

Indirect monitoring of ultralow dose API content in continuous wet granulation and tableting by machine vision

Máté Ficzer^a, Lilla Alexandra Mészáros^a, Lajos Madarász^a, Márk Novák^a, Zsombor Kristóf Nagy^{a,*}, Dorián László Galata^a

^aDepartment of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rakpart 3, H-1111 Budapest, Hungary

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ABSTRACT

This paper presents new machine vision-based methods for indirect real-time quantification of ultralow drug content during continuous twin-screw wet granulation and tableting. Granulation was performed with a solution containing carvedilol (CAR) as API in the ultralow dose range (0.05 w/w% in the granule) and the addition of riboflavin (RI) as a coloured tracer. An in-line calibration in the range of 0.047–0.058 w/w% was prepared for the measurement of CAR concentration using colour analysis (CA) and particle size analysis (PSA), and the validation with HPLC resulted in respective relative errors of 2.62% and 2.30% showing great accuracy. To improve the technique, a second in-line calibration was conducted in a broader CAR concentration range of 0.039–0.063 w/w% utilizing only half the amount of RI (0.045 w/w%), while doubling the output of the granulation line to 2 kg/h, producing a relative error of 4.51% and 4.29%, respectively. Finally, it was shown that the CA technique can also be carried on to monitor the CAR content of tablets in the 42–62 µg dose range with a relative error of 5.20%. Machine vision was proven to be a potent indirect method for the in-line, determination and monitoring of ultralow API content during continuous manufacturing.

1. Introduction

The United States Food and Drug Administration (FDA)'s Process Analytical Technology (PAT) initiative, which was released in 2004, defines PAT as “a system for designing, analysing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality”. This document encourages pharmaceutical manufacturers to improve their development, manufacturing and quality assurance processes (Food and Drug Administration, 2004).

Continuous manufacturing is also a novel approach in the pharmaceutical industry, which can increase efficiency and flexibility, while lowering costs compared to traditional batch technologies (Pauli et al., 2019). Furthermore, continuous manufacturing requires improved analytical sensors, which provide real-time information, and also demands a solid understanding of the used operations (Mali et al., 2016). Quality by Design (QbD) is a process of assuring the product quality by combining the proper comprehension and control of the manufacturing and formulation procedures with fast and accurate sensors (Pramod

et al., 2016). Behind this systematic approach lies the basic idea, that patients' needs should be met by pharmaceutical products with efficacy, safety and characteristics of performance (European Medicines Agency, 2014).

In the pharmaceutical industry, the enhancement of the flow properties of the materials is often required prior to the tableting process, so the press could be evenly filled, therefore ensuring that tablets with a narrow weight distribution can be manufactured. This can be achieved with the process of granulation, which has two implementations: dry and wet granulation (Bacher et al., 2008). Wet granulation can be performed using either batch or continuous processes. Continuous wet granulation can be executed with a twin screw granulator (TSG), which has gained significant interest in the last one and a half decade within the pharmaceutical industry (Dhenge et al., 2010). Twin screw wet granulation (TSWG) has been studied using various approaches, and as a result numerous publications focused on process settings (Meier et al., 2017; Vanhoorne et al., 2016; Vercruysse et al., 2012) and implemented screw configurations (Portier et al., 2020; Vercruysse et al., 2015). Recently, to completely exploit the advantages of continuous manufacturing, TSWG has been examined with PAT tools and various

* Corresponding author.

E-mail address: zsknagy@oct.bme.hu (Z.K. Nagy).

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sensors. Near-infrared spectroscopy (NIRS) was one of the first examined in-line measurement tools, which could be used for monitoring the moisture content of the granules (Veracruz et al., 2014).

In addition, the granule size can be measured using a high-speed imaging camera and image analysis, from which the momentary liquid/solid (L/S) ratio can be deduced. Sayin et al. (2015) and El Hagrasy et al. (2013) coupled the Eyecon® imaging system (2020) to the outlet of a TSG, and found that the particle count and d_{10} data of the granules were the most sensitive parameters to the introduced alterations in the L/S ratio, while d_{50} and d_{90} values were not reliable, because of their greater variation. Meng et al. (2019) used the Eyecon® in addition to Raman and NIR spectroscopy to analyse various characteristics of a TSWG process. They found that NIRS can predict some physical properties of the granules, Raman spectroscopy can monitor the drug content of the product and the d_{10} value measured by the Eyecon® system can be used in process monitoring and process control. Madarász et al. (Madarász et al., 2018) used an in-line particle size analysis tool to predict the liquid/solid (L/S) ratio of the granules. They were the first to apply the data from the in-line gathered images to set up a feedback-control of a TSWG system. The system could correct disturbances in real-time, therefore creating a Process Analytically Controlled Technology (PACT) (Nagy et al., 2017). Because of the correlation between the applied L/S ratio and the API content, it might be possible that the latter could also be measured with the image analysis techniques described here. However, so far, no technique has been proposed which used an in-line high-speed imaging camera to directly or indirectly measure the API content of the product during TSWG.

There were some cases in the past, where the API content was monitored directly in-line during TSWG. Based on the research of Kumar et al. (Kumar et al., 2014), others investigated different analytical techniques and APIs. Rehrl et al. (Rehrl et al., 2018) used an in-line NIR spectrometer to analyse granules containing 4 w/w% Na-saccharine, a placebo API. They developed a partial least squares (PLS) regression model to predict the Na-saccharine concentrations, based on 50 NIR spectral observations of a set of API concentrations from 0 w/w% to 7.5 w/w%. Their model had a root mean squared error of prediction (RMSEP) value of 0.36 w/w% API. Harting et al. (Harting and Kleinebudde, 2018) also developed an in-line API quantification method for TSWG based on Raman spectroscopy. They used ibuprofen and diclofenac sodium in concentrations from 5 w/w% to 50 w/w% as model APIs. They built separate PLS models for the two APIs, employing pre-blends of 8–8 binary mixtures, registering 20 Raman spectra for each, making it a total of 160 spectra per drug. The RMSEP for ibuprofen and diclofenac sodium was 0.59 w/w% and 1.5 w/w% respectively. They also applied their calibration models during a split feeding process with concentrations ranging from 7.5 w/w% to 50 w/w%. They stated that the model could successfully predict the API content but had some limitations at low and high concentrations. Later the authors (Harting and Kleinebudde, 2019) refined their method by optimising the measurement setup, achieving an RMSEP of 0.3 w/w% for their new PLS calibration model based on blends containing 5 w/w% to 25 w/w% diclofenac sodium. Nonetheless, no method capable of measuring API content of granules below 4 w/w% in real-time has been described previously in the literature.

One of the most crucial evaluation factors of solid oral dosage forms is content uniformity (CU). Therefore, CU tests are regulated and conducted through different phases of pharmaceutical research, development, and manufacturing. CU is especially critical during the production of low dose preparations (Rohrs et al., 2006). A drug product is considered low dose, when its active pharmaceutical ingredient (API) content is below 2 w/w% (European Medicines Agency, 2016). Over the past few decades, many low dose products were developed, and many were marketed in the form of tablets. However, the manufacturing of low dose tablets remains a very difficult task, due to the strain of accomplishing CU, low efficacy on account of manufacturing loss and instability, and because of the huge ratio of excipients to API and thus

the lack of reliability (Huang et al., 2013).

