PROCESS ANALYTICAL TECHNOLOGY IN TABLET MANUFACTURING

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CHINNAKRIT PRASERTWONGSA

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UNIVERSITY OF STRATHCLYDE

STRATHCLYDE INSTITUTE OF PHARMACY AND BIOMEDICAL SCIENCE

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Signed: _____

(Chinnakrit Prasertwongsa)

Date:

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Abstract

Process Analytical Technology (PAT) is the important tools to understand the process, obtaining critical process parameters and used as process monitoring following by quality by design (QbD) concept. It has been applied to the pharmaceutical manufacturing especially in every step of tablet production until final product in order to ensure the product quality. Otherwise, it would be an essential part of a scientific-based pharmaceutical quality assessment due to the food and drug administration announcement.

The purpose of this research is to show the application of PAT tools through tablet manufacturing process; blending by cube mixer, granulation by twin-screw extruder and including finished product properties. Critical process parameters were identified through design of experiments (DoE), and then data of each step was recorded by widely accepted PAT approaches (e.g. near-infrared spectroscopy, particle size analysers) before initiation of downstream process to strengthen scientific data of design space. Moreover, multivariate analysis software (SIMCA), was involved in order to extract multivariate spectroscopic data to be comparable form such as Principal component analysis (PCA). This formulation contained two active pharmaceutical ingredients (API), Paracetamol and Caffeine, while others studies contain one which was challenging of this study. Nevertheless, this formulation based on high shear wet granulation method, however it was successful to be granulated with twin screw extruder. However, more studies with screw configuration are still required to improve shapes on granules.

Blending uniformity was firstly studied by ATR-IR spectroscopy and PCA showed similarity of data after 10 min of blending in all 3 batches. Therefore, DoE was employed to optimize three critical process parameters; solid feeding rate, liquid feeding rate and extruder speed. EYECON and QICPIC were utilized for obtaining particle size distribution during experiments; consequently, one configuration which provided the best unimodular-shaped PSD was selected and full batch granulation was performed. Near Infrared spectroscopy (NIR) was equipped in order to monitor the

product content uniformity along batch granulating. Spectrum were analysed to be Hotelling T^2 plot against time and used as process signature for the future batches and scale enlargement. Finally, after tablet compression, ingredient mapping on the tablet surface was illustrated by Raman Microscope to ensure the uniformity of 2 active pharmaceutical ingredients on the tablet. Maps showed uniformly spreading of each API on the table surface.

This studies were demonstrated that PAT tools can be involved along the process development, obtain data of attributes and be a part of decision tools. It could be a sample for involvement of PAT tools in the whole manufacturing process and could be further applied for an industrial production which corresponded to QbD concept to make better strategy for drug quality assurance for the next recent years.

Chapter 1 - Literature Review

1.1. Introduction

Pharmaceutical manufacturing process is a complicate system that consists of many processes along the stream to make a convenience and effective product, such as mixing, granulating and tableting. Therefore, many factors such as time, force and fine adjustment are involved and subsequently related to product's quality. Moreover, not only the process but also raw material, operational variable and environmental have an effect on variations on the final product [1]. At the present time, quality-affected factors are not well characterised, understood and controlled, therefore, current process regulations which based on time-indicated procedure and narrow-ranged adjustment are in use [2]. Due to the fact that a few scientific based studies can support the process, root cause investigation when batches are failed cannot be performed effectively. Consequently, corrective action and prevention cannot be performed at the root cause; eventually, the manufacturer continues wasting resources for failure batch since many problems are not correctly solved [1, 2]. However, if the solution for reprocess batch was found, manufacturers would have to change procedure or product requirement and report any action to Food and Drug Administration (FDA) [1, 2]. This step requires supportive documents including process repeatability and validations which consume resources in order to ensure the quality of product that would not be complicate if the process was studied enough during research and development (R&D) steps.

Quality of drugs plays important role in their efficacy on patients and pharmaceutical factories have responsibility to ensure the quality before distribution [3]. Currently, raw materials, intermediates and end products which are produced by fixed manufacturing process are tested through FDA submitted method and specification called Quality by Testing (QbT) [2]. In contrast, new concept called Quality by Design (QbD) introduces understanding of all along process, especially variability sources to design good system, configuration, suitable control and consequently achieve a good clinical performance drug [2, 4]. Due to the new concept, scientific based procedure will perform acceptable attributes of products which pharmacopeia based testing might be not required if sufficient data can be provided [2]. However, this useful holistic

scientific based system cannot be done without supporting techniques called Process Analytical Technology (PAT). FDA defines PAT as “a system for design, analysis, and control of manufacturing processes, based on continuous monitoring of quality and performance attributes of raw material, intermediates and products” [1]. Subsequently, some data analytical tools are involved to transform massive unorganised data obtained from PAT instrument to reveal messages from experiments [1].

In brief, QbD is scientific based concept regarding to understand the manufacturing process which assisted by PATs for approaching quality attributes in order to minimize a risk to patients [1]. Application of them on the pharmaceutical manufacturing process, involvement of PAT equipment and data analysis tools will be described in the next chapters.

1.2. Pharmaceutical Manufacturing Process

Pharmaceutical Manufacturing Process is a step to transform drugs to be in a suitable form for usage. Each manufacturing step has an effect on quality and need to be considered following the QbD concept in order to establish the goal parameters during the research and development process [1]. Firstly, expected goals of product need to be considered based on patients’ requirements by establishing of target product profile (TPP) [1]. That leads the pharmaceutical scientist to establish the quality target product profile (QTPP) which is an extension of product characteristic to deliver TPP; otherwise it is a linkage from clinical performance to drug production and expected as product performance for customers [2]. Then, understanding the effect of variables on each manufacturing process to reach QTPP leads the pharmaceutical scientists to gain expected product properties called Critical Quality Attribute (CQA), and process variables which impact those properties called Critical Process Parameter (CPP) are identified [1, 2]. Eventually, a number of experiments would be performed in order to define the configuration of CPP that give desirable CQA through organised experimental plan called Design of Experiment (DoE) and whole area of study which gives desirable product attribute, called design space, is define. This can establish relationship among process parameters and therefore flexible manufacturing

procedures and Quality Control (QC) strategy will be established [2]. However, all of these procedures and control strategies can be improved through product life cycle due to new assured quality data to develop better performance which beneficial to consumer [1,2].

1.3. Tablet Manufacturing process and techniques

Tablet is one of the most popular pharmaceutical dosage forms in consumer's view due to its convenience. Unfortunately, it requires many steps for a batch and regulations becomes more tighten in control according to pharmacopeia-based regulation. Therefore, QbD concept is a good choice to minimize complicate of drug manufacturing and QC. Moreover, understanding of factor involved and product attributes in each step due to QbD concept provides careful regulation at each critical point and result in good products regarding to PAT instrument as facilitator to find out CQA and CPP through DoE along studying.

1.3.1. Mixing

Drug powder and excipients are blended in the mixer to disperse the drug through excipients to ease downstream process; therefore, to prevent inadequate dose in a unit, homogeneity of blending and adequate time of mixing have been investigated as a critical parameter.

Near-Infrared Spectroscopy (NIR) seems to be the most applicable method for this application since it provides sensitive spectroscopic data, fast processing through time as an online measurement [5-7]. Moreover, it provides non-contact analysis which eliminates sampling procedures. Juan et al. (2013) equipped NIR camera with the blender and outcome that NIR chemical image can illustrate mixing distribution [5] while Benoit et al. (2014) placed the NIR probe at the sapphire windows of V-shape blender and NIR spectrum was given [7]. Signal will directly record to the detector in specified time and then spectroscopic data will be analysed in order to indicate homogeneity of mixing and therefore equilibrium point of mixing will be obtained.

For the data analysis, quantitative and qualitative methods are employed for homogeneity test to extract the information from complicate spectroscopic outcome.

For qualitative data, univariate analysis can be used if focused substance has got a specific wavelength for determination or data are ready to use. However, due to application of PAT instruments, results are too complicate for univariate analysis such as unique response is not presented at any wavelength. Therefore, some multivariate analysis such as Principal component analysis (PCA) and Hotelling T^2 statistic (see more at section 6.2) can be applied. Transformation of multi-wavelength data into principal component (PC) score can simplify complexity of data and difference of the most variable spectrum would be extracted.

Lee et al. (2012) showed the application of PCA for the determination of blending end point in Trimebutine tablets [6]. U-type blender was utilized and two samples were taken at the top, middle and bottom of blender for 5 times. NIR probe was directly measured mixed powder, spectra were then recorded and therefore they were analysed to be Principal component result by multivariate software. Accumulation of data on plotting PC2 against PC3 showed trend regarding time of mixing; therefore, 6 data at the last sampling time were plotted on close area which showed homogeneity of samples (Figure 1.1).

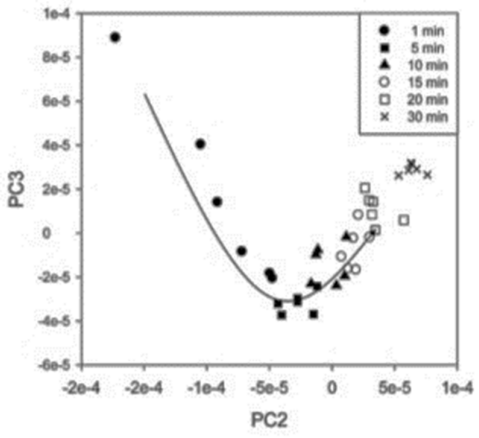


Figure 1. 1 PCA Score plot of NIR spectra from PC 2 against PC3 (Reproduced with permission; Lee et al., 2012)

Moreover, Martinez et al. (2013) used NIR method for collecting data and PCA score plot was performed to study effect for two process parameters which result in clustering of data for each group (Figure 1.2) [8]. It showed sensitivity of selected NIR wavelength regarding to loading plot of the same data set and multivariate software provided scientific-based information to researcher to study regarding QbD concept.

Furthermore, Martinez et al. (2013) also stated 3 axis plot during blending (PC1, 2 and 3) and it clearly show the phase of mixing from non-uniform mixing to steady state with the variation of critical process parameters (Figure 1.3) [9].

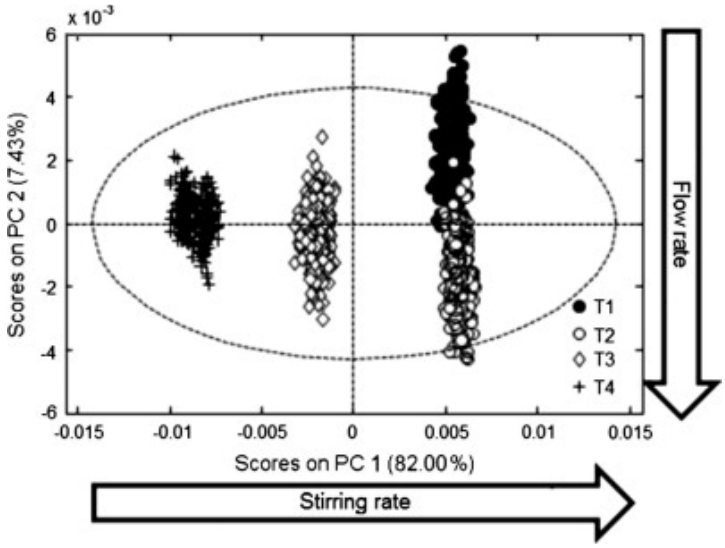


Figure 1.2 Application of PCA score plot on PC1 against PC2 for studying the effect of process parameters. (Reproduced with permission; Martinez et al., 2013)

Although this qualitative method is simple since no calibration curve required, it has been used as trend inspection. However, decision criteria could be clearer than graph inspection if some criteria were applied such as T^2 hotelling method which displays statistical acceptable area of similarity.

On the other hand, quantitative methods such as partial least square (PLS) method might be more suitable to be a process monitoring tool due to more accurate result and difference of concentration among samples can be explained [7]. Martinez et al. (2013) showed applicable of calibrated PLS model for study the effect of blending configuration in continuous blender [8]. Calibrated PLS model predicted amount of API and %SD through time and it showed the plot was sensitive to configuration changed (Figure 1.4). However, to reduce complexity, this study will employ the qualitative PCA and Hotelling T^2 as process analyser for this step.

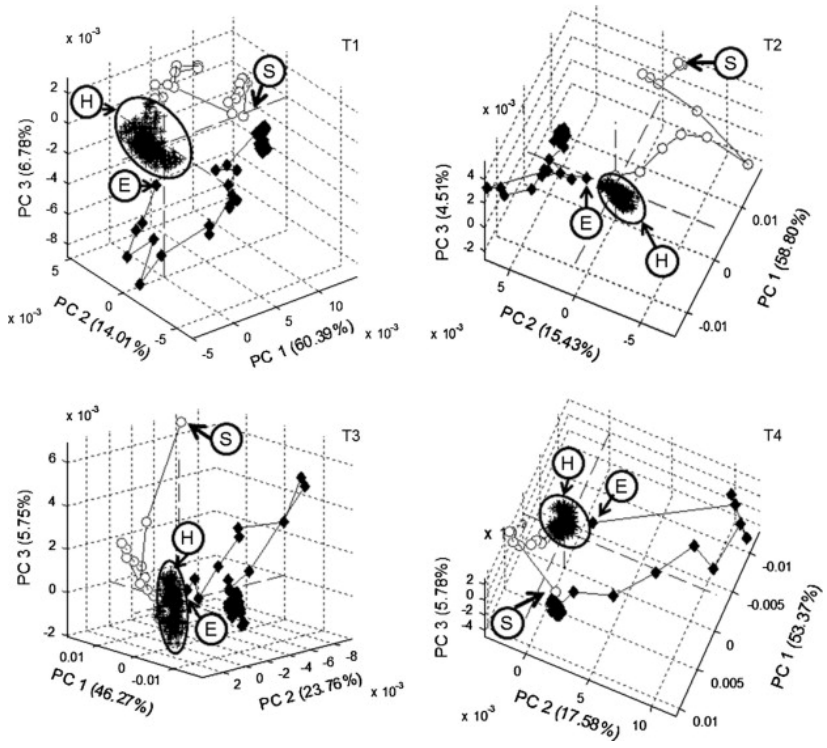


Figure 1.3 Application of 3-axis PCA in studying of process parameter; (○) means Start-up stage, (*) means steady stage, and (♦) means emptying. S indicates the first measured sample, H corresponds to the homogeneous blend and E to the emptying stage (Reproduced with permission; Martinez et al., 2013)

Moreover, mixing and segregation of mixed powder were influenced by some physical properties of particles such as particle size [10]. Bellamy et al. (2008) stated that added compounds might result in increasing of peak-to-peak noise in 2nd overtone region while the second overtone measurement is less sensitive to changing of particle size than 1st overtone [10]. The best particle size of microcrystalline cellulose (MCC) was identified for some API to achieve the best time for uniform mixing. Furthermore, this study stated importance of mixing order regarding time to reach uniform mixing. Therefore, it could help for problem solving during this study if uniformity cannot be performed due to current mixing order and properties of used raw materials.

Due to the fact that addition of lubricant to the mixture for the purpose of facilitates tableting process could affect the uniformity of mixture, dissolution property and distribution of lubricant. Jung et al. (2013) stated that presence of lubricant can slow down blending properties than absence but it required fewer rotations when active drug

was around 3 % [5]. Moreover, Lee (2012) stated that adding of lubricant even in small amount will affect to position of samples on PCA plot and therefore effect to decision making which based on accumulation of score plot [6]. Therefore, if formulation is adjusted in the future due to facilitate up-stream process such as increasing granule flowability, the calibrated model or even PCA model should be examined.

This work will employ spectroscopic methods such as NIR with a supportive of qualitative data analysis in order to define the suitable range of mixing period. Distribution of each API will not be studied but uniformity of all excipients will be analysed in general. Multivariate analysis will be employed to analysed and transformed spectrum into PC score and uniformity of mixture will be concluded if three consecutive point of sampling are placed in the acceptable range.

