulation process using a twin-screw extruder, Int. J. Pharm. 356 (2008) 224–230.

[13] M. Verstraeten, D. Van Hauwermeiren, K. Lee, N. Turnbull, D. Wilsdon, M. am Ende, P. Doshi, C. Vervaet, D. Brouckaert, S.T.F.C. Mortier, I. Nopens, T.D. Beer, In-depth experimental analysis of pharmaceutical twin-screw wet granulation in view of detailed process understanding, Int. J. Pharm. 529 (2017) 678–693.

[14] L.J. Gorringe, G.S. Kee, M.F. Saleh, N.H. Fa, R.G. Elkes, Use of the channel fill level in defining a design space for twin screw wet granulation, Int. J. Pharm. 519 (2017) 165–177.

[15] S.V. Lute, R.M. Dhenge, A.D. Salman, Twin Screw Granulation: an investigation of the effect of barrel fill level, Pharmaceutics 10 (2) (2018).

[16] R. Meier, K.-P. Moll, M. Krumme, P. Kleinebudde, Impact of fill-level in twin-screw granulation on critical quality attributes of granules and tablets, Eur. J. Pharm. Biopharm. 115 (2017) 102–112.

[17] A. Kumar, J. Vercruysse, M. Toiviainen, P.E. Panouillot, M. Juuti, V. Vanhoorne, C. Vervaet, J.P. Remon, K.V. Gernaey, T. De Beer, I. Nopens, Mixing and transport during pharmaceutical twin-screw wet granulation: experimental analysis via chemical imaging, Eur. J. Pharm. Biopharm. 87 (2014) 279–289.

[18] K. Kolter, M. Karl, A. Gryczke, Hot-Melt Extrusion with BASF Pharma Polymers, second ed., Pharma Ingredients & Services, BASF SE, 2012.

[19] 5.21. Chemometric Methods Applied to Analytical Data, in: European Pharmacopoeia, ninth ed., 2017.

[20] ICH, Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005.

[21] Nationale Institute of Standars and Technology (NIST), Strong lines, avaiable online on: https://physics.nist.gov/PhysRefData/Handbook/element_name.htm, (downloaded on 14.02.2019).

[22] M.F. Saleh, R.M. Dhenge, J.J. Cartwright, M.J. Hounslow, A.D. Salman, Twin screw wet granulation: binder delivery, Int. J. Pharm. 487 (2015) 124–134.

[23] A. Kumar, M. Alakarjula, V. Vanhoorne, M. Toiviainen, F. De Leersnyder, J. Vercruysse, M. Juuti, J. Ketolainen, C. Vervaet, J.P. Remon, K.V. Gernaey, T. De Beer, I. Nopens, Linking granulation performance with residence time and granulation liquid distributions in twin-screw granulation: an experimental investigation, Eur. J. Pharm. Sci. 90 (2016) 25–37.