In the case of low dose products, wet granulation can ensure the desired CU and also the homogeneity of the utilized excipients (Mahours et al., 2017). During wet granulation the API can be added into the product by dissolving it in the granulation liquid. This way, the CU of the product can be improved, because the API is mixed with the excipients on a molecular level. Furthermore, it can reduce the operator exposure, because the potent, low dose drug is not used in its dry state (Almaya, 2008). Démuth et al. utilized the advantageous properties of TSWG to produce homogenous ultralow dose (0.035 w/w%) carvedilol tablets, while evaluating the impact of different types of pumps on API homogeneity. They found that a continuous TSWG system can produce highly homogeneous ultralow dose tablets with excellent physical properties (Démuth et al., 2020). Fülöp et al. set up a continuous manufacturing (CM) line, consisting of a TSWG, a continuous fluid bed dryer and a continuous sieving unit to produce ultralow dose (0.05 w/w%) carvedilol tablets. They compared the efficiency of TSWG to high-shear granulation and found that the former is superior both in liquid distribution and CU. Furthermore, they achieved significant scale-up with the CM line (from 1 kg/h to 10 kg/h), which yielded granules with properties very similar to the products of previous smaller throughput granulations (Fülöp et al., 2021).

Various methods have been developed, for example in the food industry, where different coloured substances have been monitored using image analysis (Aguilar et al., 2009; Sorouraddin et al., 2015; Wrostad and Smith, 2017). Similarly, within the pharmaceutical industry several cases have been reported, where image analysis has been used to determine the concentration of coloured substances within different pharmaceutical products and process steps. Durão et al. used an RGB camera, a NIR and a light-induced fluorescence (LIF) spectrometer to monitor the concentration of different vitamins in a multivitamin blend within the feed frame of a tablet press. They concluded that the RGB camera can be used to adequately quantify the content of some components (Durão et al., 2017). Mészáros et al. utilized a digital camera to predict the API content of tablets containing meloxicam, a yellow drug. With the usage of CIELAB and RGB colour space based algorithms, they predicted the API content with a relative error of approximately 5% at the target concentration of 2 w/w% (Mészáros et al., 2020). Galata et al. used riboflavin as a model API to create a machine vision-based PAT tool for monitoring continuous low dose homogenization. They used an in-line RGB camera to determine the riboflavin content of the low dose blend leaving the continuous blender. They reported a relative error of 2.53% at 0.324 w/w% riboflavin concentration. Thereafter, they used the developed method to characterize the blending and measure the Residence Time Distribution (RTD) of the process and to apply real-time feedback control to the system. They proposed that in the case of low dose APIs, which cannot be detected directly using a camera, riboflavin could be used as a coloured tracer (Galata et al., 2021a).

The examination of existing literature revealed that the accurate, real-time measurement of ultralow dose products remains a challenging task nowadays. The purpose of this study was the development of a system, which, with the addition of a coloured excipient, could indirectly measure the API content in concentrations below 0.1 w/w%. This could be a major progress in the field of monitoring the API content of ultralow dose products. For this, machine vision can be used as a PAT tool to monitor continuous twin-screw wet granulation, where the API and the coloured tracer would be dissolved together in the granulation liquid, therefore their concentrations would change simultaneously. Furthermore, during granulation the particle size of the granules should correlate with the applied L/S ratio, therefore a correlation between the particle size and API content might also exist. These two methods should be comparable to each other. Finally, it could be possible to extend the indirect, colour analysis-based API determining method for the examination of ultralow dose tablets.

2. Materials and methods

2.1. Materials

Lactose monohydrate was provided by DFE Pharma (Goch, Germany). Corn starch was shipped by Roquette Pharma (Lestrem, France). Povidone (PVPK30) was supplied by BASF (Ludwigshafen, Germany). Riboflavin (RI), also known as vitamin B₂ and registered as a food dye under the number E101, is a powder with an intense orange colour even in low concentrations was purchased from Merck (Budapest, Hungary). Carvedilol (CAR) was also provided by Merck (Budapest, Hungary).

2.2. Methods

2.2.1. Continuous wet granulation

Ultralow dose coloured granules were prepared the following way. First, a pre-homogenized, white powder mixture was prepared by mixing lactose monohydrate with corn starch in polyethylene bags for 5 min. The granulation liquid consisted of 0.47 w/w% CAR, 0.83 w/w% RI and 27.9 w/w% PVPK30 in 2 v/v% acetic acid. Granulation was executed with a continuous TSG (Quick 2000 Ltd., Hungary), using a screw with the diameter of 16 mm (25 L/D ratio) operated at 100 rotations per minute (rpm). The screws consisted of conveying elements and kneading zones as shown in Fig. 1. The pre-blend was fed into the TSG using a gravimetric feeder (Brabender Technologie, Duisburg, Germany) operated in gravimetric mode, with a constant feeding rate of 1 kg/h or 2 kg/h. The orange-coloured granulation liquid was dosed into the system using a peristaltic pump (Watson-Marlow 120U, Wilmington, MA, USA) operated at different feed rates, which were determined previously with calibrations. Granules were dried using a continuous horizontal fluid bed dryer (Quick 2000 Ltd, Hungary). Supply air temperature of 80 °C and an airflow rate of 100 L/min/zone (24 m³/h) were set. The drying surface area of a single zone was 0.024 m². Material transport was conducted via vibration with an intensity of 50 Hz. The dried product was milled using a continuous mill (Quick 2000 Ltd, Hungary), which was operated with an oscillatory mill tool. Milling was performed with 0.8 mm sieve size and with an oscillation rate of 200 oscillations/min.

2.2.2. Measurement of coloured tracer concentration with RGB camera

The manufactured wet granules fell on a black slide (for providing the black background), which was part of an in-house built camera stand. The stand held a Basler acA4112-30uc (Basler, Ahrensburg,

Germany) area scan, 12-megapixel RGB camera, which was equipped with a Basler MEGA TS1214-MP objective (Basler, Ahrensburg, Germany). The distance between the slide and the edge of the objective was kept at 15 cm using the camera stand. An in-house built dark box was stationed around the camera stand to exclude external light, therefore protecting the setup from the alternation of the outside lightning. For illumination a ring light, which consisted of three rows of white light emitting diodes (Apokromat Ltd., Hungary), was used while operated at maximum output. The communication between the camera and the controlling computer was carried out via an USB 3.0 port. 1000 by 500 pixels images were obtained with a rate of 26.5 frames per second, and with an exposition time of 150 µs. The setup of the continuous wet granulation system can be viewed on Fig. 2.