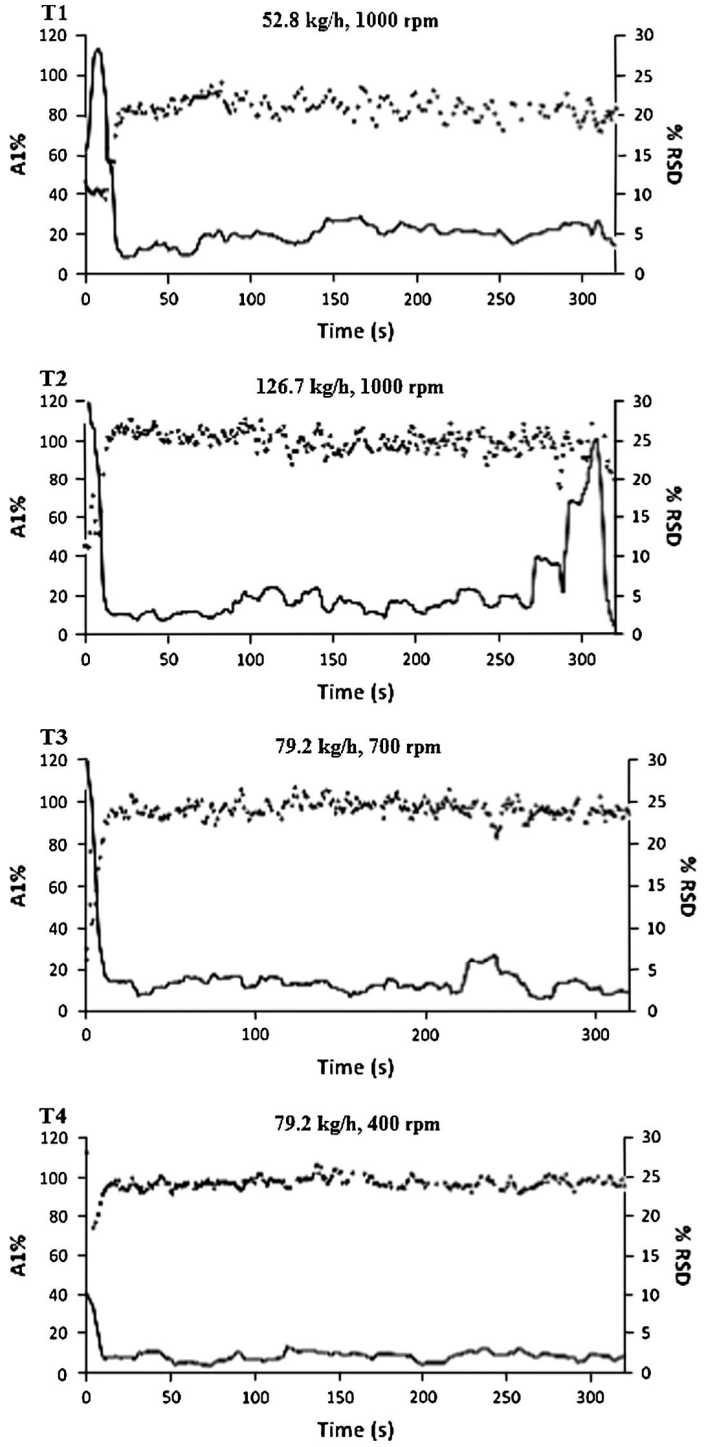


Figure 1.4 Predicted API amount for PLS model in different process settings. Dot line refers to predicted API amount and bold line refers to %RSD of predicted data (Reproduced with permission; Martinez et al., 2013)

1.3.2. Hot-Melt Extrusion (HME)

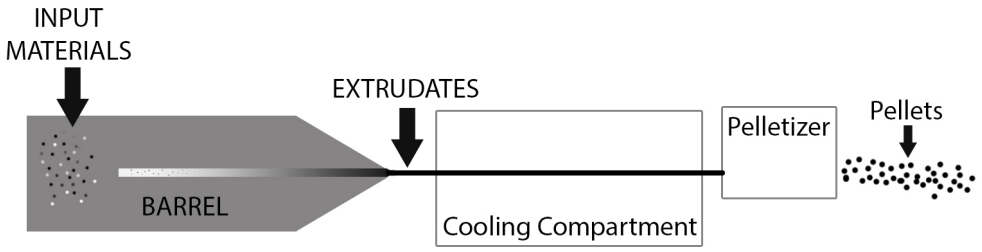


Figure 1.5 Process of hot-melt extrusion

Hot-melt extrusion (HME) is a manufacturing process applied to pharmaceutical industry by dispersing the drug in the matrix then extruded through hole and finally cut to be pellets (Figure 1.5). This process performs a lot of advantages especially preserve the amorphous form of API to enhance drug solubility and increase bioavailability. Moreover, dispersing of drug in matrix enhances uniformity of drug content, might protect water sensitive drug from environment and could modify drug release with appropriate polymer [11]. Furthermore, HME could reduce a number of operation units since it does not require drying process and could be involved continuous manufacturing process; subsequently, time consumption and operational cost were decreased [12]. Therefore, it could be applied to produce wide range of drug dosage form including tablets, transdermal patch and modified release system. [11, 13].

HME system consists of powder feeder, extruder screw, barrel, heating system, die and pelletizer (Figure 1.6). A configuration of those has an effect on product attributes due to PAT concept. The process starts from material feeding at the hopper, and then the screw in the barrel rotates and heat is applied to melt or soften excipients. For example, Wahl (2013) used Calcium stearate as matrix to disperse Paracetamol crystal while Saerens (2012) chose polyvinyl acetate and polyvinyl pyrrolidone for Metopolol dispersion [14, 15].

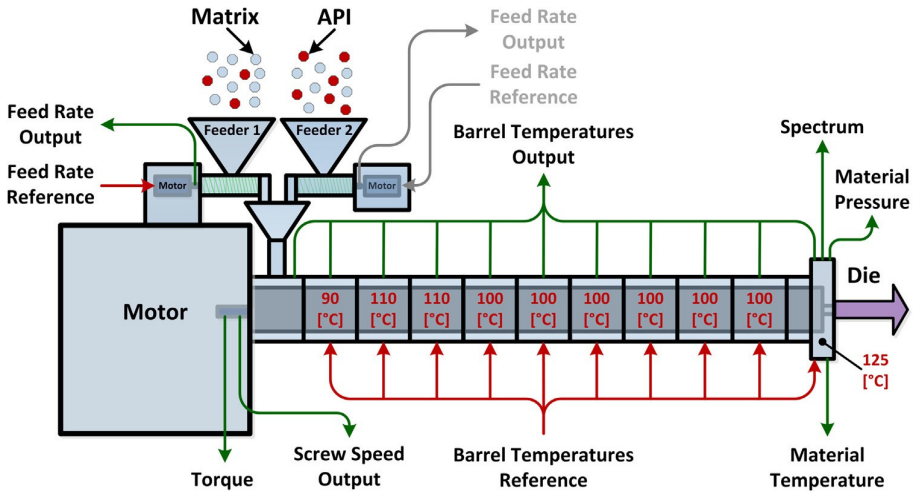


Figure 1.6 Overview of HME system and related process parameters (Reproduced with permission; Wahl et al., 2013)

Finally, the product will be extruded through the hole, cooled until dried and cut as pellets in order to be used for the downstream process such as tablet compression or capsule filling [12, 16]. PAT has been involved through process to study the product behaviour during the development process and employed to control the product attributes during the manufacturing process [17].

HME reserved API in the matrix to stabilise desirable morphologic form and therefore to enhance drug solubility. Hence, drug-polymer miscibility should be studied in term of presentation of desired drug form and drug-polymer interaction [15, 18, 19]. Polymorphic form can be obtained by X-Ray Powder Diffraction (XRPD) which can show identity of drug not only between crystalline and amorphous API but also among polymorphism form [19]. The study of compatibility can be illustrated by thermal analysis; especially, Differential Scanning Calorimetry (DSC) which is one of the most popular instruments to track changes and interaction in the extruder. Transition of heat flow through temperature is changed due to occurrence of specific intermolecular bonding such as ion-dipole or H-bond and can be compared to raw material [15, 18, 19]. Detection of new peak, depletion of some peaks and decreasing of polymer glass transition temperature might be found compare to pure substance because of molecular interaction [15, 18]. However, changes might not be occurred that indicates poor dispersion due to no interaction helps preserve drug form and

dispersion [18]. However, NIR and Raman spectroscopy can be employed to study the change of physical properties, and NIR provides greater performance in differentiate due to Saerens et al. (2012) experiment. They used different PAT instruments to confirm interaction between drug and polymer, glass transition temperature was an indicator for DSC while spectrum was employed for NIR and ATR-IR; therefore, they concluded that NIR provided the greatest ability detecting change during experiments [15].

Application of imaging technique can be employed to study drug-polymer miscibility and product morphology. Scanning electron microscope (SEM) has been used to elucidate surface morphology since excipients are compatible if no drug crystal present on the surface [19]. Furthermore, image analysers such as EYECON™ and FBRM have been employed to evaluate the effect of process parameter on pellet morphology [16, 20, 21]. Treffer (2014) stated the performance of EYECON™ camera to estimate size and shape of pellets when it was equipped after cooling compartment; therefore, detection of chance helped study of effect of cutter speed on particle size distribution (PSD) [16]. The result expressed in distribution ratio and shape of the product and showed that increment of speed makes a smaller pellet [16]. Good agreement of particle distribution results between off-line and on-line methods at grater size of particle at percentile 50. However, the difference occurred at percentile 10 which can be caused from reflected light and shadow projection as well as the small number of analysed sample but overall result was slightly different and therefore a big picture of distribution was not distorted. Eyecon™ can be used as the qualitative tools with the recommendation that sample size should be increased or change to lesser reflect sample could make a narrower gap [16]. However, application of usage to detect product appearance in the other manufacturing process which products are detectable such as Twin Screw Extrusion (TSG) is possible and it will be mention at the next section (3.3). Furthermore, the study can be extended by focusing on the other variable which fixed in this experiment, such as raw material input rate and therefore the relationship between current process parameter, product attribute and process parameter of downstream process could be established.

One of the most important settings is barrel temperature which directly affects the melting of matrix and therefore dispersion. Applied temperature should be above the glass transition phase temperature of the matrix in order to transform to liquid phase which can be disperse easier through screw. All of ingredients especially APIs should be thermostable at applied temperature to avoid unpleasant degradation and maintain the best therapeutic performance. However, barrels are sections and therefore their temperature are controlled individually; consequently, excipients can be melted and disperse with the higher temperature at the early section and then some thermo-labile drug can be adding at cooler later barrel segment [12]. Keen et.al (2015) shows that decreasing barrel temperature near the end of barrel below the binder melting point can also prevent occurrence of wet mass [13].

The geometrical design of screw is widely study because various screw types, order and sequence created wide application served different user requirements. Screw design has a direct effect on extrusion process such as the solid dispersion, melting and downstream throughput rate. Generally, screw consists of mixed small elements; conveying elements which help transportation of melted mixture and kneading element which help mixing and blending of excipients. Twin screw design becomes more popular since it performs greater conveying and mixing properties than single screw. Furthermore, co-rotational twin screw provides shorter residence time with high output rate while counter rotation is trended to generate uncontrolled overheat because of higher shear force and therefore can cause unexpected destruction of thermo-labile components [12]. Furthermore, screw shape and sequencing have directly effect on product attributes but this study will focus on twin-screw extrusion.

To investigate the differences and make a decision for the most appropriate setting, pharmaceutical analyses with PAT tools are involved. Content of uniformity is one of the best parameter described wellness of dispersion regarding employment of NIR or Raman spectroscopic method [14, 15, 18]. They usually be equipped after the extrusion screw and before the downstream extender; consequently, spectrum is obtained and be analysed to extract quantitative or qualitative data. For qualitative analysis, not only the content uniformity and homogeneity can be evaluated but also physical properties such as drug form or occurred chemical interaction can be detected

as well [14, 15, 18]. Otherwise, spectrum can be calibrated and quantitative data could be extrapolated with a confirmation with conventional offline method such as High Performance Liquid Chromatography (HPLC) or Ultraviolet (UV) Spectroscopy. Eventually, API content of each setting is comparable in the term of standard deviation (SD) and setting which makes low SD preferable [14].

Compatible drug-excipient formulation is the first requirement for an appropriate formulation for HME, and then it required specific process configuration to generate appropriate process parameter and consequently the good attribute of product will be produced [11]. PAT instruments provide the data of product attributes and could be involved along the process to help making decision. Although this experiment does not perform HME, the fundamental knowledge regarding extruder machine and PAT tools such as imaging technique and geometrical screw design can be applied for twin-screw extruding method since they used the same instrument but different operation.

1.3.3. Granulation

Granulation is the process of mixed particle agglomeration assisted by binder liquid and then force will be applied to form granules which assisted downstream process especially tablet compression [20, 21]. Good attributions of tablets are significantly induced from granule properties; therefore, some granule attributions should be mentioned such as shape and size distribution. Spherical shape is identified as the most desirable since it provides good flow ability and therefore uniformity of weight. Modular size distribution provides homogeneity of ingredients and therefore effect on uniformity of dosage unit which is one of common regulation for tablet quality testing.

Wet granulation is a common granulating method used in pharmaceutical industries while granulating machine takes a part on production line including TSG which provides an advantages of robust continuous manufacturing process with the acceptable throughput rate. There are two main compartments, the conveying which mix and transfer a fed powder into the next compartment and the kneading compartment which force a shear fiction into the granules [18]. Involved granulating mechanism was identified as nucleation and layering small particle and then coalescence breakage and shear-elongation were applied due to configuration of

kneading elements [22, 23]. It makes the advantage over using high shear wet granulation for decrement of over-sized granule and particle aggregation since particles are crushed over the time [21, 24]. El Hagrasy and Litster (2013), state that kneading element configuration affects the granule attribution through the growth mechanism. They also conclude that forward configuration whether 30^{o} or 60^{o} act like a conveying element which minimize barrel wall smearing and chopping and allows agglomerated particles passes [22]. Otherwise, these activities happened less when reverse configuration or neutral (90^{o}) are applied [22, 25]. Vercruysse (2015) showed the effect of added number of kneading elements to be 12 kneading elements in 2 kneading zones could induce shifting of PSD to be higher particle size since they induced both shear and compressive forces during extrusion which result in particle densification [24]. Furthermore, there are some more elements to be applied to optimize screw performance such as tooth-mixing-element, screw mixing element and distributive mixing element but this study will focus on two fundamentals, conveying and kneading elements to reduce complexity of experiment variables [24, 25].

Spreading of binding liquid convinces agglomeration of particles from the nucleation to layering of small particles [38]. Amount of liquid applies was studied as the term of liquid and solid ratio (L/S) which is the proportional of amount of applied liquid divided by amount of solid to facilitate researchers to organize amount of binding liquid to make the most desirable granules. This experiment is expected to find L/S ratio that give product to be granules. Vercruysse (2015) studied the effect of L/S ratio when screw configurations were fixed and lower L/S ratio which means smaller amount of liquid provided larger fraction of particles due to insufficient water for particle attachment around wet powder while agglomerated particles were found in the larger fraction at high L/S ration (Figure 1.7) [24]. However, the ratio is varied depends on the formulation and counted as the one of parameters which should be studied before continuing of experiment.

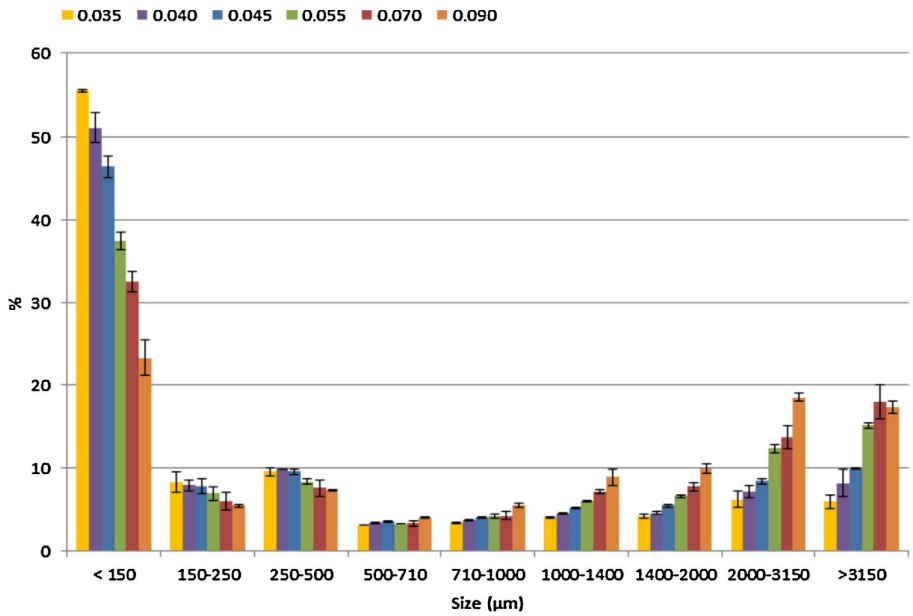


Figure 1.7 Particle size distribution from different L/S ratio (Reproduced with permission; Vercruysse et al., 2015)

Spreading of the binder is also considered as the parameter which affect granule size uniformity since insufficient liquid distribution through powder makes unequal granule nucleation. To measure the liquid binding distribution, dye was dissolved into binder solution and they are measured from granules in the term of recovered dye concentration. Vercruysse (2015) also studied dye distribution and percentage of dye recovery among ranges of particle size showed the best ability to spread out binding liquid in the configuration of 12 kneading elements in 2 kneading zones which was the same configuration that performed the largest particle size [24]. Therefore, changing of screw elements will affect generation of particle in difference ways and screw pattern should be customized due to the formulation to make the most appropriate product attributes.

Distribution of particle size can indicate the quality attribute in this step since particles with large distribution tends to segregate more and subsequently have poor content uniformity when compression is applied. Control of particle size variation could be beneficial than addition of milling process since too small particles can be generated which enhances poor content uniformity and could not be practical for industrial use [24]. There are PAT instruments provide this type of information such as FBRM and

Eyecon™ [21, 24, 26]. Sayin (2015) illustrated application of Eyecon for detection of PSD which affected from L/S ratio (Figure 1.8) [23]. The result corresponded to Vercruysse's studied (Figure 1.6) that these granules trends to be agglomerated and result as bigger particle size from the greater L/S ratio.

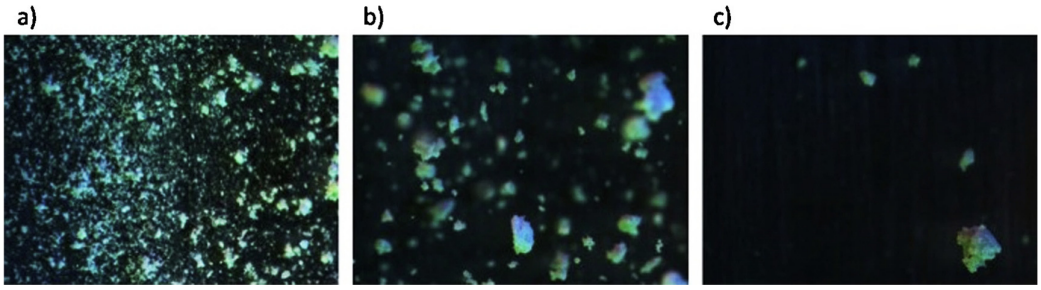


Figure 1. 8 Captured granule from Eyecon camera during TSG experiments with variation of L/S ration (Reproduced with permission; Sayin et al., 2015)

Nonetheless, without considering critical attribute factor as describe above, there are variable factors which have an effect on the quality of granule such as length of the compartment, type and position of spray nozzle in the granulator [21]. Although some process parameters do not show a significant effect to the analysed data, it does not mean it has no effect but the system has robustness. Moreover, some variables which remain unclear should be fixing and monitored during the manufacture is processing as well as quality impacted parameter.

1.3.4. Tableting

Tablet compression is the important step to transform drug as the granule to usable form with desirable performances as tablet. Product attributions are mainly described as pharmacopeia-based specification; for example, the content uniformity, dissolution and hardness. Although they have been used in the traditional quality by testing, PAT instruments provides information regarding critical parameter which help manufacturers moves from rigid procedure to flexible process based on QbD concept.

