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A quality by design (QbD) case study on enteric-coated pellets: Screening of critical variables and establishment of design space at laboratory scale

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ABSTRACT

The study aims to prepare naproxen enteric-coated pellets (NAP-ECPs) by fluid-bed coating using QbD principle. Risk assessment was firstly performed by using failure mode and effect analysis (FMEA) methodology. A Plackett–Burman design was then used for assessment of the most important variables affecting enteric-coated pellets characteristics. A Box–Behnken design was subsequently used for investigating the main, interactive, and quadratic effects of these variables on the response. By FMEA we discovered that eight factors should be considered to be high/important risk variables as compared with others. The responses of acid resistance and cumulative drug release were taken as critical quality attributes (CQAs). Pareto ranking analyses indicated that the coating weight gain (X_7), triethyl citrate percentage (X_1) and glycerol monostearate percentage (X_2) were the most significant factors affecting the selected responses out of the eight high-risk variables. Optimization with response surface method (RSM) further fully clarified the relationship between X_7, X_1, X_2 and CQAs, and design space was established based on the constraints set on the responses. Due to the extreme coincidence of the predicted value generated by model with the observed value, the accuracy and robustness of the model were confirmed. It could be concluded that a promising NAP-ECPs was successfully designed using QbD approach in a laboratory scale.

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1. Introduction

Pharmaceutical development involves traditional and systematic approaches. The former mainly depends on empirical evaluation of product and process performance, which pays little attention to the impact of multivariable on formulation and the process understanding. Product quality is identified mainly by restricting flexibility in the manufacturing process and end product testing (so called quality-by-testing, QbT) [1].

There exist some lags to ensure product quality during the pharmaceutical development. The latter is pharmaceutical Quality by design (QbD) philosophy, which is a more scientific, risk-based, holistic and proactive approach with proper feed-forward and feed-back control strategies to pharmaceutical development. The approach builds quality into product during the pharmaceutical development but not merely testing for it [2], which helps to thoroughly understand the root-cause, such as critical material attributes and process parameters that impacting the predefined quality attributes [3]. The following equation clearly illustrates what affects the product quality: Pharmaceutical Quality = F (drug substance, excipients, manufacturing, packaging, et al). The function F in the equation contributes to understand how the formulation variables and process parameters influence the end product profile [3], hence developing accurate and robust product. Building from the QbD paradigm, methods can be used upstream at the beginning stages of the research, development and design phases [4], meanwhile the product quality should be proactively controlled in the manufacturing process. By developing a product with QbD principles, end product testing would be only used for the confirmation of product quality [1].

QbD is concerned with the certain predictable quality through linking the critical material attributes (CMAs) and critical process parameters (CPPs) into the critical quality attributes (CQAs) of drug product. The application of QbD concept in pharmaceutical development is presented graphically in Fig. 1. Firstly, the potential risk factors are determined by risk assessment in the initial design during product development. Then, to improve process knowledge, multivariate experiments are carried out using design of experiments (DOE). DOE method can link the inputs to the outputs,

as such, the relationships between CPPs and CQAs are well understood in mathematical form and the design space (DS) is further established [5]. The most common used of DOE is Plackett—Burman, which can quickly screen the main factors among numerous inputs variables [6]. However, the disadvantage of Plackett—Burman design is that interactions between variables are generally confounded and cannot be easily determined, as there are not enough degrees of freedom. Box—Behnken design were usually used to establish DS for it can determine the variables range which requires fewer runs than a central composite design [7].