2.2.3. Continuous granulation runs

In the placebo experiment, the pre-mixed powder was fed with the gravimetric feeder at a constant feeding rate of 1 kg/h, while the pump's feeding rate was changed every 3 min to the following L/S ratios: 0.101–0.110–0.119–0.128–0.137–0.146–0.155–0.164 (4.5–4.9–5.3–5.7–6.1–6.5–6.9–7.3 rpm). The TSG was operated at 100 rpm. During the experiment, images were taken of the produced granules for 2 min after 1 min of waiting for steady state, at every calibration step.

During the first run with API, a calibration was set up with a constant powder feeding rate of 1 kg/h, the TSG was set to 100 rpm. The pump's feeding rate was set to the following L/S ratios: 0.119–0.128–0.137–0.146–0.137–0.128–0.119 (5.3–5.7–6.1–6.5–6.1–5.7–5.3 rpm) and was operated at each step for 3 min. At each step, after one minute the recording was started, and three samples were taken from the system. The sampling lasted for 3–3 s and was conducted at 90, 120, 150 s from the start of a step. After that a validation run was performed during where the initial L/S ratio was 0.119, this was increased to 0.133 after 5 min and to 0.142 after 10 min. Five samples were taken at every 30 s from the 120th second of each step.

The second run with API was conducted similarly to the previously described one. This time the mass flow rate and TSG rotation speed were doubled resulting in settings of 2 kg/h and 200 rpm, respectively. Furthermore, the amount of RI in the granulation liquid was halved. During the calibration run, the flow rate of the peristaltic pump was set to the following L/S ratios: 0.099–0.109–0.118–0.128–0.138–0.147–0.157 (8.8–9.7–10.5–11.4–12.3–13.1–14 rpm). Each step lasted for 2 min, with sampling at 30, 60 and 90 s for 3 s at every step and the collection of whole, non-sampled product in separate boxes step by step. During the validation run, 25% of relative difference was set between

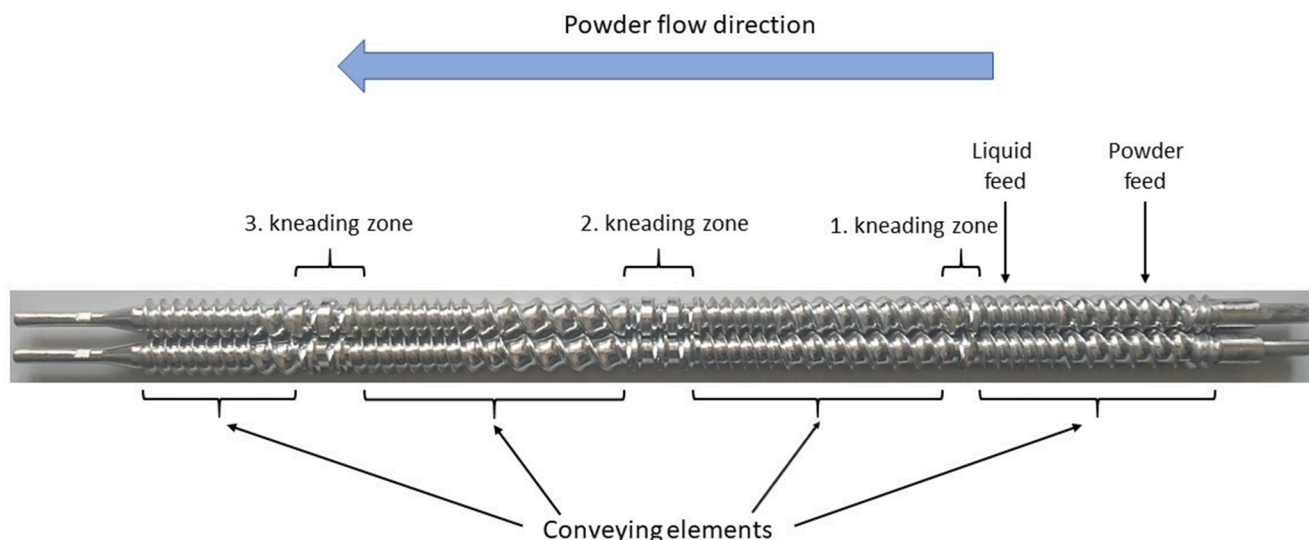


Fig. 1. The screw configuration of the TSG.

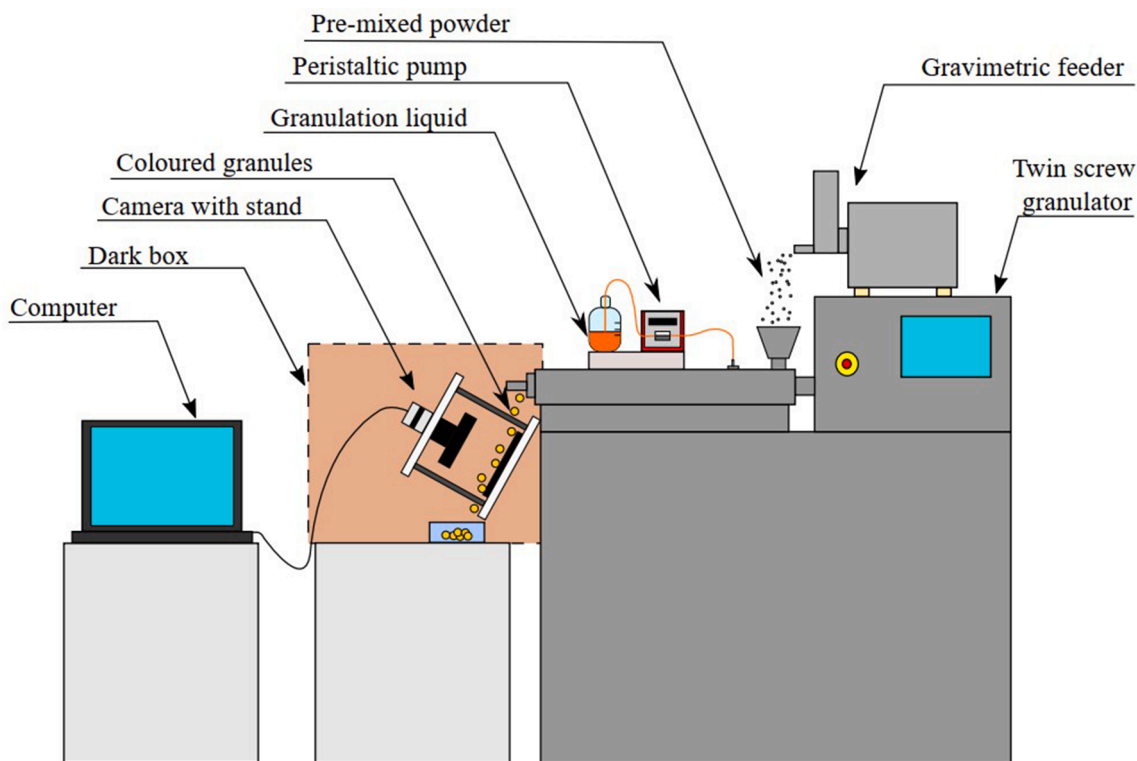


Fig. 2. The continuous wet granulation system.

each step, resulting in the setting of 0.099–0.124–0.155 L/S values. Each validation step lasted for 5 min, with sampling at 150, 180, 210, 240 and 270 s. The non-sampled granules were again collected in distinct boxes for each step. The separately collected granules of this run were compressed into tablets.