Uniformity of dosage unit which is one of issues for tablet manufacturing is performed on intact tablet by NIR spectroscopic method (see more detail at section 4.1.1). Šašić (2015) reported the application of NIR probe at the feed frame on continuous process that it could detect the moderate change occurred during transferring from blending compartment [27]. Kristina (2013) states that equipped multiple NIR probe next to

multiple compressing punch in the direction of ejected compressed tablet could be the way of 100% of batch inspection at full machine performance [28]. Wahl (2014) raise the issues regard to increment of sample size from traditional method to be entire manufacturing time and current US pharmacopeia regulation is not compatible with sampling size more than 30 units since acceptable area is too narrow [29]. However, the solution was described in the European Pharmacopeia chapter 2.9.47 that the criteria would be changed due to the sample size; thus this application would be possible to utilize in manufacturing processes.

Wahl (2014) also showed application of multivariate analysis for tabletting process which processes are done as the same as granulation [29]. For quantitative application, principal component (PC) score plot was generated from online spectrum could detect the process abnormality such as emptying and fluctuation of API-contained mixture or drug segregation [29]. In quantitative view, the data set of calibrated sample complying with PLS model generates the accurate calibration model to determine drug amount during process which therefore are calculated as the uniformity of dosage units [29]. Therefore, the use of these can detect chance occurred during process and can help researcher for study, optimisation and making control specification of process.

The study from Abe (2012) also showed application of NIR spectroscopic method to present disintegration and dissolution properties [30]. Relationship between calibrated NIR data on the tablet surface was established and the dissolution time as the time of 50% dissolution (T50) were obtained with the confirmation from UV-Vis spectroscopy [30].

Nevertheless, chemical imaging (CI) technologies was utilized to study distribution of API and excipients by Raman microscopic method. Vajna (2009) stated that univariate Raman mappings are able to illustrate components distribution on the tablet surface [31]. It was used in 7 difference tablet configuration regarding to granulation method, liquid binder, drying method and compaction force. Thus, Raman mapping provided analysis of comparison among configurations and therefore help for decision of the most appropriate granulating technology and settings which would help to study of any studies regarding tablet manufacturing.

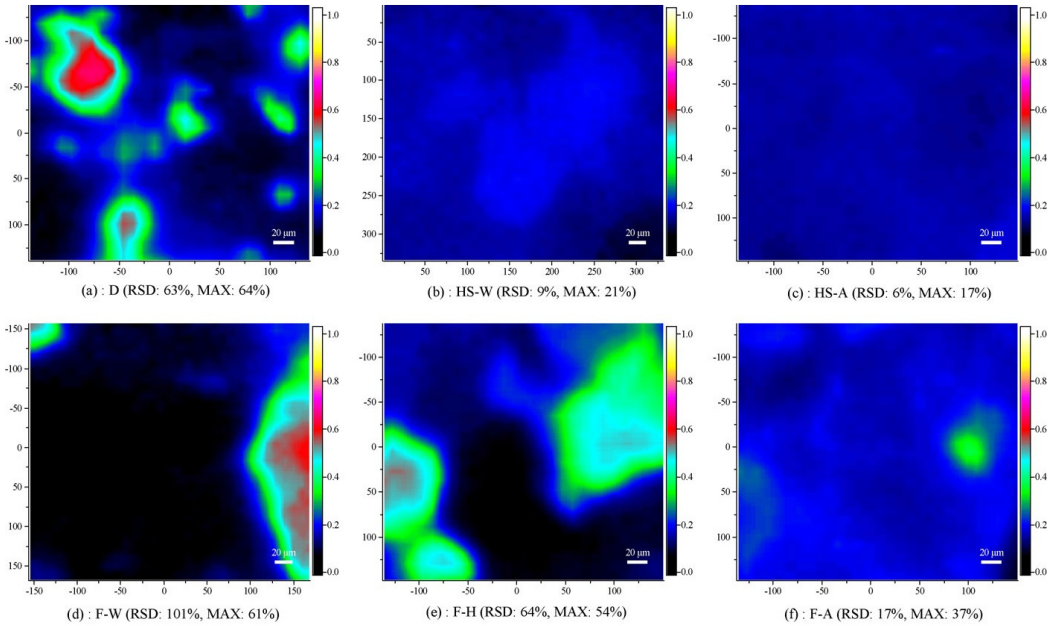


Figure 1. 9 Distribution of API from Raman microscopic method; scale bar illustrated amount of detected API while black showed space that API was not detected. (Reproduced with permission; Vajna et al., 2009)

To conclude, attributes of tablets such as content uniformity and dissolution time can be obtained from difference ways during tableting process. Data required an extraction process including calibration. Applications of PAT have been reported both research and an industrial setup and pharmacopeia suggested the solution that would be occurred when quality concept is changed could be a good sign for commercial use.

1.4. Process analytical technology (PAT)

The QbD concept leads new analytical technology called PAT involved in pharmaceutical quality assurance system as the process analyser in order to support data from studying process [1, 17]. PAT could be utilized from the designation of experiments, supply information of product and process attributes during study, and help extraction of information which consequently making conclusion of study [1, 2, 17]. Moreover, application of PAT tools involves over product life cycle from research and development step to the routine industrial production as one of product specifications to be process control strategy. [1, 2, 12]. The outstanding advantages, non-destructive and fast informative interpretation, facilitate the detection of any effect on the product attribute regarding any change applied. Moreover, for the routine work,

out of specification product could be found and take any actions earlier than using of traditional QbT analysis tools [2, 16, 17].

Generally, PAT are known as quick information supplier for process analyser during experiment. They are generally divided regarding the way of taking sample into 3 types; online, offline and in-line measurement. The most of available PATs are categorized as in-line measurement which samples are removed from system and they can be returned while the most comfortable is online which samples are continuous monitoring and not necessary to be removed from the system [17]. There are many type of information provided by PAT tools. Firstly, product morphology analysis can complement the influence of process factor on their shape, size and distribution [16,20,21,26]. Secondly, surface mapping and quantitative measurement provides the distribution of interest on the surface of product which indicate the uniformity of dispersion [5, 6, 8, 27, 28, 29]. Lastly, drug-matrix interaction and formulation stability can be illustrated to fulfil understanding of process and can be used to choose compatible excipients [13, 14, 15, 18, 19, 32]. To conclude, various information can be obtained due to variation of available PAT tools; therefore, researchers should know the suitable PAT to be used in their study and can be able to extract data efficiently.

1.4.1. In-line PAT

PAT tools which can acquire informative data while process is still running without removing sample are counted as in-line tools due to FDA definition [17.] Part of analyser could be invasive or non-invasive but real time data could be recorded through process running. Analysis interval could be all processing period or could be a duration which provided enough data to see changing through process. Most of the In-line PAT instruments provides an imaging data such as particle size, shape and distribution while some require post-processing operation as give more complex analysis such as pattern recognition analysis.

a) Near infrared spectroscopy (NIR)

NIR has been used for a hundred year as an identification method which displays the Infrared energy absorption for the vibrational movement in the dipole moment-

changeable molecules. NIR is located at the overtone of infrared radiation between 12800 – 4000 cm^{-1} and their spectrum can be obtained without sample preparation; therefore, the tested sample is not destroyed and becomes one of the greatest reasons for choosing this method since it is time-efficiency. Fast and continuous acquiring data of NIR spectroscopic method are also an outstanding performance; moreover, continuous data acquisition can be performed as long as power still supply and probe window is still clean. Consequently, NIR has been widely used as online PAT instrument which provides the practical use in the QbD concept with real-time product quality analysis.

Although NIR and FTIR are very close analytical technique, NIR has advantages over FTIR which made it seems to be more popular; deeper penetration through sample, transparent of water signal and especially longer distance allowed for data transferring which allows NIR has a longer probe cable, made it can be reached manufacturing machines and becomes In-line PAT tool.

Qualitative application for NIR usually employs as detection of product homogeneity or segregation along processes. Beer (2010) reviewed the application of NIR through in-line monitoring of blending, fluid bed and high shear granulation and moreover content uniformity of tablet manufacturing [17]. Product behaviour can be also understood from the NIR-tracking such as mixing behaviour, structure transformation which could help during process studying period [6, 17]. Moreover, Li (2007) showed semi-quantitative blend uniformity without calibration model in granules and pellets can be utilised in the early stage of development with some restrictions regarding drug level and some functional group such as amine [33]. Furthermore, some product properties could be predicted without performing physical test. Abe (2011) showed prediction of dissolution time from NIR spectra in order to study the effect of lubricant while Otsuka (2005) who the possibility of predict tablet hardness by analysing NIR spectra [30, 34]. However, all applications of NIR spectrum required post-processing and also validation of predictive model, multi-dimensional analysis software (e.g. SIMCA) is necessary.

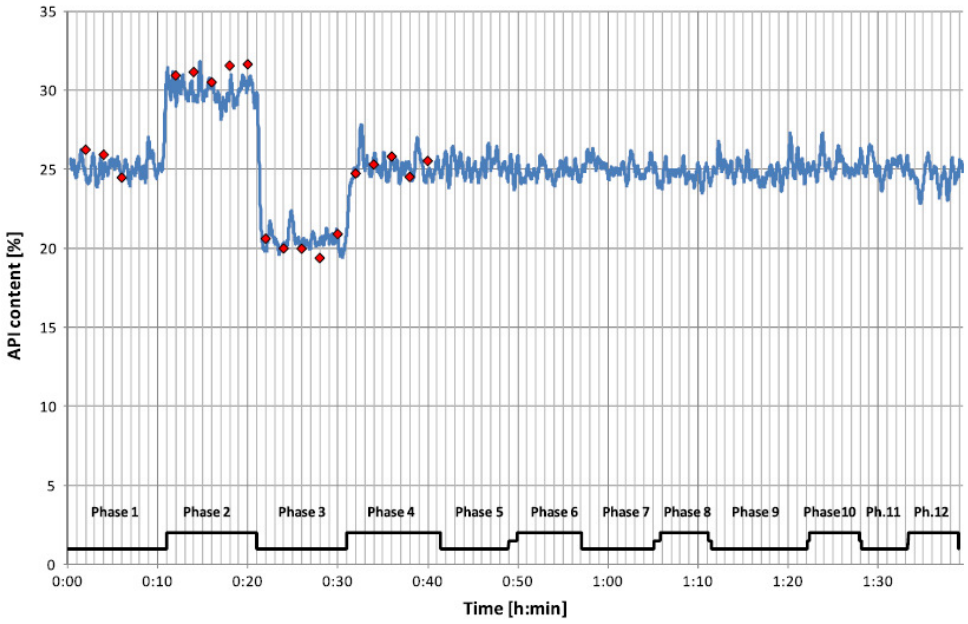


Figure 1.10 Example of process monitoring by predicted API content via NIR method, dots referred to UV-vis analysis (Reproduced with permission; Jarvinen et al., 2013)

NIR probe can be equipped at working area as process monitoring instrument via API content. Probe could be equipped at tablet feed frame to analyse the consistency of flowed mixture to control the uniformity of active component as staged in Wahl (2014) and Sasic (2014) [27, 29]. Moreover, Jarvinen (2013) located probes at the mixer and tablet presser and they were also utilized as process monitoring tools (Figure 1.9) [28]. Consequently, real-time process monitoring could be possible and practicable, critical parameter of the process can be monitored during the process which consequently produce good acceptable quality of product. Surprisingly, application of granulation on process monitoring of TSG has not been found; therefore, this study will employ NIR spectrometry as product homogeneity to present the application of process monitoring system in TSG method.

b) Eyecon®

Eyecon® provides information of particle size and shape via the photometric image analysis which is a novel method regarding 3 colour light source, red green and blue [26]. It helps particle edge detection and preventing false analysis from overlapping particles even in dynamic condition [16, 26]. Internal calibration requires for white light, camera focus and corrected background to reduce noise and sharpen sample edge.

This technique provides true shape and texture from camera capturing unlike other techniques such as projection or diffraction [16, 26]. Captures were then calculated particle diameter with the assumption that particle is being spherical called best fit ellipse and finally information through time can be presented. Consequently, it can be used for particle size control measurement for process monitoring as in-line or online PAT tool. Sayin (2015) stated an application of Eyecon as in-line PAT tool to investigate the effect of L/S ratio on particle size and their distribution on 11mm diameter twin screw extruder which is the same granulator used in this experiment (see illustration at figure 1.7) [23]. Moreover, Daniel (2014) compared the particle size distribution of HME-obtained pellet with QICPIC and good agreement are performed [16]. However, some restriction of this technique are reported that black sample, transparent or strongly reflecting particle cannot be analysed [16]. Moreover, there are some issues when comparison to the others PAT especially at D50 and D90 value; therefore, data comparison to reliable particle size analyser is still necessary [16]. This study will place EYECON as an in-line measurement for detection of particle appearance and size distribution during granulation and then the result confirmation with QICPIC would be further tested.

1.4.2. At-line PAT

PAT tools which are equipped around the manufacturing area and required stolen sample from process are counted as at-line PAT according to FDA definition [17]. Generally, tools would not require any complicate operation or complex post-processing software; moreover, taken samples could be returned to the process due to non-destructive analysis. Therefore, product attributes will be obtained as soon as sample is fed but not in real time as in-line tools did.

a) Particle size analysis

Particle size of raw materials or intermediates plays an important role on product attribution since it acts as the substrate of subsequence process; therefore, their control specification can be establishing as quality target [26].

Sieving method is one of the most accurate method for particle size analysis due to direct measurement of particle size through sieve, therefore many studies still use it as product analyser for studying and reference method compare to in-line and at-line tools. For example, Vercruysse (2015) studied the effect of screw elements and L/S ratio in TSG method and sieve particle size distribution was used and compare to make a conclusion (See figure 1.6) [24].

Due to the fact that particles can be irregular or non-spherical shape, the size cannot be determined as the diameter but it could be presented as chord length distribution data. They are subsequently transformed to relative particle size in the term of D-value by sorting of mass in the ascending order with the assumption of the same density. The number after "D" describes the size at the particular percentage of relative mass; for example, D50 refers to size at the median of relative mass. Some in-line PAT tools for measurement of particle size during manufacturing such as Particle Vision Measurement (PVM) and Focused Beam Reflectance Measurements (FBRM) are available but comparison between in-line analysers which also used D value showed difference at some size range [20, 21, 26]. Although those techniques could be more reliable if good relationship was proven with trustable offline methods such as conventional sieve analysis. Sieve analyser are limited by porous of sieve tray which might not cover over sample range. Therefore, this study will employ a reliable at-line method which is QICPIC as reference particle size analyser instead of in-line tools to monitoring of manufacturing process.

b) QICPIC®

QICPIC® is one of PAT tools providing both particle size distribution and also particle shape. It was developed from dynamic range analysis which can obtain particle size and shape when samples are in fast moving air stream and 2D image can be captured [10]. Moreover, motion blur from capturing moving particle could be solved by employment of pulse light in the analysis compartment [10]. QICPIC is equipped with particle dispensing device and fast compressed air stream system which vibrate dry powder to be dispensed to a tube and then accelerated into an analysis compartment by compressed air to make a suspension for minimized effect of unfocused particle [10]. Therefore, particles will be moved through pulsed illumination lighting area

which minimized motion blur from capturing moving particle [10]. Then, transmission of light is captured regarding to equipped high speed camera and subsequently transform to the illustration of analysed particles.

Yu (2008) compared PSD result with conventional reference laser diffraction instrument called Helium-Neon Laser Optical System (HELOS) using microcrystalline cellulose particles [10]. QICPIC performs an agreement with spherical-shaped sample but overestimation was found with rod-shaped particle due to intrinsic difference of method. However, the mixer of binary particulate showed good agreement when apparent density was input instead of true density since volume fraction was involved in calculation [10]. For instance, QICPIC could be used to study the impact of process parameter on mixing powder, particles; therefore, this study will employ QICPIC as an at-line PAT tool in order to obtain size distribution of produced granules which is an important part of DoE and batch manufacturing. Moreover, it will be used to compare the data with Eyecon as the same as Treffer's experiment (2013) [16].

1.4.3. Off-line testing

Some PAT instruments cannot be processed rapidly due to small working scale as micro or nanoscale of studied surface phenomena or the nature of analysing method. However, valuable data which facilitate studying and provide understanding of process are provided from these off-line methods.

a) Mid infrared spectroscopy (MIR)

Infrared spectroscopy has been used for a hundred year which corresponds to Infrared energy absorption from the vibrational movement in the dipole moment-changeable molecules. Infrared radiation (IR) can be found at the wavelength between 10 – 14000 cm^{-1} but mid-IR (MIR) which is its subset ranges from 1000 – 4000 cm^{-1}. Involvement of multiple component analysis in the past decade has moved IR's position in field of pharmaceutical analysis from identification method to quantitative analysis which was previously applied in food analysis and subsequently support QbD research [33]. Moreover, there are reasons support Infrared spectroscopic method to be popular since it does not require sample preparation and also is sample non-destructive method. In

addition, involvement of Chemometrics in vibrational spectroscopy including MIR can expand bounder of application as NIR did. Unfortunately, MIR application is not popular as in-line NIR since restriction of available cable length of probe; however, it can still be used as offline PAT tool with better spectrum intensity. This study will utilize this method during the mixing period since light cannot pass through the mixer's wall and samples have to be taken; eventually, NIR probe or camera cannot be employed but offline MIR would be more suitable.

b) Raman spectroscopy and Raman mapping

The Raman spectroscopy has been a useful method for pharmaceuticals in the quantitative as well as qualitative applications. Gaining or losing small energy of incident photon when applied to samples makes stroke or anti-stroke scattering which present different energy from incident photon called Raleigh scattering [17]. Raman spectra displays stroke and anti-stroke response compared to Raleigh light in the unit of wavelength as the same as infrared [17]. Raman-active compound usually posse π-electron that can be induced to be polarized when suitable monochromatic radiation is applied; therefore, incident light gains or loses energy and scatters as stroke or anti-stroke light [17]. Both of them are sensitive to structural change; hence, it can be used in the solid state study as well as the spatial dimension [9]. The main advantage over infrared spectroscopy is that Raman spectrum gets weak response from water; therefore, aqueous-contained sample could not interfere the Raman spectrum. On the other hand, excipients which mainly contains σ-bond as the same as water will give weak Raman response too. However, Fonteyne (2013) studied difference of solid form in Theophylline monohydrate and anhydrous due to shifted band; therefore, detectable changes would be beneficial to understand the process especially the effect of applied variable or process to drug form [35].