Solid dosage form owns a majority share in the market and pellets are achieving increasing attention as multiple units preparations for possessing remarkable advantages, including less effect by gastric emptying rhythm, homogeneous distribution in gastrointestinal (GI) tract thus maximizing drug absorption, reducing the risk of local GI tract irritation [8,9]. Film coating processes are widely used due to its outstanding functions in oral solid drug delivery system, such as masking unpleasant taste, improving stability, enhancing appearance, adding an active compound and controlling release rate [10]. Based on the above, the coating pellets dosage form is compelling and desirable, besides, and the fluid-bed technique is a valuable approach to obtain coating pellets. Enteric-coating is the most common method for manufacturing oral solid preparation especially when the drug acid stability/dissolution or irritation to gastric mucosa is an issue. The coating pellets process consists of two phases: firstly, the pellets core containing drug should be obtained, and then the pellets are coated with enteric-coating materials. To date, the application of QbD for pharmaceutical development of enteric-coated pellets has yet been reported. Naproxen (NAP),
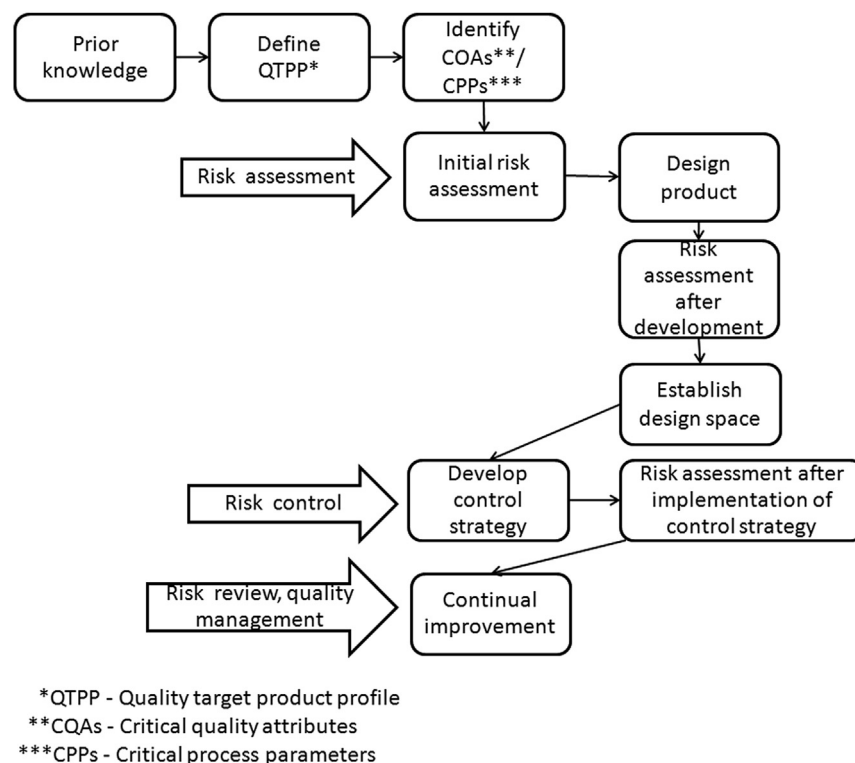

*QTPP - Quality target product profile
**CQAs - Critical quality attributes
***CPPs - Critical process parameters

Fig. 1 — QbD, risk management and quality management in formulation development.

a non-steroidal anti-inflammatory drug (NSAID), the solubility of which has a positive association of pH, meanwhile along with local gastric irritation, is used as a model compound to develop Naproxen enteric-coated pellets (NAP-ECPs). In previous work, we had successfully prepared NAP-loaded immediate-release pellets (NAP-IRPs) starter cores by extrusion-spheronization by using QbD concept (the related contents are to be published).

The ultimate purpose of our study is to comprehensively link the CQAs, variables among DOE batches, to upstream manufacturing process and material attributes so as to identify factors affecting the CQAs. The elements in QbD are fourfold: (1) a risk assessment was performed to identify the main variables influencing the selected quality attributes of NAP-ECPs; (2) a Plackett–Burman screening design was used to determine the most significant factors affecting formulation composition and process parameters on acid resistance (Y_1) and cumulative drug release (Y_2); (3) a Box–Behnken optimization design was applied in the response surface method (RSM) study to obtain the exact relationship between the preparation CQAs and various factors. DS was established following the obtained response surface, control space (CS) was further achieved. (4) moreover, verification experiments were carried out to identify the robustness and accuracy of the generated model.