2.2.4. Tableting of granules and image acquisition

Tablets with 100 mg target weight were manufactured with a Dott Bonapace CPR-6 single punch tablet press (Limbiati, Italy). The applied compression force was 15 kN and 9 mm diameter concave punches were used. 5–5 tablets were manufactured from the separately gathered granules (second run) of the calibration (drug content at 7 levels) and validation (drug content at 3 levels) steps, resulting in 35 calibration and 15 validation tablets. The punches of the tablet press were lubricated externally using Mg-stearate. The tablets were photographed off-line, with usage of the dark box and the ring light. 500 by 500 pixels images were obtained with an exposition time of 100 μ s.

2.2.5. Measurement of API concentration using HPLC

CAR content of the samples was measured using high-performance liquid chromatography (HPLC). From each taken sample, 3–3 HPLC samples were prepared. 100–100 mg of sample was measured into 10.0 mL volumetric flasks, then they were dissolved in a 1:1 mixture of methanol and phosphorous acid (1:200 phosphorous acid – water solution) inside an ultrasound bath for 10 min. Afterwards, the solution was filtered through a 0.45 μ m polytetrafluorethylene (PTFE) syringe filter. The measurements were executed using a reverse-phase Gemini® NX-C18 3 μ m 100 $\times$ 4.6 mm HPLC column (Phenomenex Inc., Torrance, CA, USA) with gradient elution at 25 °C, while the flow rate was set to 1.0 mL/min. The concentration of carvedilol was determined based on the injected solution's ultraviolet (UV) absorption at 285 nm. During the HPLC measurement of the tablets, the sample preparations were slightly altered. The average CAR content of calibration tablets was determined by dissolving all 5 tablets belonging to the same level together in 50 mL solvent. The validation tablets were measured individually by dissolving

them in 10 mL volumetric flasks.

2.2.6. Image analysis

The acquired images were analysed with Matlab 9.8.0.1451342 (Mathworks, Natick, MA, USA). Two different algorithms have been used to retrieve the required data from the images. The colour analyser algorithm examined the amounts of the coloured tracer RI in the granules. If a granule had deeper yellow colour, then it comprised more RI containing granulation liquid, therefore more API. As the first step of the image analysis, the coloured pixels had to be separated from the background. For this, a number has been set as threshold and if a pixel's R value (red in RGB colour space) was higher than that, it was considered as a part of the sample, otherwise it belonged to the background. Thenceforth the sample pixels were converted into the CIELAB colour space. Due to the orange colour of RI, the b^* value of the pixels, which represents the colour from blue to yellow in the CIELAB colour space, was used to determine the RI content of the granules. Finally, an average b^* value was calculated for all pixels per 10 images. However, the averaged values still had a high deviation, so a 20-item moving average was calculated. This new value consisted a total of 200 images acquired from a time interval between its time of recording and the approximately 8 s preceding it (with an average frame per second (FPS) rate of 26.5).

The particle size analyser algorithm examined the size of the granules present on the images. If the applied L/S ratio is higher, then usually the average size of the granules is also larger, thus the PSD of the granules can possibly be used to predict L/S ratio and consequently API content. After applying a grayscale filter for background segmentation, the particle size analyser algorithm binarized the image, setting each background pixels value to 0 and the remaining to 1. Then it excluded the granules at the edge of the image, and after recognizing the edges of each particle, it removed the remaining noise. After that, the minimal and maximal Feret-diameter (Galata et al., 2021b) of each granule of the image was determined, then the two were averaged, producing the particle's average Feret-diameter (Madarász et al., 2018). Finally, the

average Feret-diameter of all the granules of 10 images were put together and a particle number based d_{n50} value was determined. It was found that the d_{10} and d_{90} values do not correlate well with the data.

After the determination of the CAR content of the samples using HPLC, the measured values were used as dependent variables and a polynomial was fitted to the average b^* value or the d_{50} value and to the API content measured by HPLC. Afterwards, by using the calculated b^* or d_{50} values of the images and the fitted polynomial, the API content of the validation samples was determined.

For the purpose of evaluating the presented method's ability to accurately predict the API content of the samples, the RMSEP was calculated using the following formula (Eq. (1)):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (y_{predicted} - y_{measured})^2}{n}} \quad (1)$$

where n is the number of samples, $y_{predicted}$ is the value predicted using image analysis and $y_{measured}$ is the API content determined by HPLC.

3. Results and discussion

3.1. Preliminary experiments

To study the properties of the proposed system, non-continuous preliminary granulation experiments were conducted. The pre-mixed powder blend was manually granulated in a mortar using different amounts of coloured granulation liquid. The amounts of added liquid and the calculated L/S ratios are shown in Table 1.

The images were acquired from each product with the camera inside the black box. Fig. 3. presents a compilation of the taken images.

As seen on this image, it is easy to differentiate the colour of the granules on the images using only the naked eye. The particles became darker yellow when a higher L/S ratio was applied, i.e. a larger amount of granulation liquid was added. This matched the expected result, so it was considered worthwhile to test this measurement method under in-line conditions as well.

For the determination of the L/S ratio during continuous TSWG, two calibrations were set up using the colour analyser algorithm and the particle size analyser algorithm. This placebo experiment was conducted like it is described in Section 2.2.3.

A first order polynomial was fitted to the average b^* values calculated by the colour analyser algorithm and the set L/S ratios (Eq. (2)), which is shown on Fig. 4.

$$L/S = 0.002868 \cdot b^* + 0.09005 \quad (2)$$

An R^2 value of 0.926 was obtained. Based on these results, it can be concluded that during continuous TSWG the applied L/S ratio might be precisely traced with the addition of a coloured tracer.

The images were also evaluated using the particle size analyser algorithm. A first order polynomial was fitted to the d_{50} values of the average Feret-diameter of the particles and the set L/S ratios, resulting in Eq. (3) and presented on Fig. 5.

$$L/S = 0.0002971 \cdot d_{50} + 0.05068 \quad (3)$$

The obtained R^2 value was 0.835, which shows that particle size analysis might also be a suitable tool for monitoring the L/S ratio during continuous TSWG.

3.2. Determination of API content using colour analysis

After verifying the direct correlation between the applied L/S ratio and the colour/size of granules, experiments containing the active substance, CAR, were conducted in order to examine whether the technique can be extended to measure API content as well. CAR was dissolved in the granulation liquid. This run was conducted in the way it is presented in Section 2.2.3. HPLC was used to determine the CAR content of the samples.

A second order polynomial (presented on Fig. S1.) was fitted to the calculated average b^* values and the CAR concentrations measured by HPLC, where Eq. (S1) was obtained, with an R^2 value of 0.956. It was used to predict the momentary CAR concentration during the validation run. Fig. 6. shows the predicted and the measured CAR contents next to each other. Table 2. contains the values and the relative errors of the predictions at the sampled points.