Raman spectroscopic method required large intensity of incident light to make stroke and anti-stroke response detectable; consequently, it required close space to prevent the exposure as a safety issues and it would make complication of equipment settings. Allan (2013) reports some applications of Raman transmission mode can be used for homogeneity and quantitative determination on the tablet as the same as NIR spectroscopy [36]. Nevertheless, collaboration with a microscope elucidates the

content on the defined surface called 'Raman Mapping'. Condensed light is reflected on the sample which placed on the pan of microscope; then, scattered light will be recorded and expressed as colour in the particular pixel. Subsequently, each pixel is combined into the picture and called Raman Image or Raman Mapping (see details at section 5. 2). Gordon (2011) reviewed the application of Raman mapping on pharmaceuticals and found that mapping can be evolved along granulation process including blending, granulation and tablet surface to illustrate the distribution of excipients and API [9]. Vajna (2010) stated that Raman imaging method can detect differences from applied granulation technologies, liquid and compression force [31]. Chemical images were obtained by Raman microscope at 100x lens on the surface of tablet without sample preparation. Pure components were scanned to obtain unique reference Raman spectrum to be used for API determination. Finally, Raman images performed moderately precise estimation of relative quantity; unfortunately, this method could be more accurate and precise with increment of sample size and resolution of picture [31]. To conclude, Raman spectroscopy can be used to analyse the component of sample as a Raman Mapping which acquired from the process when the research is processing. This study will utilize this application to study the distribution of both API on the surface of finished tablet for a brief estimation of selected configuration.

1.5. Physicochemical characterization of the tablets

1.5.1. Solid state Characterization

Solid State Characterisation provides information of physical properties which consequently effect on performance of the product. Generally, physical characterisation of tablet regarding pharmacopeia standard are essential test. Appearance of tablet including its dimension is considered as well as uniformity of weight which can indicate amount of API on each unit. However, some issues which are not described in Pharmacopeia but they affect product attributes such as drug polymorphism are considered through production line; therefore, thermal analysis and X-ray diffraction (XRD) are employed at this process to ensure the polymorphism of

pure drug substance [15, 37]. Moreover, NIR, Differential scanning calorimetry (DSC), Attenuated total reflectance (ATR) infrared spectroscopy and Raman spectrum are employed in order to detect formula compatibility, interaction of mixture and changing affected by process [15]. Any differences are showed as shifting of peak, appearing of new peak and also spectrum pattern. Furthermore, Zhao (2013) detected changes in different way using image analysis due to change of appearance of polymer forms detected by Particle vision measurement (PVM) for visual image or focused beam reflectance measurement (FBRM) for changed particle size [38]. Unfortunately, these methods require sample in form of powder; therefore, if these applications would be applied with finished tablet since they have to be broken before analysis.

These measurements illustrate some physical properties in order to help researchers to detect a change and allow them to achieve desirable process control procedures. These could be utilized if polymeric characterisation has to be proven in this study.

1.5.2. Chemical Imaging

Component distribution of desirable substance could be illustrated in various form of sample; therefore, it can be used in the study of process parameter through process stream including tablet compression. NIR camera which was used for illustration of excipient's distribution in the mixing cube; consequently, this method could be adapted in the tablet compression if camera is located on the ejection pathway after die compression [6]. Obtained result would be the distribution of sample over the cube at particular time. Furthermore, chemical mapping could be applied in the static sample as an offline PAT tool; a pixel of defined point is scanned by spectroscopic methods and spectrum is individually recorded on the map [9]. Then, the next pixel will be treated the same and so on; finally, all defined areas are scanned and each pixel of maps contains data covered scanned wavelength is illustrated. Eventually, particular wavelength which represent studied component will be selected and showed the intensity as colour on the maps. This can be applied in the study of solid state distribution of API on tablet surface even in potent drug [9]. Moreover, implementation of microscope on chemical mapping makes a fine scale of maps in micrometres; therefore, small area of sample is analysed. However, the big picture

could be obtained if the repetition is made on the different area and they will be gathered to be a big picture [39]. Unfortunately, chemical imaging can provide the data on the surface of sample if a deeper layer has to be analysed. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) would be one of the best choice due to ability to destruct the surface layer while spectroscopic method has restriction of light penetration [40]. To conclude, chemical imaging has an advantage over the conventional method such as information provided and time spending. Therefore, it could be utilized in this study to display the dispersion of API on the finished tablet's surface.

1.5.3. Uniformity of Content

Uniformity of content is one of the most important attributes which indicate dispersion of drug and excipients on the product which subsequently affect to consistency of drug amount in a dosage unit. It would be affected by step through production line from mixing to tableting; therefore, this parameter has to be considered as one of product attributes during process not only tableting but also mixing and granulation.

Blending uniformity is a uniformity of content in the mixing process and there are many PAT tools that are able to reveal this information such as NIR spectroscopic method. Principle component analysis (PCA) and hotelling's T^2 (mentioned in section 6.2) will be utilized as process analyser to extract information and reveal uniformity of content [6, 7, 17]. Osorio (2014) showed a choice of process monitoring by image analysis since NIR camera is equipped and illustrated the component dispersion through the process [5]. Due to available instruments and time in this study, NIR and PCA usually work together to obtain the uniformity of mixed sample to ensure that the inhomogeneity mixing will not interfere to the downstream process.

Dispersion of drug during granulation process and also HME can be obtained by data from uniformity of content with utilization of PLS model [14, 18]. However, if the quantitative data is not required in this process, transformation might be done by PCA as qualitative PC score and calibration is not necessary [18]. This study will employ PCA as the analyser of uniformity during granulation to minimize the complexity of experiment.

Uniformity of content in tablet has been focused in the conventional QbT concept and also described in pharmacopeia specification which requires data from small number of acquired samples compared to a batch. However, further extend of this attribute is considered during the production in QbD concept as in-line monitoring with PAT non-invasive instrument; consequently, large sample size will be involved in analysis and full batch information would be obtained. NIR and Raman spectroscopic method are currently used regarding to PLS data analysis method [17, 29].

1.6. Design of Experiments (DoE) and Data Analysis

1.6.1. Design of experiments

To obtain the sufficient data to make conclusion for each study, large number of experiment from a number of process variable could be employed in order to prove the assumption; hence, non-economical, unorganized or insufficient amount of experiment could lead inefficient experiments. Therefore, Design of Experiment (DoE) could be utilized during the experiment designation to help researchers obtaining the optimum combination of trial which will perform sufficient data of the output to draw a conclusion. DoE consists of planning, designing and analysing in order to minimize experimental bias and avoid an incorrect conclusion and is counted as part of PAT instruments [41]. There are many pattern of designs that can be utilized but full factorial with centre point repetition has been widely used because it covers all range of every input parameter which consequently establish effective design space [21]. Generally, interested factor and range of each are required and then number of experiment to be performed including repetition of value at centre point will be calculated. It obviously helps experiment designation especially in granulation study which usually consists of process parameters; consequently, it has been widely applied among pharmaceutical process study including TSG method. For example, Fonteyne (2013) use DoE to simultaneously study an effect of 4 process parameters; screw speed, powder feed rate, water content and barrel temperature on D50 value [35]. Moreover, DoE can be employed for chemometric calibration of quantitative in-line NIR measurement with ternary mixture (Figure 1.10); 1API and 2 excipients, to obtain a

qualitative in-line measurement of API in mixture during tablet compression [29]. Furthermore, not only in TSG but also in other process such as continuous milling, granulation and HSWG [20, 21, 42]. In brief, it can help researchers to make an effective plan to make reasonable experiments and DoE would definitely be employed in this study.

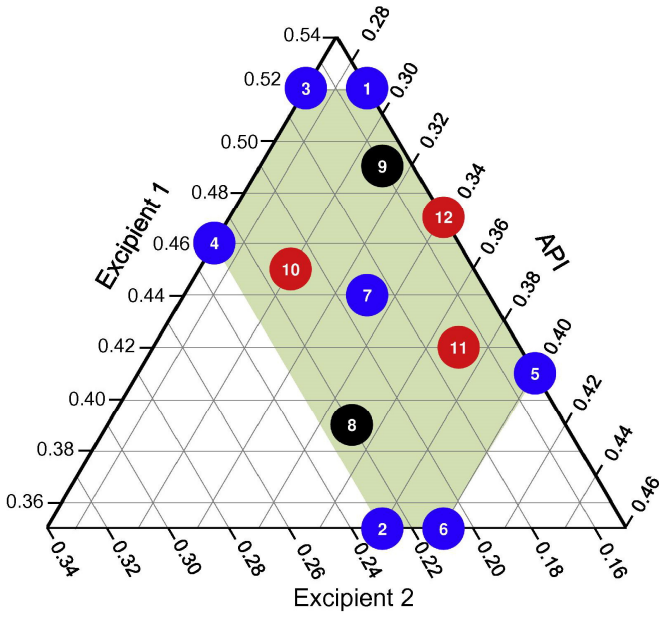


Figure 1.11 Example of DoE for NIR calibration of Ternary mixture (Reproduced with permission; Wahl, P et al., 2014)

1.6.2. Multivariate statistic

Due to application of QbD, Process analysers have been developed from measurement of simple univariate process such as pH to those that provide complicated multivariate information regarding physical and chemical attributes. Moreover, PAT tools, especially spectroscopic method, usually contains multi-dimensional variable which current statistical quality control (SQC) such as control chart cannot be applied [1]. Consequently, multivariate data analysis has been utilized in order to extract complicate information to be understandable and comparable. It can reduce the dimension of variable to extract the effect of applied process variables and also prediction of product attributes during both development process and the commercial manufacturing [1]. Common analysers to be applied in this field are principle

component analysis (PCA) and partial least square method (PLS). Generally, multi-dimensional data are delivered as matrixes consist of input variables (X) and response of each variable (Y). Multivariate method transforms the data matrix into principal component (PC) which is the most variable latent; therefore, variability is presented in the term of score plot and responsive of input variables on PC is showed as loadings plot. First PC (PC1) delivered the greatest variances while the second PC (PC2) showed the next most variances; consequently, score plot of PC1 against PC2 can explain how close of data in group through the most and the next most variable axis. To analyse the significant value, Hotelling T^2 is applied as a P-value do in univariate student t-test [1]. The distance between centroid value and sample are observed and therefore acceptable space of the plot is generated. Observations which are located outside the acceptable space are decided as different or failure; subsequently, this acceptable range can be applied to detect failure product and monitor the process output through time [1].

The raw spectrum would be treated by spectral pre-processing method before analysing to diminish any noise and make more comparable data. Common spectral treatment used in this study were Standard Normal Variate (SNV), 1^{st} and 2^{nd} derivative which are utilized for different aspect [33]. SNV can reduce the noise which might interfere data processing while derivative can magnify difference which can detect the small change [33]. The 2^{nd} derivative detects smaller difference than the 1^{st} derivative but the result would be interfered by magnified noise; therefore, choosing the spectral pre-processer would depend on the user's consideration.

The application of multivariate analysis is widely used according to Beer's article review (2010) about application in NIR and Raman spectroscopy [17] (See details at section 4.1.1 for NIR and 4.3.2 for Raman). However, this study will used NIR spectroscopy as In-line PAT to monitor 2 active ingredients and PCA will be employed to transform data aimed to monitor process of granule production using twin-screw extruder.

1.6.3. Partial Least Square Method

Partial Least Square (PLS) is one of multivariate regression tools which can indicate relation between X and Y; therefore, relation of process variable and product attribute can be established to make understanding of process [1]. PLS model can be used to predict the API concentration from input spectra and known concentration of substance; consequently, some essential product attribute such as uniformity of drug can be tracked through experiment as in-line monitoring (Figure 1.11) [15, 18, 28]. To predict API in unknown sample, the calibration model has to be establish from known concentration sample with a sufficient number of collection point covered range of prediction. Then, the model with two chosen PLS was challenged by addition of the third component and the goodness of model prediction (Q^2) is not dramatically increased. Correlation coefficient (R^2) indicates the fitness of established model if the value approaches 1 while root mean square error of prediction (RMSEP) showed the small error of model prediction if the value was low. However, in application of spectroscopic-based instrument such as NIR and Raman, the nature of spectrum has an effect on prediction since broad band and overlapping of API peak might worsen validity of model especially in low concentration [15]. Researchers might take the spectrum of studied mixture to recognise this issue in the period of formula selection. For this study, there are two APIs with the difference concentration in the formula, PLA model could be involved in the experiment for quantitative data of uniformity of dosage unit.

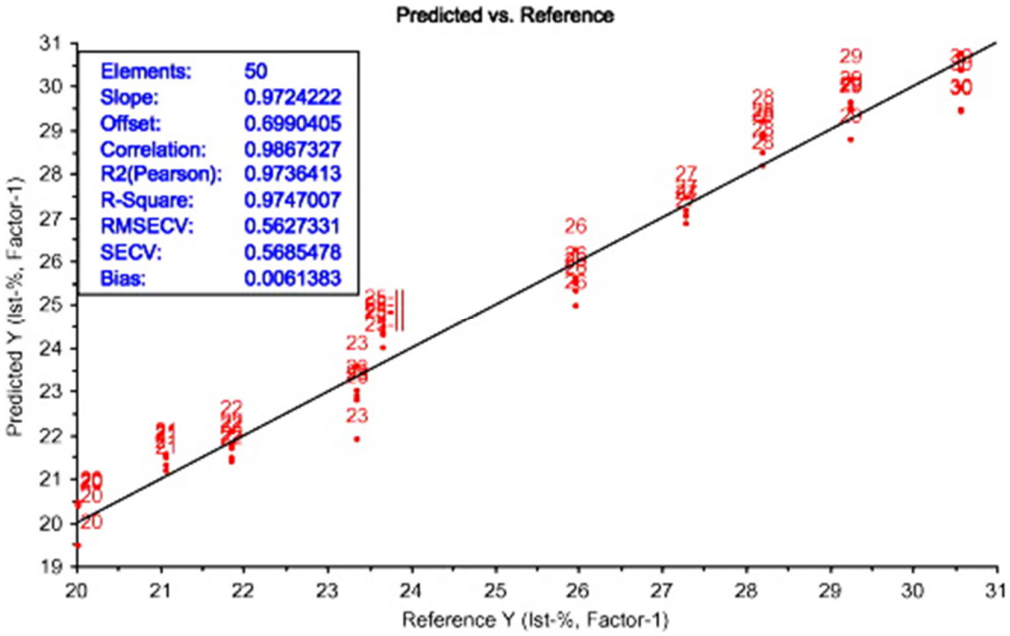


Figure 1. 12 Example of application of PLS model to calibration API amount during manufacturing (Reproduced with permission; Järvinen et al., 2013)

1.7. Aims and Objectives

This study is to investigate the most appropriate TSG configuration using DoE and PAT tools for granulation. Mixing uniformity will be investigated first to ensure that it will not affect the upstream process. Then, the effect of three TSG critical parameters will be studied with experiment plan from DoE and product attributes will be obtained from in-line and offline PAT instruments. Then, results will be compared and the best configurations will be selected and produce in the bigger scale and granules will be compressed in order to prepare tablets. Finally, offline PAT will be utilized to illustrate uniformity of API on tablet surface.

Chapter 2 - Materials and methods

2.1. Materials

Paracetamol (solid, >98.0%) was purchased from Sigma-Aldrich and Caffeine (solid, >98.5%) was purchased from Fluka. Lactose monohydrate 200 mesh (Pharmatose®, DFE Pharma), Microcrystalline Cellulose (Avicel® PH101, FMC Biopolymer), Polyvinylpyrrolidone (PVP K-30, Fluka) and Croscarmellose sodium (Ac-Di-Sol®, FMC Biopolymer).

2.2. Formulation

Although there are many papers studied about process parameter of twin screw wet granulation, many of them used placebo as samples to reduce complexity but they cannot be used for industrial formula. This study decided to start with the formulation from high shear wet granulation (HSWG) which could be counted as the closest technique compared to TSG due to process parameters regarding liquid binder such as L/S ratio and impeller speed. Furthermore, formulations contain hydrophilic excipients which can be used in TSG; consequently, this study will transfer HSWG formulation to be TSG. Lastly, those techniques used the same product attributes since they also give granules as product, result can be comparable. Similarities and difference between them are showed in table 2.1

On the other hand, this study required combination API for studying and due to author's knowledge, it has not found in TSG study. Subsequently, combined formulation was reviewed at HSWG study. Cavinato (2011) was successful producing uniformly granules with difference API but the same excipients by HSWG without detectable problem such as occurrence of sticky granule [42]. Although they did not use combine API formulation, combination of two successful studies will be more likely possible; therefore, their formulation was chosen for this TSG experiment. However, adding two API makes formulation to be slightly different from reference method; details of formulation are compared in table 2.2

Table 2.1 Difference of manufacturing process, product attributes and process parameters in this study vs in the literature [42]

Subject	Article Review	This Experiment
Mixing Method	High Shear Mixer	Cube Mixer
Granulating Method	High shear wet granulation	Twin screw extrusion
Product Attribute	Particle size, distribution, morphology	Particle size, distribution, morphology
Process Parameter	Impeller Speed Liquid Flow Rate, Force	Extruder Speed Liquid Flow Rate, Torque Solid Feeding Rate

Table 2.2 Difference of the formulation in this study vs with literature [42]

Name	Article Review*	This Experiment	Function
Active Ingredient	50.00%	44.25%/ 11.75%	Active Ingredient
Number of API	1 (Paracetamol)	2 (Paracetamol and Caffeine)	
Lactose Monohydrate (150 mesh)	23.50%	23.50%	Diluent
Microcrystalline Cellulose PH101	20.00%	20.00%	Binder/Diluent
PVP-K30	5.00%	5.00%	Binder
Croscarmellose Sodium	1.50%	1.50%	Disintegrant

Table 2. 1 showed the similarities and differences between our proposed method vs to literature. Both are studied the process parameters which affect granule attribution; therefore, study the process parameters should be newly initiated while the outcome could be comparable since the same attribution was used. However, the study in this research was more complicate since APIs consisted of 2 materials while literature has used one.