2. Material and methods

2.1. Material

Naproxen (Volume average particle diameter of 32.822 μm) was purchased from Zhejiang Charioteer Pharmaceutical Co. Ltd. (Taizhou, China). Naproxen standard was purchased from National Institutes for Food and Drug Control (Beijing, China). Microcrystalline cellulose (MCC, Avicel® PH 101) and Croscarmellose Sodium (CCMC-Na, Ac-Di-Sol®SD-711NF) were kindly donated from FMC Biopolymer (Newark, U.S.A.). Lactose Monohydrate (GranuLac®200) and Polyvinylpyrrolidone (PVP K29-32) were gifts from Meggle GmbH (Wasserburg, Germany) and by China Division, ISP Chemicals Co. Ltd (Shanghai, China), respectively. Tween-80 was the product of Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Triethyl citrate were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). Glycerol monostearate were purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). Eudragit L30D-55 was kindly provided by Evonik Industries (Darmstadt, Germany). Hydrochloric acid was purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). Sodium phosphate dibasic were purchased from Shanghai Ling Feng Chemical Reagent Co. Ltd (Shanghai, China). Sodium dodecyl sulfate were purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China).

2.2. Experimental methods

2.2.1. Preparation of NAP-IRPs starter cores
NAP-IRPs starter cores were prepared by extrusion-spheronization method. Briefly, NAP, MCC, PVP, GranuLac®200, CCMC-Na and Tween-80 were uniformly mixed at

the weight ratio of 17.00: 9.00: 1.80: 5.78: 1.19: 0.17. Distilled water was then added in a slow manner and mixed until a homogeneous and cohesive wet mass was obtained. The wet mass was then extruded and spheronized in an extrusion-spheronization apparatus (JBZ-300 multifunctional pelleting and coating machine, YILIAN new-drug research institute, China). The resulting pellets were dried in hot air oven at 40 °C for 24 h, afterwards screened through 24–30 meshes.

2.2.2. Preparation of NAP-ECPs
Adding Tween-80 and TEC to the 40% of the total amount deionized water which is preheated to 80 °C and the mixture was dispersed 2 min at 6000 rpm by homogenizer (XHF-D high-speed homogenizer, Ningbo Scientz Biotechnology Co. Ltd, China). Then GMS was added and being dispersed 8 min at 6000 rpm under 80 °C water bath, following by adding the residual water and continuously stirred to the ambient temperature. Thereafter, the above suspended liquid was added to Eudragit L30D-55 aqueous dispersion slowly, stirring at middle rate for 1 h. Prior to the film coating procedure, the blend aqueous dispersion was sieved with a 60 meshes. Deposition of enteric materials on the NAP-IRPs starter cores was performed in a fluid-bed granulator and coater (JHQ-100, Shenyang, China). And next coating aqueous dispersion was bottom-sprayed onto NAP-IRPs starter cores from a 1.0 mm diameter nozzle attached to a peristaltic pump (HL-2, Shanghai, China) under the condition of 31–32 °C coating temperature, 100–150 ml min^{-1} air flow rate, besides, the various spray rate and atomizing pressure by reference to Plackett–Burman design. The coating weight gain was calculated using the following equation F = (Wb − Wa)/Wa × 100%. In which, Wa and Wb are the pellets weight before and after coating respectively. The content of coating pellets was 47.92%. NAP-ECPs containing 250 mg of NAP were sealed in hard gelatin capsules with a manual capsule filling machine (CapsulCN, Zhejiang, China).

2.2.3. Determination of NAP
Concentrations of NAP in dissolution medium were quantified by UV–visible spectroscopy (spectrophotometer WFZ UV-2000, Nanjing, China) at wavelengths of 331 nm. The linearity of the method was studied in the range of the drug concentration 2.0–120.0 μg/ml ($r = 0.9997$). The RSD of the intraday and interday precision for NAP were less than 2%. The recovery rates for NAP were in the range of 98–102%, and the RSD were below 2%.