The RMSEP value was 2.62%, which makes this technique a potent tool of pharmaceutical quality assurance (Sulub et al., 2008). It can be observed that the prediction closely follows the trend of the concentrations measured by HPLC. All RSD values are below 5%, only the last setting surpasses 2% in the case of two methods. Also it must be noted again, that this was achieved in continuous ultralow dose manufacturing (50 µg CAR / 100 mg granule).

After the success of this experiment, the introduction of further improvements to the method was considered. The first possibility is lowering the amount of applied tracer material, as in the first run it was not utilized in the lowest possible quantity. Secondly, a broader range of L/S ratios should be explored. Lastly, the mass flow rate of the granulation system can be increased, this has two great benefits. This way the measured signal becomes stronger, as more particles are present in each image. Testing the method at higher throughputs is also useful because it yields more relevant information about its applicability in higher scale industrial manufacturing scenarios.

Based on these proposals, a second experiment was conducted with some modifications in the process parameters. The most significant change was the halving of the amount of coloured tracer dissolved in the granulation liquid and replacing the taken-out part with PVPK30. For the investigation of increased output, the feeding rate of the gravimetric feeder (from 1 kg/h to 2 kg/h), the speed of the granulator (from 100 rpm to 200 rpm) have been doubled. As a result of this, the feeding rate of the pump was raised and the L/S range was widened from 0.119 to 0.146 to the extended range of 0.099–0.157 (from 5.3 to 6.5 rpm to 8.8–14 rpm). The other applied parameters can be found in Section 2.2.3.

The captured images were first processed using the colour analysis algorithm. Eq. (S2) shows resulted second order polynomial, which had an R^2 value of 0.940. Fig. 7. presents the CAR concentrations that were predicted using Eq. (S2) and the HPLC measured ones. Table 3. contains the values and the relative errors of the predictions.

A relative RMSEP value of 4.51% was acquired, which is higher than in the previous run, but it should be kept in mind that the amount of RI was halved and also that a wider concentration range is covered. RSD values were also below 5% in this experiment, however it is noteworthy that the third setting resulted in significantly higher numbers. This is not surprising, as the concentration curve shows larger deviations in that area. During the second validation step, an unintended anomaly occurred. Most likely due to environmental vibrations the gravimetric feeder's feeding rate changed from the set 2 kg/h to around 2.1 kg/h and it could not set itself back to the target value for around 3 min, until when the next step was set on the pump. Both the predictions and the

Table 1

The added amount of liquid to 30 g pre-mixed powder blend and the calculated L/S ratios.

Weight of granulation liquid	L/S ratio
[g]	[-]
2.61	0.087
3.15	0.105
3.71	0.124
4.68	0.156
5.29	0.176

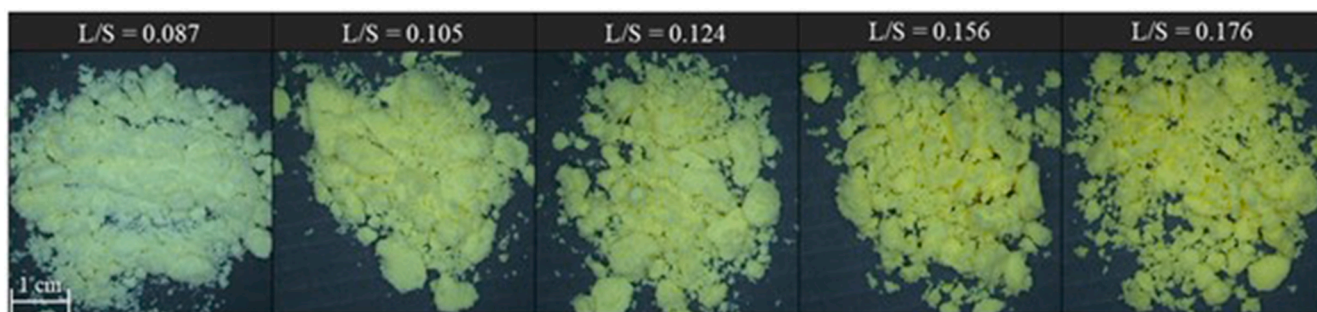


Fig. 3. Images of the preliminary products at different L/S ratios.

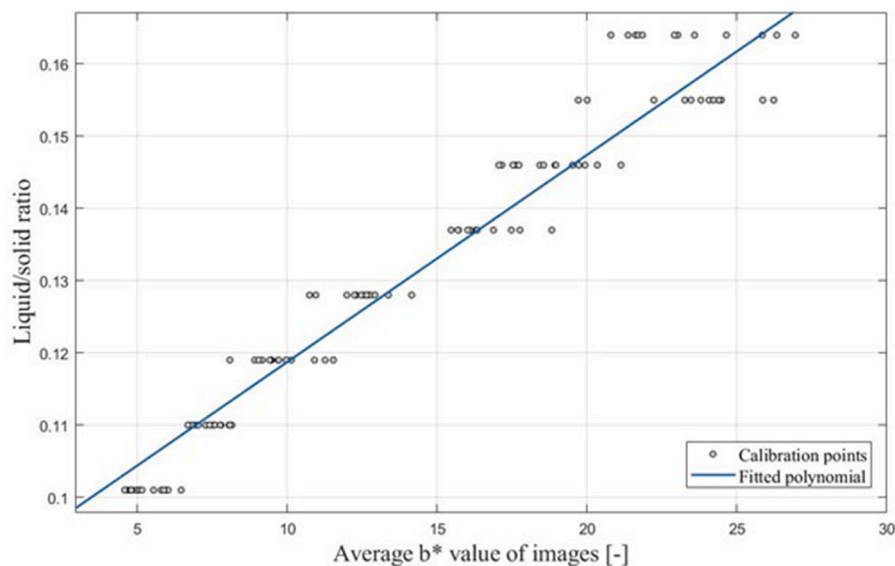


Fig. 4. The L/S ratio as a function of the average b^* value of images acquired during the placebo experiment.

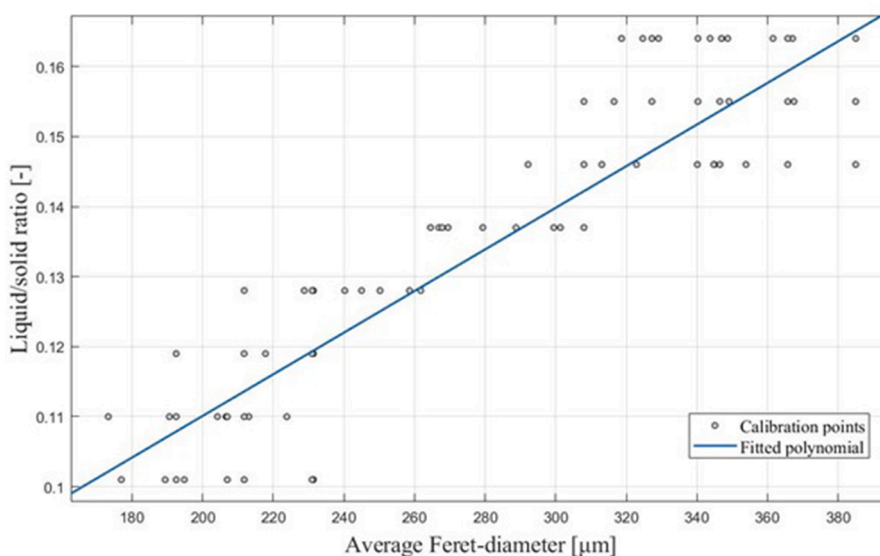


Fig. 5. The L/S ratio as a function of the average Feret-diameter of the granules produced during the placebo experiment.