2.3. Production Methods

2.3.1. Mixing

The settings of this step are newly developed since mixing processes are different from the article reviewed [42]. Product attribute of this step could be the uniformity of mixing, therefore process parameter which has an effect should be considered. For this study, cube mixer (GmbH KB30, Erweka) was employed with the lowest rotational speed, 20 RPM, in order to prevent spreading of mixed powder when the cap is opened. Moreover, the cube positional angle was observed and found that 90° of angle facilitates the best flow during mixing and the retained powder at the corner of mixer retained the least.

The mixing order was created regarding geometric dilution to promote good mixing uniformity. Small amounts of excipients were mixed first and then blended with the greater amount of excipient. Finally, the mixture of excipient met the mixture of API in the cube mixture (Figure 2.1).

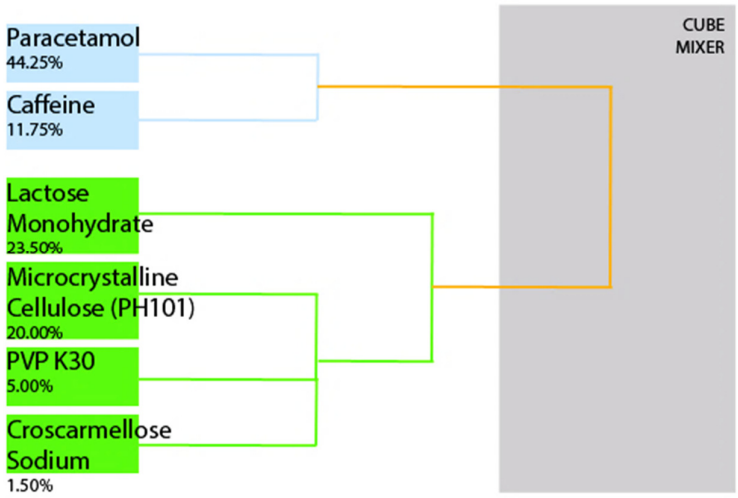


Figure 2.1 Mixing order of the formulation beyond the mixing step

Three samples were taken from the stopped cube after 1 rotation at the position of top-centre, middle-right and bottom-left. Sampling Procedure was described in Table 2.3. Then, the cube was stopped every 5 min in order to collect 3 samples until 15 min. The last sample set was obtained after 30 min of rotation and labelled as T30. In total, 6 set of samples and each set contains 3 samples from different position was collected.

Table 2.3 Sampling Procedure for mixing process

Points of Sampling Time	Sampling Position
0 Minute (T0) 5 Minutes (T5) 10 Minutes (T10) 15 Minutes (T15) 20 Minutes (T20) 30 Minutes (T30)	top-centre middle-right bottom-left

2.3.2. Granulation

The twin extruder axis was equipped in the extruder barrel whose configuration was shown in Figure 2.2. Moreover, the system of granulation part contained solid feeder (Mini Twin Loss in Weight Feeder, Brabender), liquid feeder (Masterflex® P/S 955-0000, Thermo Scientific or PHD Ultra, Harvard Apparatus) and the 11 mm extruder (Process 11, Thermo Scientific) (Figure 2.3).

On the other hand, analytical part consisted of NIR spectroscopic probe and online particle size analyzer (Eyecon®, Innopharm). The NIR probe was connected to Spectrometer (NIRQUEST512, OceanOptics) and Light Source (HL-2000-FHSA, OceanOptics). The position of analysers was showed in Figure 2.4.

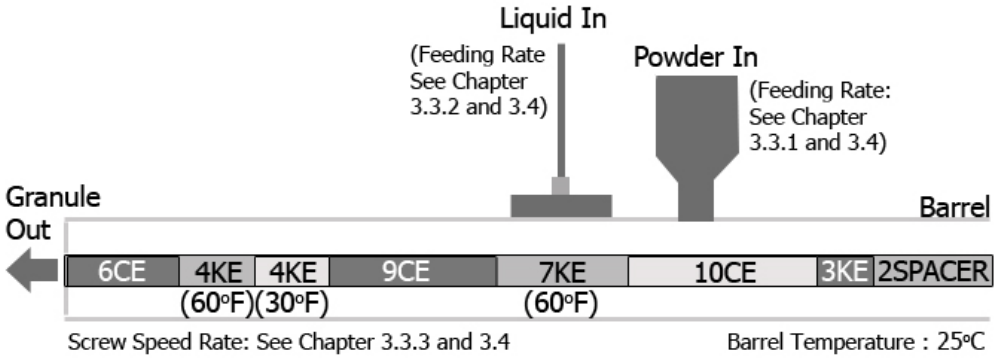


Figure 2.2 Configuration of extruder axis; where F forward configuration, CE s conveying element, KE s kneading element.

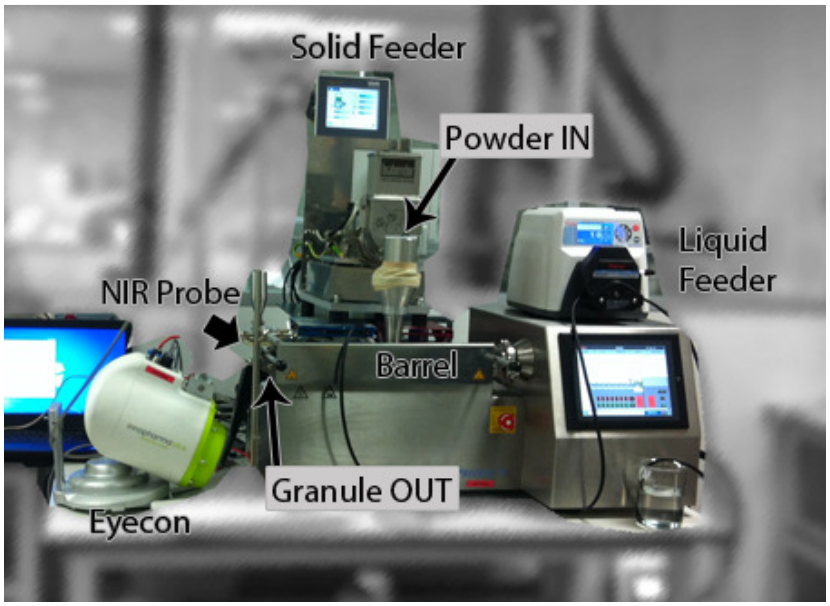


Figure 2.3 Equipment setting for granulation process

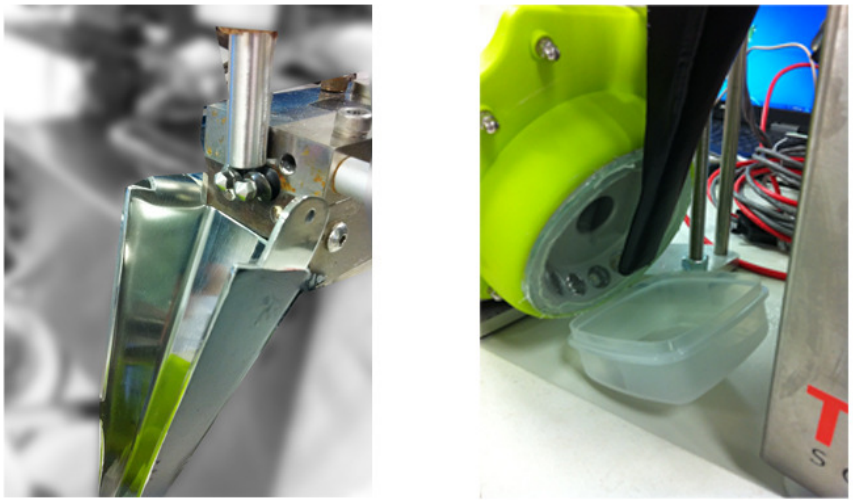


Figure 2.4 Left: Position of NIR probe, Right: Position of Eyecon camera and collector

To perform the batch experiments, the solid feeder was firstly initiated followed by the extruder and liquid feeder. The sample was taken after 1 min for system equilibration.

2.3.3. Tableting

The granules were compressed in order to produced tablets by Tablet Pressing Machine (Oystar Flexitab® Single Punch Tablet Press). The configuration was set as follow: Force 7.0 kN, die depth 9.5 mm and 3 feeding repetition. Sample tablet was taken after the fifth tablet of each batch.

2.4. Analytical Method

2.4.1. ATR-IR

All samples were taken from the process and analysed separately with ATR-IR (Tensor II, Bruker). The powders were scanned offline over a wave number range of 4000-400 cm^{-1} over 16 scans at a resolution of 4 cm^{-1}. All ATR-IR spectra were then analysed using a multivariate analysis software (SIMCA 14.0, Umetrics).

2.4.2 NIR

All granules were analysed at the end of extruder barrel using online NIR spectrophotometry (NIRQUEST512 Spectrometer, HL-2000-FHSA Light Source, OceanOptics). Reflectance mode was used over the range of 900-1700 nm at a resolution of 4 nm and every measurement was recorded. NIR spectrum was subsequently obtained through time of study and multivariate analysis software (SIMCA 14.0, Umetrics) was subsequently employed in order to extract the information regarding to principal component analysis method.

2.4.3 Eyecon®

The Eyecon® Particle Characterizer was used for the in-line granule size analysis. Granule images were recorded while the TSG running and collecting the granule samples. Figure 2.4 shows the experimental setup with the integrated TSG-camera system.

2.4.4 QICPIC®

Particle size and distribution of mixing and granules were determined by particle size analyzer (QICPIC®, Sympatec) as an offline PAT in order to compare the data from online PAT tools such as Eyecon. The powder dispenser used RODOS method, 0.5 bar, 20% with 1.5 mm raising of cone. The analysis was done over the particle range 4-2888 µm with 175 Hz, Start >0.1%, Stop < 0.1% of trigger condition.

2.4.5 Light Microscope

Light microscopy (Leica® STP6000 for Controller, DM6000M for Microscope and MC170HD for Connector) was used to obtain the "true" shape of granules. Although light cannot be passed through the granules, polarized light was utilized by a mode of an incident POL 90^{o} with intensity 175, aperture 11 and field 1. Smallest magnitude of lens, 2.5x, was employed over all experiments.

2.4.6 Raman Microscope

Each ingredient was scanned for obtaining Raman spectrum in general and then unique peak of Paracetamol at 1164.80 cm^{-1} and Caffeine at 556.04 cm^{-1} were considered to be selected for mapping study (See the selection at section 3.6.2 a). Consequently, Raman mapping mode was used for qualitative determination on the tablet surface which is divided into 9 areas as illustrated in figure 2.5.

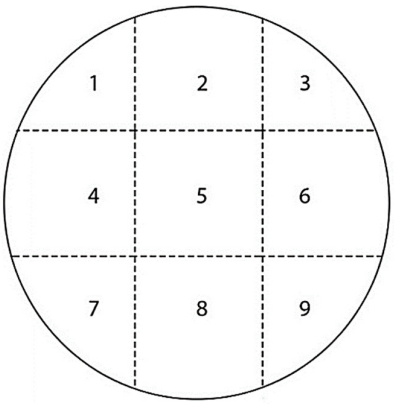


Figure 2.5 Area divided on the tablet for determination of uniformity of content by Raman microscopic method

Raman Spectrum were recorded on a Raman Microscope (XploRA® plus, Horiba Scientific) with an acquisition Range of 50 - 3600 cm^{-1}, 4 sec of acquisition time, accumulation 2 and 2 sec of delay time. Laser at 542 nm was used with a filter of 50 %, 1200 of grating, 100 µm of slit and hole.

The same equipment was utilized for the Raman Mapping over the 400 – 1700 cm^{-1} of acquisition Range, 4 sec of acquisition time, accumulation 2. Objective lens of 10x magnification was used in order to get an area appearance while the laser at 542 nm with 50 % filter, 1200 of grating, 100 µm of slit and hole was used in order to obtain the Raman

spectrum on the marked area. Mapping size was set differently; 7x7 (49 data points) for Batch 3014 while 8x8 (52 data points) for Batch 3018. Step was automatically calculated by the instrument and showed as Y = 23.2 X = 28.6 for Batch 3014, Y = 54.1 X = 52.4 for Batch 3018.

2.4.7 Tablet weight variation

Ten tablets of each batch were randomly sampled from pooled compressed tablet and they were weighted on the analytical balance (LPG-6501, VWR). Average of ten measurements was calculate and then the standard variation of data was obtained.

2.4.8 Hardness Test

At least three samples of freshly compressed tablet were randomly collected and analysed by the hardness tester (2E/205, Dr K Schleuniger & Co. Switzerland).

Chapter 3 - Results and Discussions

3.1. Particle size of Raw Materials

Raw materials were analysed by QICPIC particle size analyser for comparison with granules. Results are showed in the table 3. 1 in the term of D50 value. The majority of the D50 value are close to each other except caffeine which is considerably bigger than the others due to coalescent of powder to the nature of material. Therefore, Caffeine raw material was pass through the sieve to reduce particle size before mixing. Moreover, D50 values of mixtures are slightly bigger from raw material might also cause from coalescent of powder.

Table 3.1 D50 value of each raw ingredient and the mixture.

Name	Particle Size, D50 / µm
Paracetamol	72.84
Caffeine	117.56
Lactose Monohydrate (150 mesh)	62.38
Microcrystalline Cellulose PH101	81.99
PVP-K30	87.35
Croscarmellose Sodium	52.00
Mixture – Batch 2003	87.11
Mixture – Batch 2004	90.14

3.2. Mixing Process

Uniformity of mixing during the process which is one of product attributes was carried out using ATR-IR in order to obtain the spectrum and analyse them using the principal component analysis (PCA) software as multivariate analyser. Spectra were collected from six mixing periods and three samples from different positions (Table 2. 3) were taken from each period, replicate test were done; consequently, there are 36 spectrum involved in this study. Less- variable IR region was excluded from analysis since contains less information; the selected region was 400 – 1650 cm^{-1}. Then, spectral treatment processes were applied to the data set for magnify variation and diminish the

noise; consequently, the 2nd derivative and Standard Normal Variate (SNV) were applied. This mixing configuration was utilized by 3 consecutive batch in order to ensure the precision of the method.

The result was illustrated by plotting the first principal component (PC1) VS the second (PC2) with 95% hotelling's T^2 as the acceptance criteria (Figure 3.1). A few data points from starting point (T0) and T5 placed outside the acceptable area while no points after 5 min shows that patent. PCA showed difference of collected spectrum compared to the others group; therefore, this means the uniformity at stating point and 5 minutes later did not reach the steady state and need to be further mixed. On the other hand, similarity of spectra from 10 min of mixing showed by PCA allowing conclusion that mixing reached the steady state and be mixed uniformly. Moreover, this experiment was repeated with another two batch, Batch 2003 and 2004, data from the starting point (T0) was also placed outside the acceptance criteria but the others were not. Subsequently, it is a confirmation that the mixing time could be more than 5 min to reach the equilibration state.

Experiments for this process were limited due to one of two process parameters, rotational speed, has to be fixed due to properties of mixed powder. When blending was finished, the small dust was generated and spread over the cube; therefore, it cannot be opened until dust was segregated which can be seen when the space in the cube was clear. This happened more and take longer period for segregation if rotational speed was increased. Therefore, this experiment keeps the rotational speed to be minimum as 20 RPM; consequently, only mixing time was studied as remained process parameter.

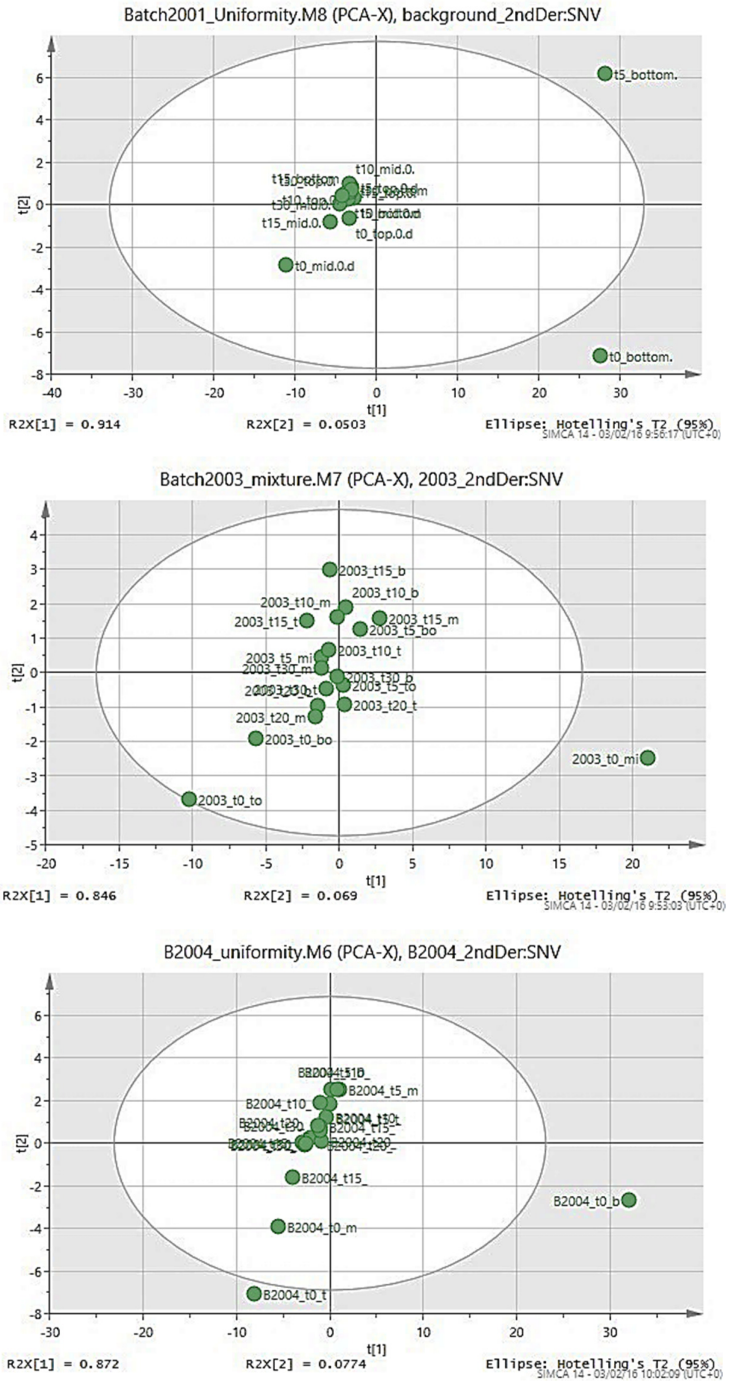


Figure 3.1 PC Analysis of mixture through time (t) 0,5,10,15,20 and 30 minutes at the position of bottom (b), middle (m) and top (t) from mixing Batch 2001, 2003 and 2004 (3 Consecutive Batch)

One issue to be discussed is usage of PAT tool for this process, this study tried to record NIR spectrum via NIR camera such as Juan (2013) experiment did for studying powder blend uniformity through blender's wall [5]. Unfortunately, wall-through recorded spectrum did not present interpretable peak while direct measurement can do. One possibility would be thickness of the blender's wall can reduce intensity of NIR signal; consequently, method was changed to be powder sampling and offline analysis from ATR-IR is chosen to be more suitable for this sampling method. Therefore, data were obtained from point of sampling instead of continuous map of distribution. Although sampling procedure in this experiment makes difference from Juan's study from continuous studying to point studying, it is enough to ensure the uniformity of mixing will not affect to downstream process. However, study of uniformity of blending could be extended to find out better configuration such as less processing time to make this process easier.