HPLC measurements show that indeed the L/S ratio and thus the CAR content was lower during this period than the expected value. This illustrates that the method based on colour analysis was capable of accurately tracking and showing real-time interferences during

continuous TSWG, thus showing its potential as a PAT tool. The proposed indirect monitoring method can be utilized considering the following limitations. The API must dissolve in the granulation liquid and it should not be incompatible with neither the coloured tracer nor

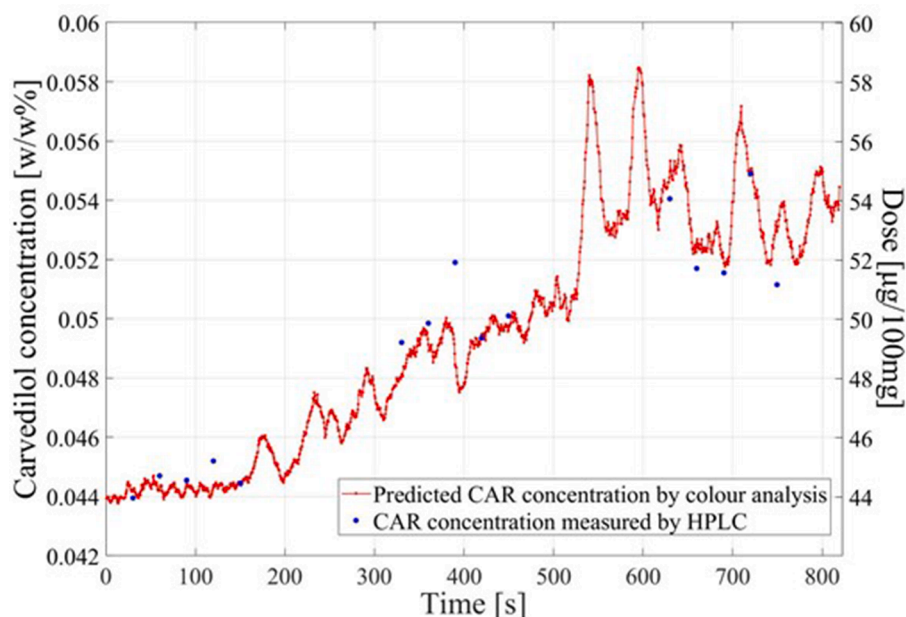


Fig. 6. CAR concentrations predicted by colour analysis and measured by HPLC during the first validation run.

Table 2

The predicted and measured CAR content and the relative errors of both predictions at the sampled points during the first validation run. CA: colour analysis; PSA: particle size analysis. Average and relative standard deviation (RSD) values are calculated from all measured point of the time intervals.

Time [s]	Predicted CAR using CA [w/w%]	Predicted CAR using PSA [w/w%]	CAR measured by HPLC [w/w%]	Relative error of the CA's prediction [%]	Relative error of the PSA's prediction [%]
150	0.0442	0.0438	0.0440	0.57%	−0.34%
180	0.0442	0.0446	0.0447	−1.13%	−0.22%
210	0.0444	0.0449	0.0446	−0.34%	0.79%
240	0.0444	0.0452	0.0452	−1.80%	0.00%
270	0.0444	0.0452	0.0445	−0.11%	1.69%
Average (RSD)	0.0443 (0.34%)	0.0449 (1.32%)	0.0446 (0.90%)		
450	0.0481	0.0496	0.0492	−2.29%	0.81%
480	0.0491	0.0497	0.0499	−1.53%	−0.30%
510	0.0485	0.0506	0.0519	−7.01%	−2.50%
540	0.0495	0.049	0.0494	0.30%	−0.71%
570	0.0496	0.0512	0.0501	−1.01%	2.20%
Average (RSD)	0.0491 (1.29%)	0.0503 (1.55%)	0.0501 (1.93%)		
750	0.055	0.0526	0.0541	1.73%	−2.68%
780	0.0526	0.0507	0.0517	1.71%	−1.93%
810	0.0519	0.0522	0.0516	0.67%	1.26%
840	0.0551	0.0522	0.0549	0.36%	−4.92%
870	0.053	0.0519	0.0512	3.49%	1.47%
Average (RSD)	0.0536 (2.74%)	0.0520 (1.12%)	0.0527 (2.86%)		

the excipients. The coloured component should have an intense colour, should not be toxic and it should be stable under the conditions of manufacturing.

3.3. Determination of API content using particle size analysis

As shown in Section 3.1., there is a significant correlation between the applied L/S ratio and the size of the produced granules. During the previous experiments described in Section 3.2., the changing of the API

content of the granules was executed by changing the L/S ratio during granulation. Therefore, it should be investigated whether PSA could also be used to accurately measure the API content of the product in our setup in real-time.

The particle size analyser algorithm was used to analyse the images captured during the first calibration-validation run. The algorithm calculated the d_{50} values of the average Feret-diameters of particles from every 10 images. Then a second order polynomial was fitted to the calculated d_{50} values of the calibration images and the CAR concentration measured by HPLC (presented on Fig. S2.), with which Eq. (S3) with an R^2 value of 0.911 was obtained. Eq. (S3) was utilized to predict the API content during the validation run. Fig. 8 presents the measured and the predicted CAR concentrations and the d_{50} values of the granules. Table 2. contains the values and the relative errors of the predictions.

A relative RMSEP value of 2.30% was acquired, which is similar to the one produced by colour analysis. Therefore, it can be said that in these conditions both algorithms can accurately predict the API content of the produced granules. However, by using the particle size analysis algorithm, the size and API content of the granules could be determined simultaneously, which are both CQAs of continuous TSWG. It also can be noted that the particle size analyser algorithm's prediction has a higher deviation and better describes the validation points in the middle range, while colour analysis seemingly traces the CAR concentration more precisely when the L/S ratio is higher.

The images captured during the second experiment were also evaluated with the particle size analyser algorithm, and the second order polynomial, shown in Eq. (S4), was used to predict the API content during the validation run, presented in Fig. 9 along with the d_{50} values. Table 3. holds the predicted and measured values and the relative errors.

The prediction's relative RMSEP value was 4.29%. Several phenomena can be observed while investigating these predictions. Firstly, the PSA was also capable of detecting and accurately monitoring the disturbance caused by the feeder malfunction described in Section 3.2., just like CA. It can also be said that in the lower API content range, colour analysis appears to be a better option for implementation. It is most likely due to that in low L/S ratios, the presence of the small fraction can cause a higher variability, thus this measurement method becomes less reliable. It still can be said that in the middle zone the particle size analyser algorithm is more accurate than its colour-based counterpart. In the higher L/S area, the methods have similar accuracy.