Due to employment of qualitative PCA and hoteling T^2 statistic as data analyser, similarity among data set in the same batch was showed in plotting. However, the exact amount which required for further optimization such as shorten mixing time, adjusting rotational speed cannot be showed. Therefore, this experiment could be more advance if quantitative method such as PLS can be employed as Benoit's study (2014) [7] since not only the amount of API but each excipient's distribution can be obtained through time. This makes trials to be quantitative measurements and it would be easier to draw design space of blending for this formulation from DoE of this study. However, this study just required the validity of mixing ability since at least 3 point of sampling time located in the same area according to PCA's plot (see Figure 3.1). More advance data analysis would be necessary if the formula will be used for industrial scale.

3.3. Granulation: condition finding

An experimental was planned for study the effect of process parameters on product attributes to get the most appropriate setting which gives desirable product attribution. Process parameters were solid feed rate, liquid feed rate and extruder speed while particle size distribution was selected to be the product characteristic for decision.

3.3.1. Solid feeding rate

Solid feeding rate was studied since feeding capacity will limit the rate of solid input. The study was started at 0.6 kg/h following the study by Treffer's (2013) [16]. However, it did not work for this experiments since it reached instrument output limit. Therefore, the rate was decrease by 0.1 kg/h until 0.2 kg/h with the ability to perform continuously. Moreover, this method was continued to expand the range of setting rate and found that the machine can dispense properly from 0.15 to 0.225 kg/h. Therefore, the range of solid feeding rate was established to be 0.15 to 0.225 kg/h or 2.50 – 3.33 g/min and it would be used in DoE experiment at the section 3.4

3.3.2 L/S Ratio

The next study was the appropriate liquid feeding rate in terms of liquid and solid ratio (L/S) which can provide granules as product. Solid feed rate, which obtained from 3.1.1, was set at 0.150 kg/h (2.50 g/min) and extruder speed was set as 75 RPM. The experiment was set following table 3.2, starting for the lowest common ratio, 0.1, and then increasing by 0.1 until wet mass comes out. Unfortunately, due to the limitation of liquid feeding equipment, values were slightly changed to 1 digit and eventually effected on the ratio.

Table 3.2 Configuration of TSG for determination of suitable feeding rate

L/S Ratio	Liquid Feeding Rate (Theory)	Liquid Feeding Rate (Actual)	Solid Feeding Rate	Extruder Speed	D50 (μm)
0.1	0.25 ml/min	0.3 ml/min	0.15 kg/h (2.5 g/min)	75 RPM	65.66
0.2	0.50 ml/min	0.5 ml/min			63.90
0.3	0.75 ml/min	0.8 ml/min			64.50
0.4	1.00 ml/min	1.0 ml/min			185.96
0.5	1.25 ml/min	1.3 ml/min			319.32
0.6	1.50 ml/min	1.5 ml/min			388.74

The particle size distribution and D50 value was reported as product attributes. It can be seen from table 3.2 that the granule size did not change at low ratio (0.1-0.3) and products were appeared to be fine powder; therefore, these configurations cannot be

used. At L/S ratio 0.4, D50 was increased more than 2 times compared to mixture (87.11 and 90.14µm, from table 3.1). Unfortunately, wet mass was generated after 150 secs due to excess of liquid amount at ratio 0.6; therefore, this setting cannot be used and this ratio was excluded. However, two available L/S ratio, 0.4 and 0.5, might be too narrow for the design of experiment which often prefer 3 levels of each parameter, ratio 0.3 which cannot generate granules due to this setting was included for the design of experiment in case that variation of others process parameter might affect its performance of granulation. Therefore, this ratio was counted as the lower range and the L/S ratio to be studied was 0.3-0.5.

3.3.3 Extruder Speed

The study of suitable extruder speed was investigated with 0.4 of L/S ratio which made from the liquid feeding rate at 0.1 ml min^{-1} and solid feeding rate at 2.5 g/min. The study started at the lowest common speed which is usually set for this extruder, 50 RPM and then granules was produced. The speed was increased by 25 RPM to 75, 100 and 125, respectively. Finally, the powder came out at the speed of 150 RPM because the duration for granule formation in the barrel was too short due to fast extrusion; therefore, the highest speed that can produce a granule was 125 RPM. Consequently, it would be concluded that the suitable extruder speed would be 50 – 125 RPM.

Beyond this point, the 3 parameters which are considered as process parameters are discovered as described in Figure 3.2.

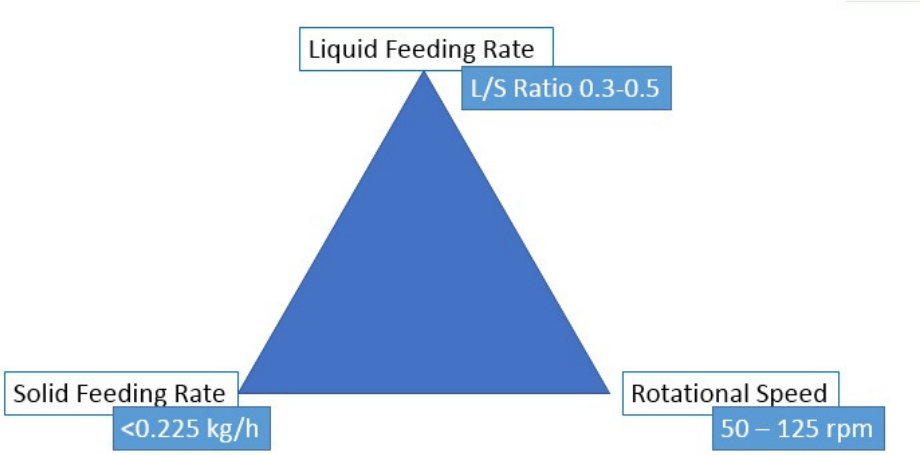


Figure 3.2 Process parameters for TSG granulation

3.4. Granulation: fine adjustment

Three levels of each process parameter were inquired to MODDE 10.1 software in order to generate Design of Experiments (DoE) which would be help to perform the most suitable experiments and get suitable data. Equilibration period was left for 1 min after each running and then the product was collected for 3-4 min while granules were analysed by Eyecon as in-line measurement. Then, granules were left to dry for 1 day and then moved to analysed by QICPIC as at-line measurement. The data were reported in Table 3.3 as appearance of granule and D50 value from both instrument. Some experiments produced agglomerated granules length more than 2888 µm which were too large to be analyzed by QICPIC; consequently, their data were showed as N/A and the experiment which generated sticky mass were excluded from D50 measurement as present as dash sign on the table. Difference of D50 value between two methods which were presented will be discussed at section 3.5.1.

Table 3.3 Design of experiments for the step of fine adjustment and D50 value obtained from Eyecon and QICPIC. (N/A mark meant no data available due to restriction of analysis range)

Exp.	Solid Feed Rate (g/min)	L/S Ratio	Extruder Speed (RPM)	Appearance	D50 (µm)	
					Eyecon	QICPIC
N1	1.67	0.3	50	Large Granule	1670.1	1273.9
N2	1.67	0.4	90	Granule	433.3	1412.3
N3	1.67	0.5	125	Granule	520.4	812.1
N4	2.50	0.3	90	Granule	204.4	727.5
N5	2.50	0.4	125	Granule	273.9	945.2
N6	2.50	0.5	50	Large Granule	154.6	N/A
N7	3.33	0.3	125	Granule	384.9	817.9
N8	3.33	0.4	50	Granule	219.8	861.2
N9	3.33	0.5	90	Sticky Mass	-	-
N10	2.50	0.4	90	Sticky Mass	-	-
N11	2.50	0.4	90	Granule	332.0	827.2
N12	2.50	0.4	90	Granule	220.7	804.3

According to Table 3.3, experiments 1, 6, 9 and 10 were determined as failure setting since generating of sticky mass and too large granules; therefore, 8 settings remained. Consequently, particle size distribution was then involved to be another decision criteria and uni-modular distribution is a desirable graph shape. The graph between distribution density and particle size was generated by QICPIC to find out and experiments 5 and 8 perform the most similar uni-modular shape (Figure 3.3); consequently, they are selected to be used in a full batch manufacturing.

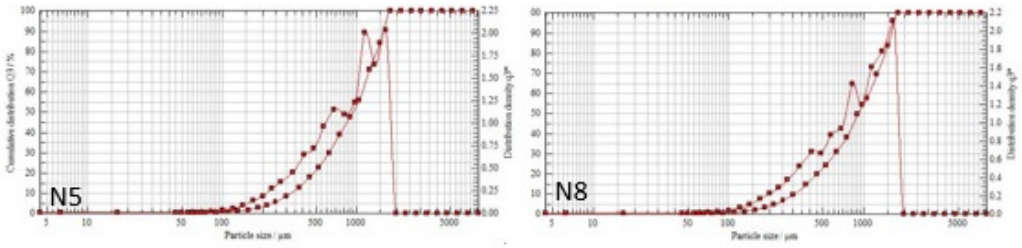


Figure 3.3 Particle size distribution of experiment 5 and 8.

Due to the fact that, this formulation was previously performed by HSWG following Cavinato (2011) [42] and showed that the mode of particle size of finished granules appeared around 200 and 250 µm for Paracetamol-based and Caffeine-based formulation, respectively (Figure 3.4). Formulation which has been used through this study contains both Paracetamol and Caffeine, and the size is expected to be in the similar range, however, QICPIC reported the particle size was three to four times bigger (Figure 3.5). Screw configuration was considered as one of possible reason and it required further study to be compatible with product attribute of tablet compression; however, they were analyzed following product attributes in the section 3.6.

The granule size could be reduced using the same configuration if the kneading element of extruder axis configuration was modified to be reverse direction according to El Hagrasy and Litster's study (2013) [22]. They stated that the forward configuration of 30° and 60° act as conveying element which minimized barrel smearing and chopping [22]. Consequently, if reverse configuration could be applied, the granule would be chopped and smeared, and smaller granule could be provided as result without any chance of studied process parameters.

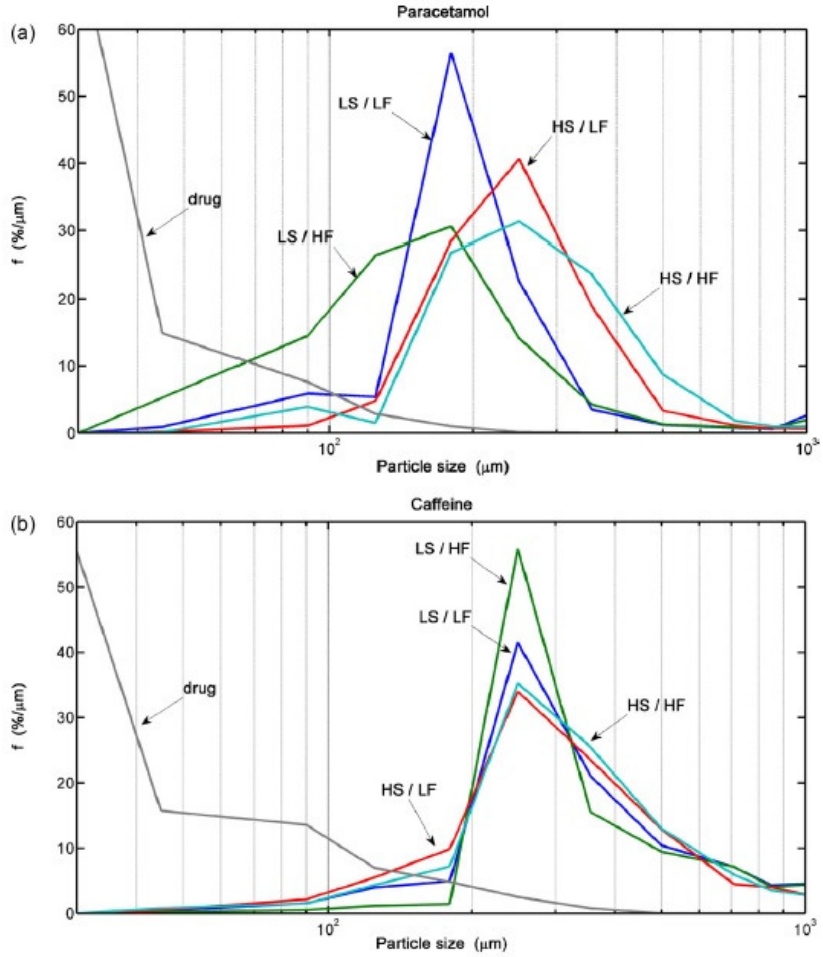


Figure 3. 4 Particle size distribution measured by sieve analysis of the final granules with difference configuration (25% moisture content) for (a) paracetamol, (b) caffeine. (Cavinato, Mauro et al (2011). LS refers to lower impeller speed, HS: Higher impeller speed

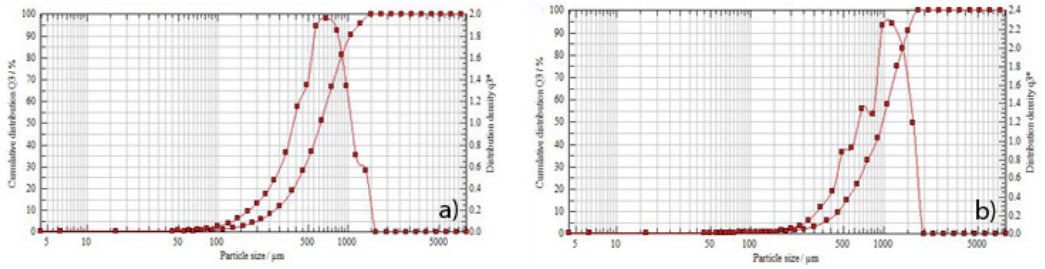


Figure 3.5 Particle size distribution of granule Batch 2103 (Left) and 2303 (right).

3.5. Granulation: Batch production

Experiment 5 was fully run with the setting from 2.2.2 and named as Batch 2103 and 2304. However, the liquid feeder machine was change and diameter of feeding tube was reduced for Batch 2304 to improve feeding accuracy. On the other hand, the experiment 8 performed granules only for short time since the wet mass occurred after 10 min running. Therefore, it was excluded from the study and there remains only one setting as a result.

To run experiment 5, equilibration period was extended to be 5 min to ensure the uniformity of first fraction and sample was then collected after that. Sample collection of each interval was shortened from every 10 min in Batch 2103 to be 5 min in Batch 2304 to magnify difference of product during processing, collecting protocol are described in table 3.4. Unfortunately, the production periods are not equal due to performance of solid feeder; therefore, production time of two batches are not equal, it was 45 min in Batch 2103 but 35 min in Batch 2304. In short, both sample fraction and production interval in 2 batches are not the same as described in table 3.4.

Table 3.4 Interval of data and sample acquired from Batch 2103 and 2304

Interval	**Action**	**Batch 2103**		**Batch 2304**	
		Time (min) (Start – Stop)		**Time (min) (Start – Stop)**	
0	Equilibration	0	5	0	5
1	Data acquired	5	15	5	10
2	Data acquired	15	25	10	15
3	Data acquired	25	35	15	20
4	Data acquired	35	45	20	25
5	Data acquired	-	-	25	30
6	Data acquired	-	-	30	35

3.5.1 Granule morphology

Granule morphology was obtained by both online (Eyecon) and offline (QICPIC) methods. As can be observed in table 3.5, differences of data at the same interval in the same batch obviously showed in Batch 2013. This was happened since Eyecon system cannot clearly focus moving granules, this issues will be discussed in the next chapter.

Table 3.5 D50 value for each interval of batch 2103 and 2304 production from both analytical instrument.

Interval	D50 Value			
	Batch 2103		Batch 2304	
	Eyecon (µm)	QICPIC (µm)	Eyecon (µm)	QICPIC (µm)
1	377.4	688.7	955.5	980.4
2	123.4	728.5	1114.5	966.2
3	485.9	685.0	905.2	1176.9
4	780.1	747.5	874.0	764.6
5	-	-	1199.9	953.3
6	-	-	1524.6	972.0

Light microscope was used to illustrate granule morphology in figures 3.6 for each collection interval in Batch 2103 and figure 3.7 for each interval of collecting in Batch 2304. Generally, granules are not spherical and their size are still varying. Although improvement of PSD is still required, granules will be used for the next downstream process due to limitation of time.

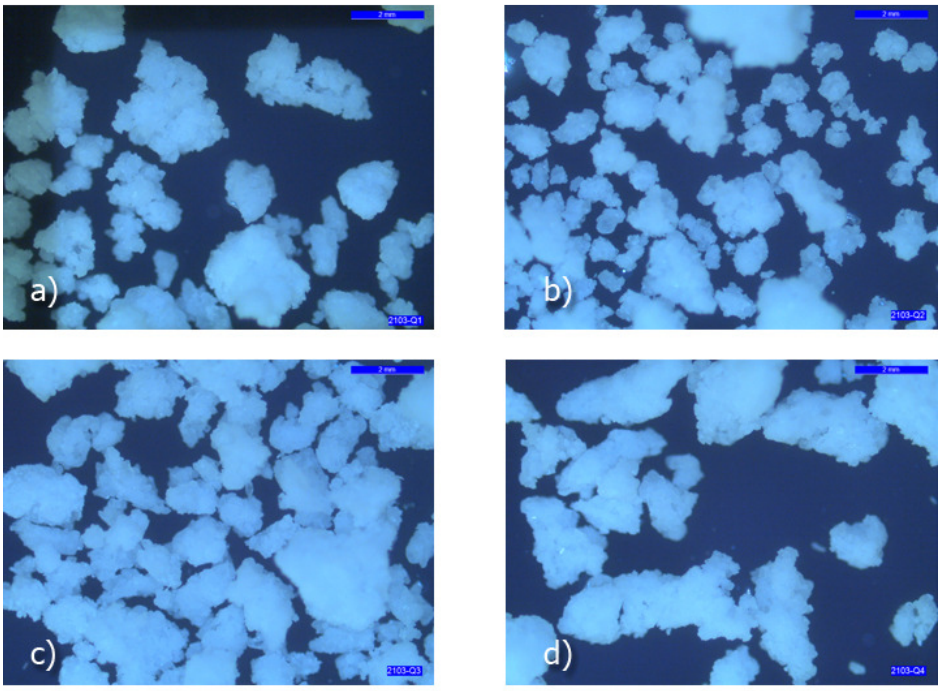


Figure 3. 6 Granule morphology of each interval of batch 2103 by light microscope; a) to d) refer to intervals 1 to 4, respectively. The bar on the up-right corner indicates 2 mm.

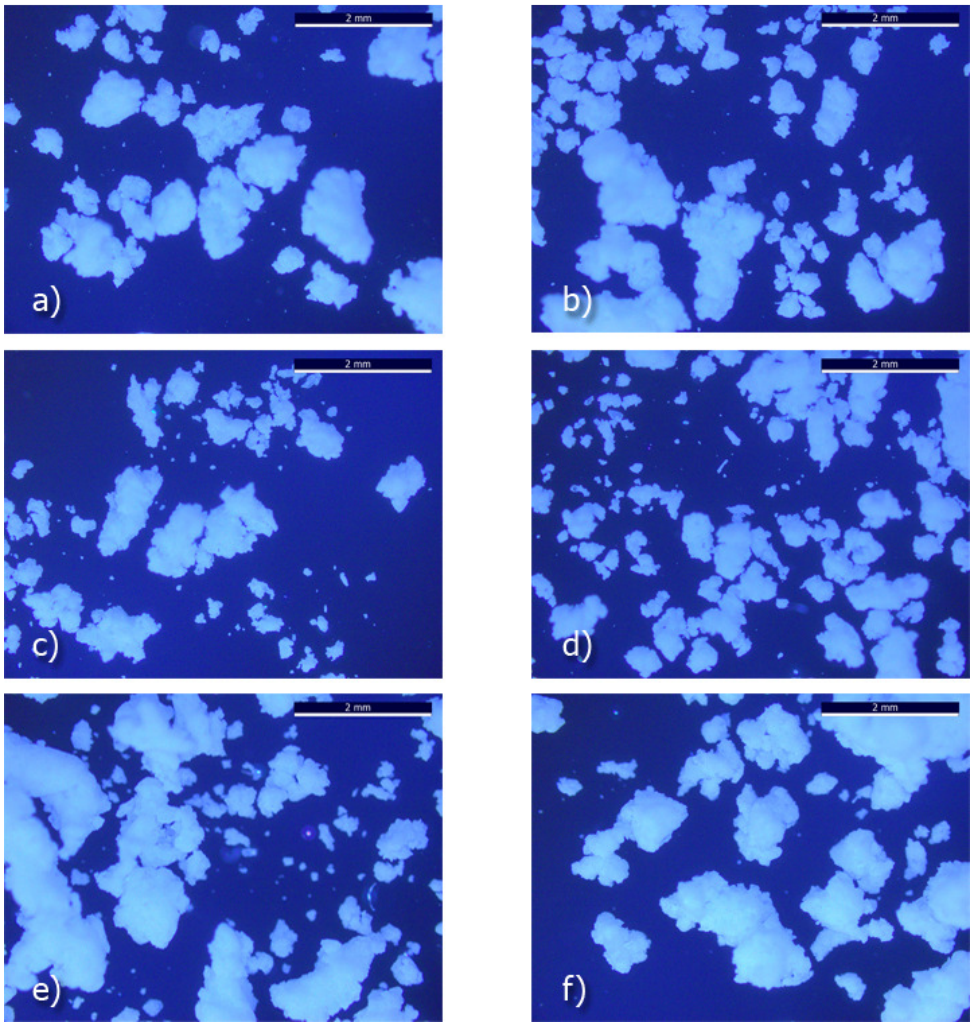


Figure 3. 7 Granule morphology of each interval of batch 2304 by light microscope; a) to f) refer to interval 1 to 6 respectively. The small bar on the up- right corner indicates 2 mm.

One more issue to be mention is difference of reported Particle size via Eyecon camera compare to QICPIC. According to table 3.3 and 3.5, it can be seen that the D50 values acquired from Eyecon have high variation during the continuous running batch; however, that it cannot be observed by an appearance. The investigation had been moved to acquired images and find difference of image quality among periods of batch running (Figure 3.8). The shape and detail of granules cannot be clearly illustrated in 3. 8a due to noise and the edge of granules cannot be accurately determined due to blurred picture in 3. 8b. Therefore, this could be the reason that D values between Eyecon and QICPIC differ significantly. On the other hand, at 3. 8c, the details of

granules surface can be seen, but the edge is still unclear while in 3.8d the granule shape and edge are obviously clear. Consequently, the result from period ‘d’ and QICPIC displayed to be very close.

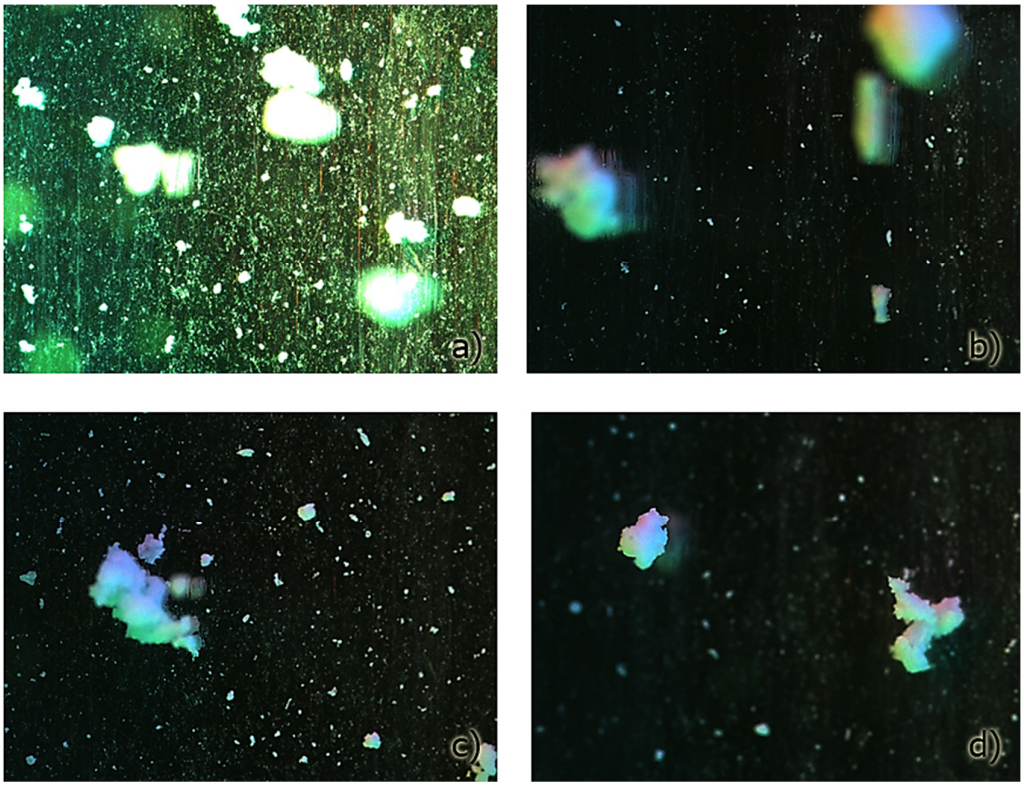


Figure 3.8 Eyecon Picture during interval 1-4 (a to d) of Batch 2103.

The difference on the quality of capture and edge sharpness were explained by the inline focus calibration which was done before starting every single period. Calibration when the extruder stopped could result in the background focus and blurring of granule since calibrated condition did not present true condition. Therefore, calibration during process running helped Eyecon® calibrated the right focus length easier. Although one calibration does not guarantee the exactly right focus length, the best focus can be decided visually from granule appearance on the software and correction was done with Batch 2304 which results were closer than that of the other batch (see table 3.5).

Last issue to be discussed is changing of feeding tube between two production batch which was expected to improve feeding accuracy, consequently reduce the variation of content uniformity and the size distribution to be approach the uni-modular shape.

Batch 2304 was produced with the new liquid feeder (HA apparatus) with more than three times reduction of liquid feeding tube diameter. Although the D50 value appeared to be higher than the all period of previous batch (Table 3.5), the size distribution and the control chart of content uniformity did not show "better". The comparison of particle size distribution (Figure 3.9) was showed that Batch 2103 appeared to be uni- modular distribution than Batch 2304. It seems to be no advantageous to decrease feeding tube diameter; however, the amount of defect over-sized granules which cannot be determined by QICPIC (>3000 µm) which were occasionally found was dramatically decrease in Batch 2304. Consequently, decreasing of tube diameter could help for minimize over size granulation.

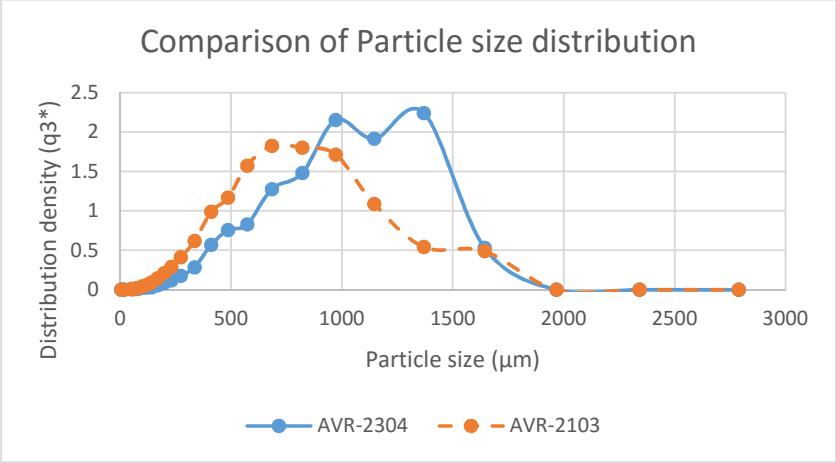


Figure 3.9 Comparison of Particle size distribution between batch 2304 (solid line) and 2103 (dash line).

3.5.2 Content Uniformity

The spectrum was taken from granules by both offline (MIR) and online measurements (NIR) in two production batch. Then they were filtered by SIMCA® software with the first derivative and SNV and finally it was analysed by PCA method to describe uniformity of samples in difference period (see samples at table 3.4). The online measurements from NIR spectroscopy gives the control chart of general component through production time (Figure 3.10).

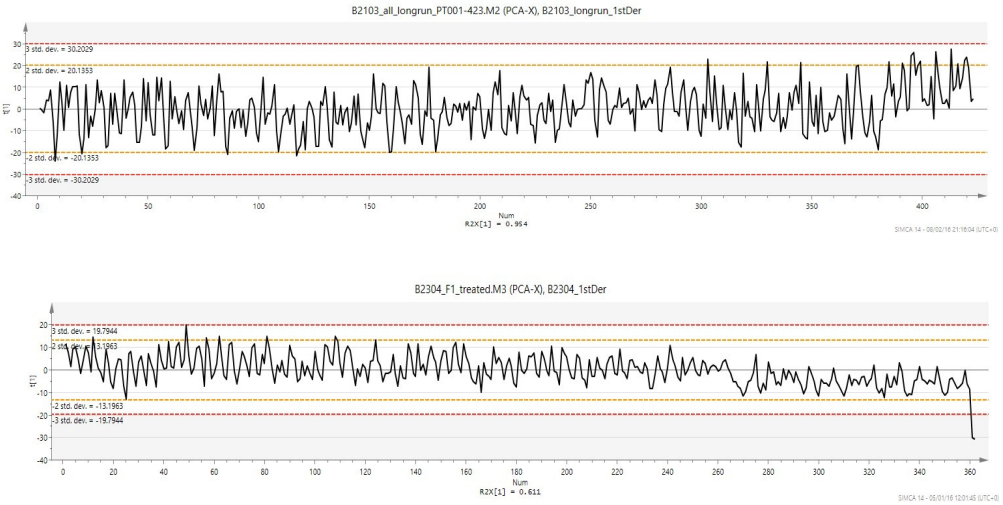


Figure 3.10 Process control chart of batch 2103 (top) and 2304 (bottom) with a treatment of 1st derivative and SNV filter.

Variation and trends can be seen at process control chart from PC1 score versus running time (sec). Batch 2103 displayed some significant data outside 2SD around the end of process but no data was outside 3SD. On the other hand, Batch 2304 showed the similar trend during the early and middle of production period but trend was fallen at the end which showed less condense of granule contents. However, granules were considered as uniform due to PC analysis from offline IR measurement (Figure 3.11) at each section (Sampling protocol was showed at table 3.5). Therefore, drifting can be considered that not caused from uniformity issue. One possibility could be issue with probe glass which was often attached by products during analysis. More condense of granule content might causes from accumulated and attached granule and made high intensity; otherwise, less condense might cause from different position after replacement from cleaning since further distance reduced intensity.

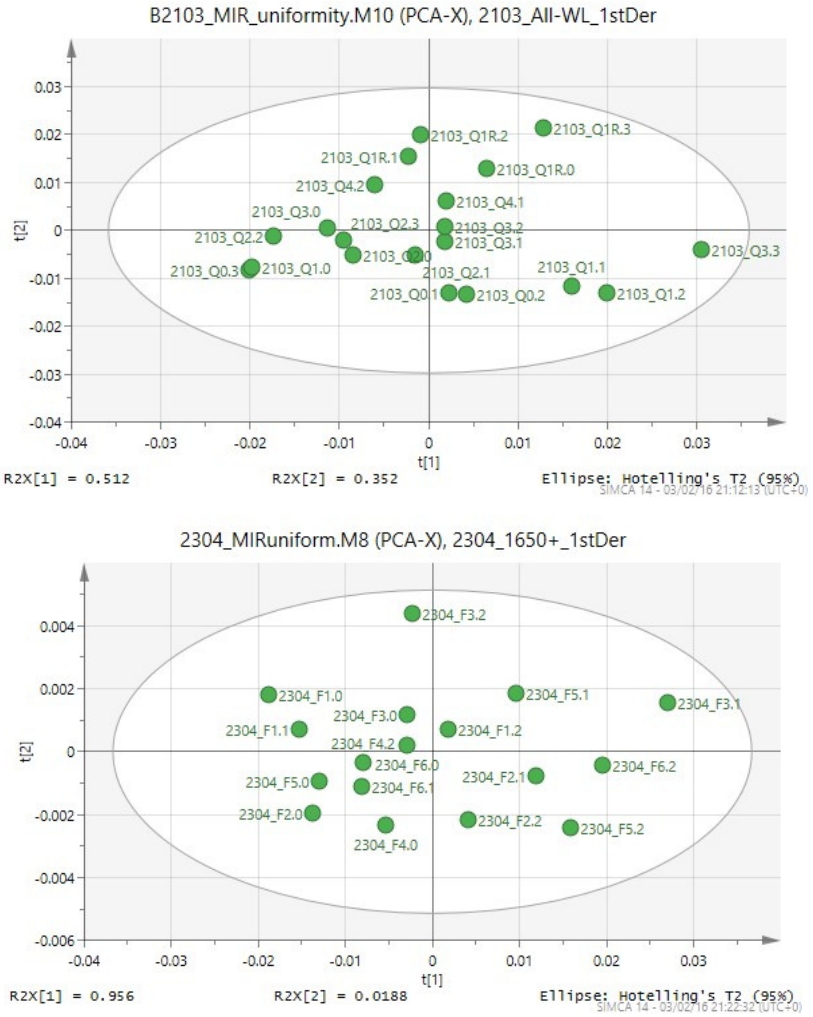


Figure 3. 11 PC analysis of content uniformity of Batch 2103 (Left) and 2304 (right) with a treatment of 1st derivative filter.

Data position in the acceptance criteria of plotting between extracted spectrum data, PC1 and PC2, show uniformly disperse of ingredients during granulation. Although offline method cannot provide trend as the same as online can do, sampling period did not show significantly different between period due to position of each data were not placed outside acceptable area. Although online data showed drift of data tendency at the end of production period, offline PC analysis did not display any difference. Therefore, the issue of NIR probe position to avoid sticking of granules have to be considered to give enough intensity and probe can be cleaned easily without movement.

Moreover, T-square hoteling plot (Figure 3.12) could be applied to be a process signature even some evaluation point was raise over acceptance limit since all product attributes are approved to be acceptable. Lastly, if quantitative calibration such as PLS is involved along production period, these data would be transformed to be labelled amount to confirm that all products in batch are in pharmacopeia's specification and could be utilized as quality control procedure instead of conventional analysis.