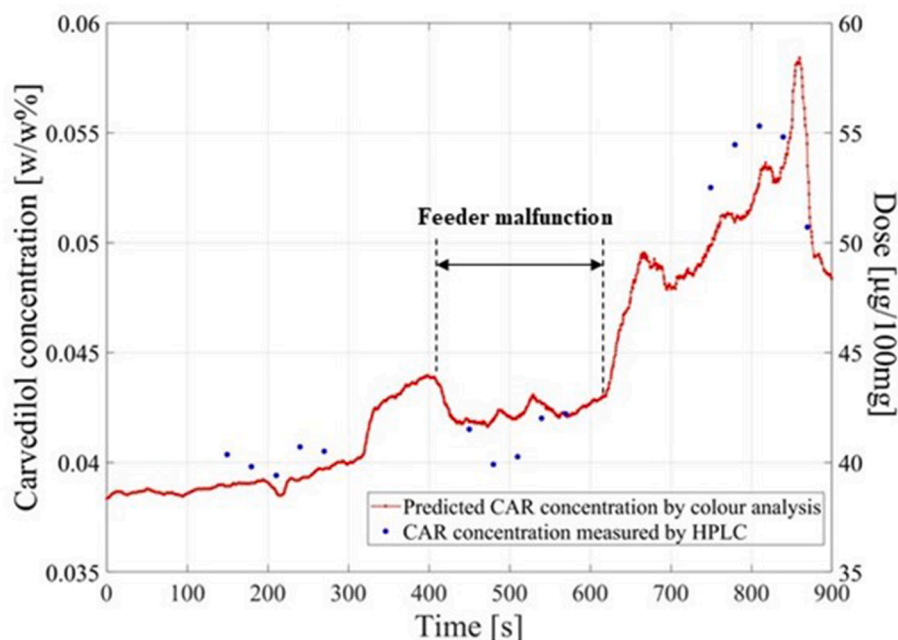


Fig. 7. CAR concentrations predicted by colour analysis and measured by HPLC during the second validation run.

Table 3

The predicted and measured CAR content and the relative errors of both predictions at the sampled points during the second validation run. CA: colour analysis; PSA: particle size analysis. Average and relative standard deviation (RSD) values are calculated from all measured point of the time intervals.

Time [s]	Predicted CAR using CA [w/w%]	Predicted CAR using PSA [w/w%]	CAR measured by HPLC [w/w%]	Relative error of the CA's prediction	Relative error of the PSA's prediction
150	0.0389	0.0380	0.0404	-3.59%	-5.82%
180	0.0390	0.0380	0.0398	-2.01%	-4.52%
210	0.0387	0.0366	0.0394	-1.78%	-7.11%
240	0.0392	0.0392	0.0407	-3.69%	-3.69%
270	0.0397	0.0393	0.0405	-1.98%	-2.96%
Average (RSD)	0.0391 (0.71%)	0.0380 (1.77%)	0.0402 (1.19%)		
450	0.0418	0.0404	0.0415	0.72%	-2.65%
480	0.0419	0.0404	0.0399	5.01%	1.25%
510	0.0421	0.0408	0.0403	4.60%	1.37%
540	0.0427	0.0415	0.0420	1.67%	-1.19%
570	0.0422	0.0414	0.0422	0.00%	-1.90%
Average (RSD)	0.0422 (0.83%)	0.0412 (1.14%)	0.0412 (2.26%)		
750	0.0498	0.0506	0.0525	-5.14%	-3.62%
780	0.0509	0.0520	0.0545	-6.52%	-4.50%
810	0.0529	0.0524	0.0553	-4.34%	-5.24%
840	0.0534	0.0549	0.0548	-2.55%	0.18%
870	0.0535	0.0487	0.0507	5.52%	-3.94%
Average (RSD)	0.0528 (3.95%)	0.0531 (3.12%)	0.0536 (3.20%)		

3.4. Indirect measurement of tablets based on colour analysis

Up to this point, this study only focused on the examination of granules, which are only a means to an end in the production of tablets. Therefore, tablets were manufactured from the product collected during the second calibration-granulation run (described in Sections 2.2.3. and 2.2.4.) and investigated with the implementation of colour analysis. The API content of the tablets was determined with the HPLC measurement method described in Section 2.2.5.

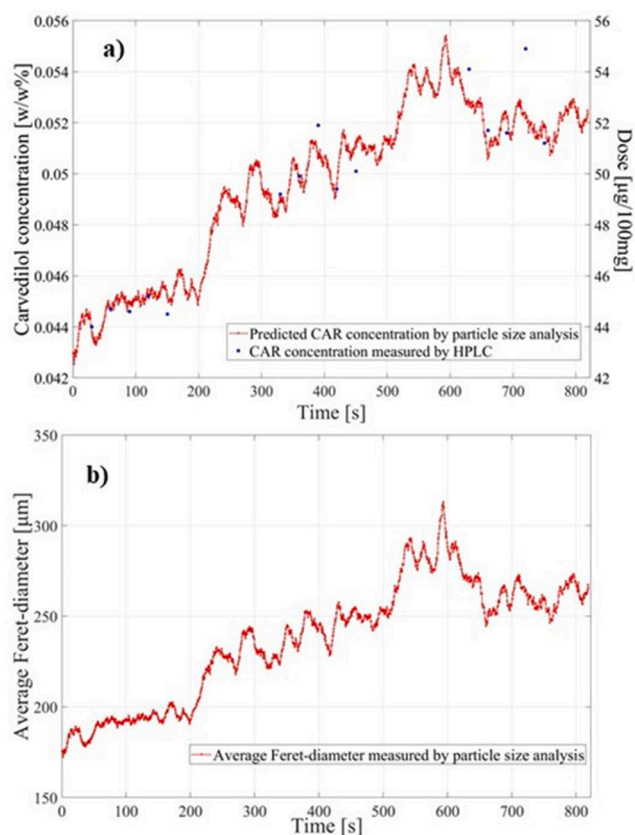


Fig. 8. a) CAR concentrations predicted by particle size analysis and measured by HPLC; b) Average Feret-diameter of granules during the first validation run.