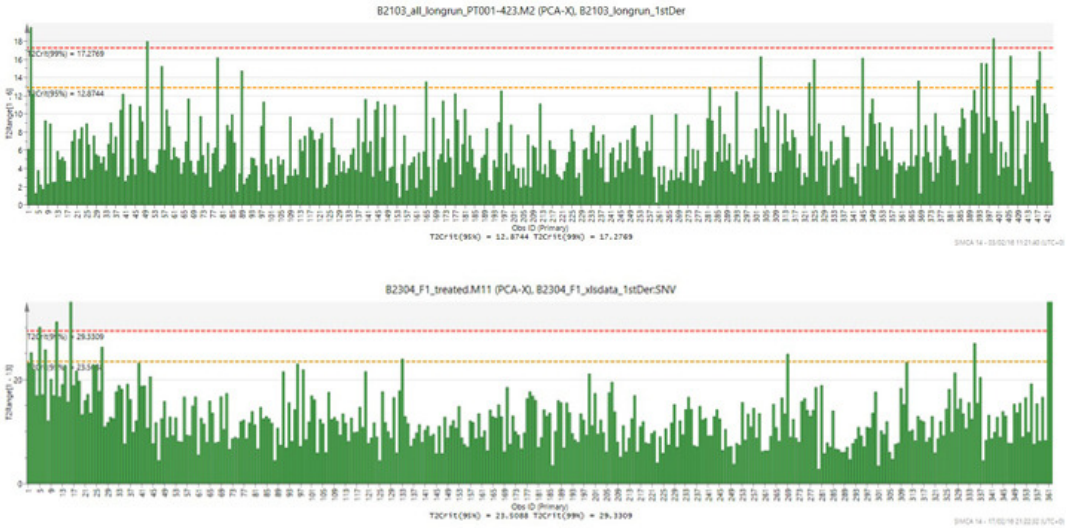


Figure 3.12 Hoteling T-square score along the time of batch processing; Top for Batch 2103, Bottom for Batch 2304, upper dash line refers to limit of T-square Hoteling borderline.

3.6. Tableting

All produced granules from both batch productions in session 3.5 are compressed in order to make tablets with the configuration in 2.2.3. Basic analysis of physical properties such as weight variation and hardness were analysed after tableting. Therefore, Raman mapping was created to illustrate uniformity of content of each API over the tablet surface.

3.6.1 Weight Variation and hardness

Ten freshly compressed tablets of each batch was weighted and then average and standard deviation (SD) were calculated. Analysis with the hardness tester was done after that and the result was showed at table 3.6. Same configuration was applied to

both granule batches; therefore, average weight was slightly difference while hardness is considerably the same.

Table 3.6 Average weight and hardness of sample of Batch 3018 and 3014

Tableting Batch	Granulation Batch	Weight / mg	Hardness / kP
3018	2103	261.3 ± 3.3	8.3 ± 0.4
3014	2304	268.4 ± 2.3	8.5 ± 0.2

3.6.2 Raman mapping

a) Spectrum of each ingredient

The Raman spectrum of each raw ingredient was observed from raw material to be used as references for this experiment. They were compared on the same window as showed in figure 3.13. Therefore, the unique spectrum of each API was found as described in table 3.7 and eventually picked for detection of API content of tablet surface.

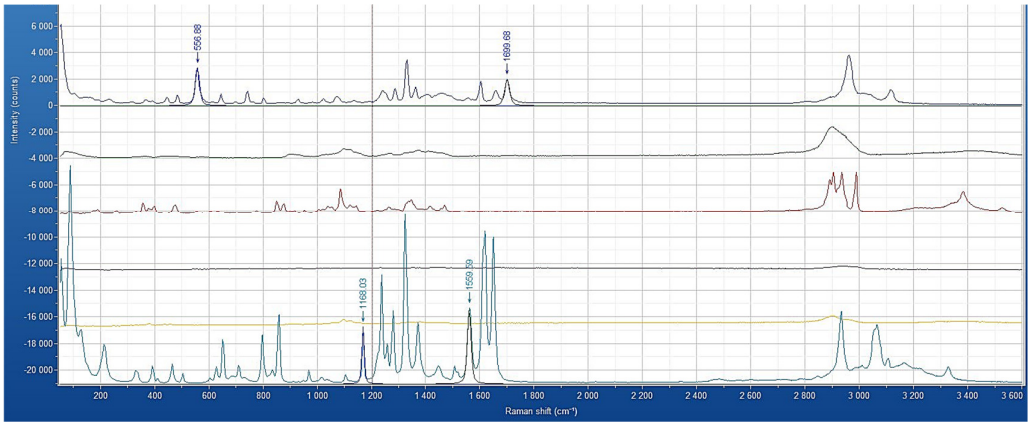


Figure 3.13 Raman shift spectrum of each ingredient. From the top; Caffeine, Croscarmellose, Lactose, PVP K30, Avicel®PH101 and Paracetamol respectively.

Table 3.7 Unique spectrum on the Raman shift of each API

API	First Choice	Second Choice
Paracetamol	1168.4 cm^{-1}	1560.0 cm^{-1}
Caffeine	556.04 cm^{-1}	1699.9 cm^{-1}

Eventually, the first choice of spectrum; 556.04 cm^{-1} for Caffeine and 1168.4 cm^{-1} for Paracetamol will be used of detection of API on the tablet surface.

b) API Mapping

Raman Mapping of tablet surface was applied to Batch 3018 and 3014. The spectrum at 1168.4 cm^{-1} and 556.04 cm^{-1} were used to interpret the uniformity of content of Paracetamol and Caffeine respectively. The determination from divided areas (method describe in 2.3.6) showed in Figure 3.14 for Batch 3018 and Figure 3.15 for Batch 3014. Red refers to high concentration of active component at particular wavelength, and blue refers to low amount detected at that area. Bar was added at the right bottom area to scale each picture.

It was obviously seen that some dark red areas which contained high concentration of API occurred in the result of all batches. However, high-content API showed to be granule shape which were illustrated from microscope in Figure 3.6 and 3.7; therefore, improvement of configuration is still required to make smaller granule and therefore more uniform API showed by Raman mapping.

According to Raman mapping (Figure 3.14 and 3.15) some regions present an intense response with an area which likely to be rod-shaped granule (Figure 3.7) It could tell that API was still agglomerate due to moderate distribution during granulation. Consequently, modifying of screw elements to be reversed kneading elements seems to be one solution for this issue.

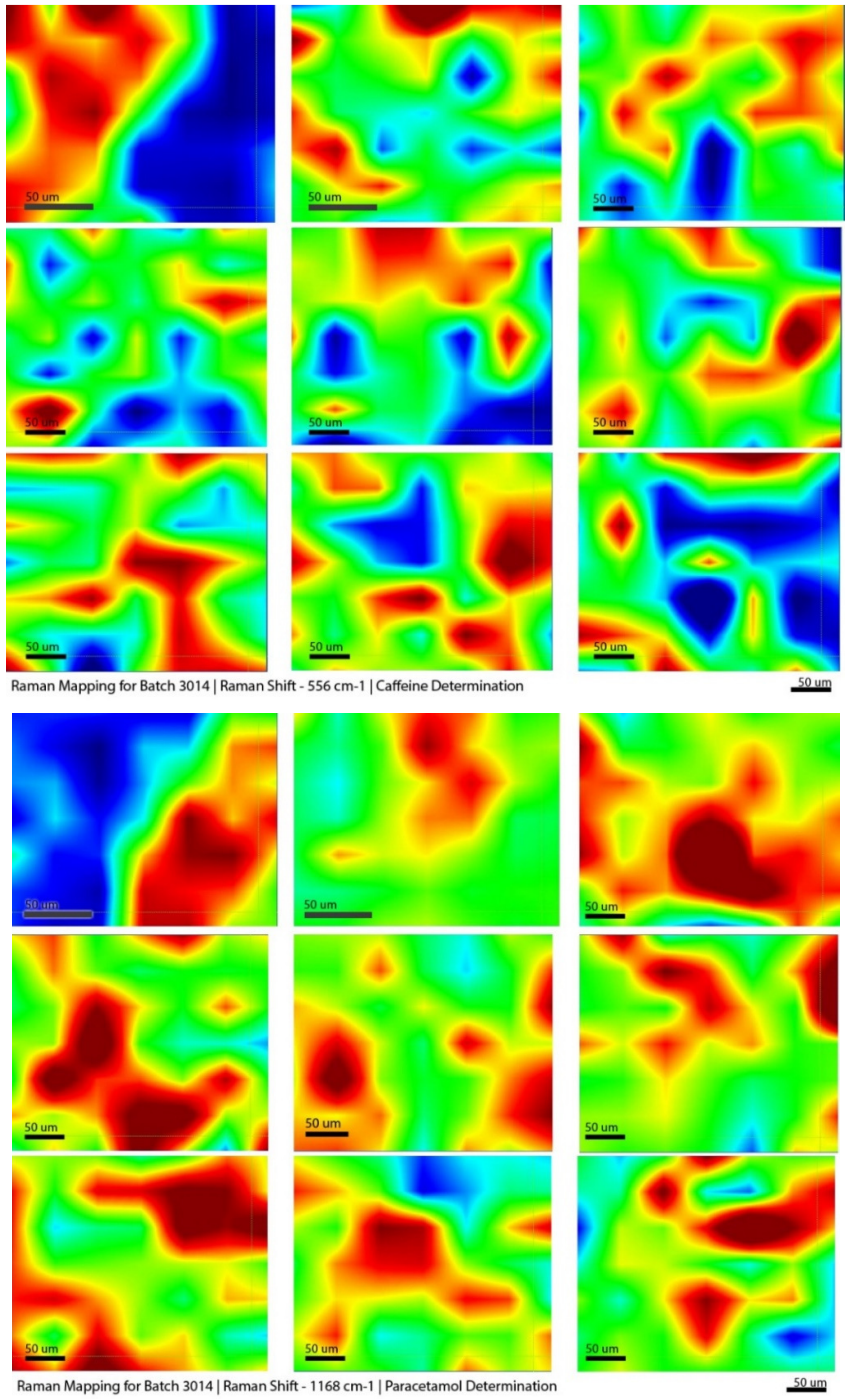
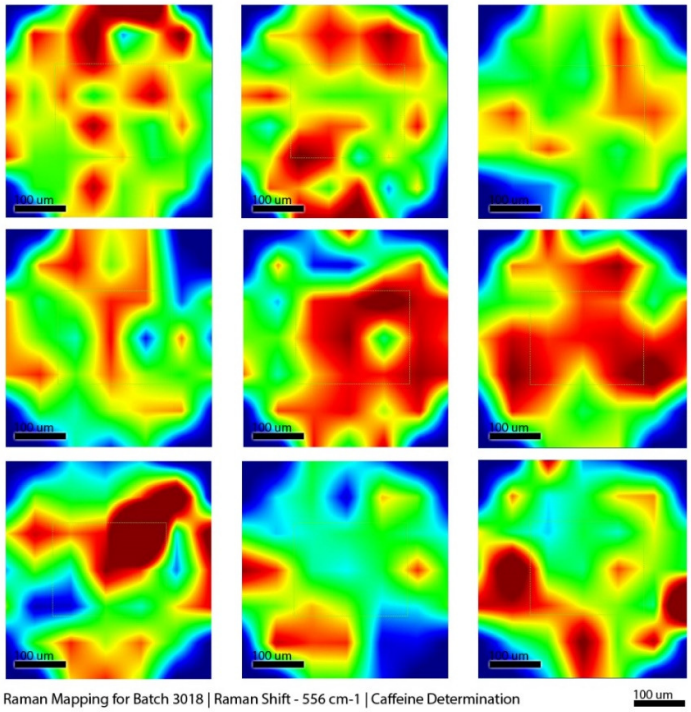


Figure 3.14 Raman Mapping for Batch 3014, Caffeine (Top) and Paracetamol (Bottom).



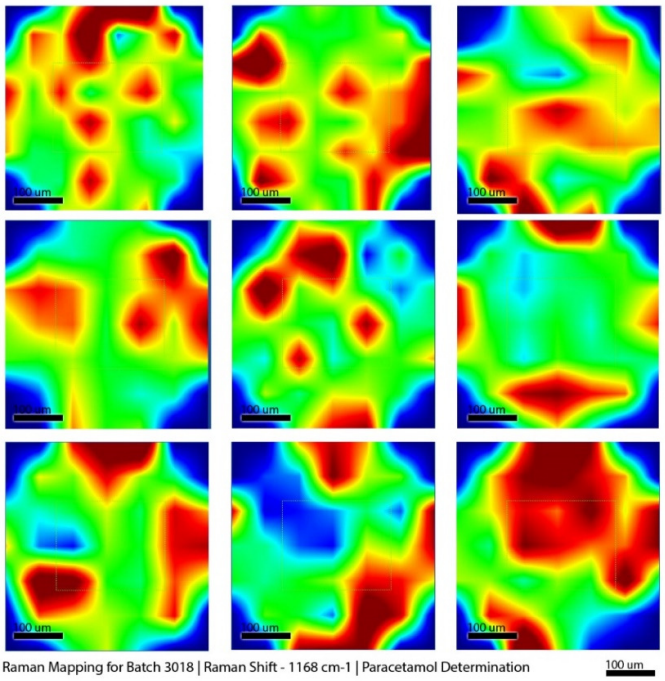


Figure 3. 15 Raman Mapping for Batch 3018, Caffeine (Top) and Paracetamol (Bottom).

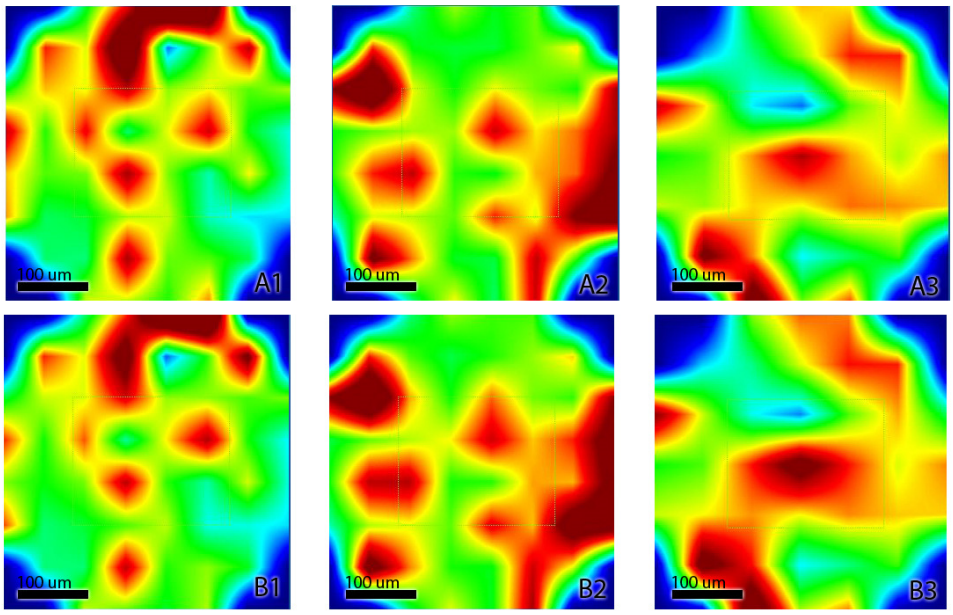


Figure 3.16 Raman Mapping of Batch 3018 for Paracetamol at the region 1-3; Top - mapping of the first choice wavelength (1168.4 cm^{-1}), Bottom - mapping of the second choice wavelength (1560 cm^{-1}).

Scanning of Raman shifting through the API showed the unique API wavelength and can be used for Raman Mapping (Table 3.7). Both Paracetamol and Caffeine presented more than 1 wavelength that can be measured. For Paracetamol, both wavelength showed similar distribution which was showed in figure 3.16. Signal intensity from the second choice wavelength of Caffeine at 1699.9 cm^{-1} appeared to be lower compare to the first choice 556 cm^{-1} but picture was not showed. However, when scale was expanded to compare maps in fine scale (Figure 3.16 bottom), majority of maps display indifference.

Chapter 4 - Conclusion

The main purpose of this project was to involve PAT tools during the research and development steps of tablet manufacturing process along the lines of mixing, granulation and tabletting. Applications could help expanding knowledge space and therefore obtain important process parameters to be used for understanding processesand consequently makes a reasonable adjustment.

For the first step of production; mixing, offline FT- IR was used to ensure the uniformity of mixing and no more information required. Samples were determined to be homogeneous since sampling from three consecutive points at difference position show insignificantly difference via PCA analyser.

Three parameters liquid feeding rate, solid feeding rate and extruder speed were studied during granulation. DoE was employed to find out the best particle size distribution. Eyecon® was equipped to be online analyser showed difference result with QICPIC reference method; however, the gap was eliminated if Eyecon® was calibrated with the condition of usage while granulator is operating. This could be the way to apply Eyecon for real- time monitoring and minimized issue of accuracy. Moreover, NIR was equipped as in- line and PCA was employed as multivariate analyser for spectroscopic data, this combination has potential use for process control during batch production. Unfortunately, variation over acceptable range of T^2 hoteling statistic requires acceptable product attributes to confirm validity of the charge that over-ranged data caused from natural variability. However, PC1 score with T^2 hoteling as acceptable range along batch production has potential to be used as online process monitoring.

Furthermore, this formulation combines 2 API but they were not separately investigated during mixing and granulation due to restriction of time but in finished product by Raman mapping. Each API mapping was generated from its specific wavelength to show uniformly spread over the surface without post- processing procedure. Some areas on the maps show high API intensity with a rod- shape which

displays insufficient uniformity. However, this issue could be solved by modifying screw configuration to be reversed kneading elements.

Although this study projected on a small batch of production, received data will be useful for scale-up study and further study still require to improve product attributes before apply to industrial batch. Moreover, methodology in obtaining scientific-based data could help for solving problem regarding quality attributes of formulation and process and will be beneficial in future for pharmaceutical research and quality assurance field.

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