100 images were acquired from both sides of the 50 manufactured tablets off-line in the dark box, which were processed with a slightly modified version of the colour analyser algorithm. Instead of applying a moving average, a first order polynomial, with an R^2 value of 0.912 (Eq. (S5)) was fitted to the 35 calibration tablets' average b^* values

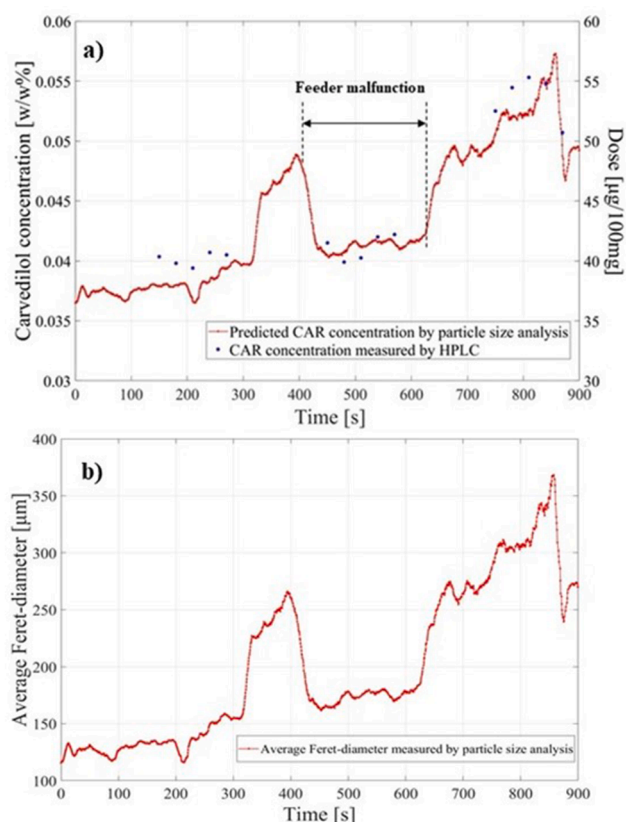


Fig. 9. a) CAR concentrations predicted by particle size analysis and measured by HPLC b) Average Feret-diameter during the second validation run.

(calculated from the separate b^* values of the two sides of the tablets) and their average carvedilol concentrations measured by HPLC.

Next, this polynomial was used to predict the API content of the 15 validation tablets. Fig. 10. presents the CAR content predicted and measured by HPLC of each side of the tablets, while Fig. S3. and Table 4. shows the average values for each tablet, RSD values are below 5% in all three settings.

It can be noted that the difference between the predicted API

Table 4

The average CAR content of the validation tablets predicted by colour analysis and measured by HPLC. CA: colour analysis, RSD: relative standard deviation.

Time [s]	Predicted CAR using CA [w/w%]	CAR measured by HPLC [w/w%]	Relative error of the CA's prediction
150	0.0423	0.0439	-3.70%
180	0.0422	0.0422	0.00%
210	0.0429	0.0438	-2.07%
240	0.0410	0.0445	-7.75%
270	0.0418	0.0448	-6.76%
Average (RSD)	0.0420 (1.45%)	0.0438 (2.05%)	
450	0.0474	0.0455	4.21%
480	0.0459	0.0453	1.29%
510	0.0477	0.045	5.91%
540	0.0458	0.0451	1.63%
570	0.0456	0.0448	1.77%
Average (RSD)	0.0465 (1.88%)	0.0451 (0.54%)	
750	0.0589	0.0601	-2.04%
780	0.0583	0.0616	-5.40%
810	0.0594	0.0612	-2.89%
840	0.0585	0.0605	-3.28%
870	0.0557	0.0604	-7.71%
Average (RSD)	0.0582 (2.19%)	0.0608 (0.91%)	

contents of each side is never greater than 0.005 w/w%, which indicates that the TSG homogenizes the materials during the process effectively.

The relative RMSEP value when separately considering the two sides of the tablets was 5.78%, while for their average it was 5.20%, which is comparable with the RMSEP values of the granule-based ones. Therefore, it can be concluded that the tracer method retains its ability to accurately measure the API content even after tableting.

4. Conclusions

In this paper, a novel PAT tool was developed for the in-line monitoring of continuous TSWG with the colour analysis and PSA of images. Two experiments were conducted with API concentrations under 0.1 w/

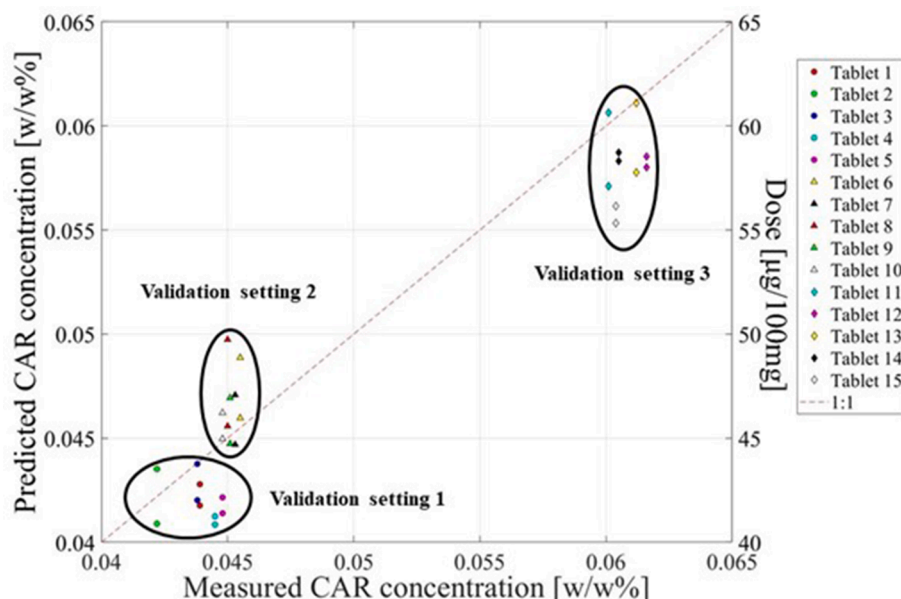


Fig. 10. CAR content predicted by colour analysis for both sides of the validation tablets versus CAR content of the tablets measured by HPLC.

w% and the amount of CAR was predicted with relative errors under 5%. The presented system was capable of successfully identifying and monitoring an unexpected disturbance caused by the gravimetric feeder, showing that the proposed method can track a continuous granulation process. Finally, colour analysis was used off-line to predict the API content of tablets, producing a similar relative error to the granule-based methods.

This method could theoretically be used with any API, where there is no harmful interaction between it and the RI. Furthermore, other substances with strong and durable colour could be implemented instead of RI. Colour analysis could be applied to improve other technologies, for example high-shear wet granulation, to help the evaluation of homogeneity.

Particle size analysis could be implemented in already existing manufacturing lines because it does not require the addition of another substance to the product. This method while being able to predict the API content, also determines the size of the particles, which is also a critical quality attribute of the granulation process.

The off-line tablet measurement method should be investigated for in-line application. It might also be possible that based on the measured API content of the granules, the content of tablets could be predicted. Further studies could be conducted to implement the gathered in-line, real-time information for the feedback control of gravimetric feeder and the granulation system, creating a PACT to ensure that a product of higher quality is obtained and consequently, the patient receives a safer and more effective treatment.

CRedit authorship contribution statement

Máté Ficzer: Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Visualization. **Lilla Alexandra Mészáros:** Formal analysis, Writing – original draft. **Lajos Madarász:** Data curation, Investigation. **Márk Novák:** Methodology, Investigation. **Zsombor Kristóf Nagy:** Conceptualization, Supervision, Writing – review & editing. **Dorián László Galata:** Methodology, Investigation, Data curation, Formal analysis, Writing – original draft.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpharm.2021.121008>.

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