2.2.4. In vitro dissolution study of the NAP-ECPs
Dissolution studies were carried out according to USP 34 XXIII, apparatus II paddle method at a rotation speed of 50 rpm and the temperature was maintained at 37 ± 0.5 °C. In brief, the total duration of dissolution was 2 h and 45 min. During the first 2 h, the preparations were subjected to simulated gastric media [0.1 M HCl solution containing 0.5% sodium lauryl sulfate (SLS)] and during the later 45 min, the preparations underwent simulated intestinal media (Buffer pH 6.8). Acid stage: the dissolution medium was 300 mL of 0.1 M HCl solution containing 0.5% SLS. Each NAP-ECPs capsule containing 250 mg NAP was put into each vessel. After 2 h, 5 mL of the

dissolution sample was withdrawn and substituted with the same amount of fresh dissolution medium. The samples were filtered through 0.45 μm filter and analyzed by UV spectrophotometer at 331 nm. Buffer stage: After 2 h operation in the acid stage, 600 ml of 0.1 M K_2HPO_4 preheated to 37 °C was immediately added into the previous fluid. If necessary, adjust by 1 M HCl or 1 M NaOH to a pH of 6.8 ± 0.05 (PBS 6.8). The operation was continued for 45 min. At the end of 45 min, 5 mL of the dissolution sample was withdrawn and passed through 0.45 μm filter and analyzed by UV–visible method for NAP as described above.

2.2.5. Risk assessment

Fish-bone diagram was constructed to identify the potential risks and corresponding causes. Specifically, acid resistance and cumulative drug release were identified as the two CQAs. Based on previous knowledge and initial experimental data, failure mode and effect analysis (FMEA) method were further applied in the risk analysis of the parameters of the pellets coating. Each variable (potential failure mode) was scored in terms of severity (S), detectability (D) and probability (P). More broadly, Severity is a measure of the possible consequences of a failure mode affecting on the safety and efficacy of the final product. Detectability defined that a failure mode can be detected. The final parameter probability is considered as the occurrence probability or the likelihood of a failure. For each risk, S, D, P scores were multiplied together to produce a "Risk Priority Number" (RPN), RPN = S × D × P, which represents the overall magnitude of the risk. We ranked S, D and P of 5 as worst-case, 1 as best-case value and 3 as moderate-case value, and then a maximum RPN of 125 and a minimum RPN of 1 are possible.

The RPN threshold was set at 60, and any formulation variable or process parameter with an RPN 60 or above was regarded as a potential critical factor, that is, potential risks are evaluated by subsequent process characterization studies since it possibly has a potential impact on CQAs and in consequence on product safety and efficacy, while factors with a lower RPN can be eliminated from further study [11].

2.2.6. Plackett–Burman design screening study

Based on the risk assessment results, Plackett–Burman study was used to screen significant factors influencing selected CQAs. The coating aqueous dispersion always contained Tween-80 as emulsifier which was 40% of the GMS amount. The Plackett–Burman design screening study with each factor evaluated at low (−1) and high (+1) levels were summarized in Table 1. The determination of the low and high values was derived from the preliminary study results. The responses evaluated were Y_1 and Y_2.

2.2.7. Box–Behnken design optimization study

Relied on the results of the Plackett–Burman screening study, RSM were applied in order to rapidly achieve the optimal NAP-ECPs with Design Expert software (Version 8.0.6). Box–Behnken design was specifically selected here for aforementioned reasons of requiring fewer runs than a central composite design [7]. The DOE details were listed in Table 2.

The relationship between the material attributes/process parameters and CQAs was delineated in the DS. DS was determined from the common region of successful operating ranges for multiple CQAs (Table 2). The successful operating ranges for the Y_1, Y_2, were determined $Y_1 \leq 10\%$ and $Y_2 \geq 80\%$, respectively. Based on the prior knowledge space, the CS was also determined. It is expected that operation within the CS will result in a product possessing the desired CQAs.

2.2.8. Confirmation tests of model

To verify the accuracy and robustness of the model, three different combinations were got at low, medium or high levels of the selected factors within CS. Formulations at those compositions were analyzed and further compared the observed responses with the predicted.

2.2.9. Statistical analyses

The results of Plackett–Burman study were analyzed via the statistical software of Minitab (version 16.1.0), the influence of each parameter on the responses was demonstrated in the constructed Pareto charts, in which the length of each bar stood for the magnitude of the impact on the response.

Table 1 – Plackett–Burman screening Design of Experiments and their results. X_1: TEC percentage; X_2: GMS percentage; X_3: spray rate; X_4: atomizing pressure; X_5: batch size; X_6: coating aqueous dispersion solid content; X_7: coating weight gain; X_8: curing time. The response acid resistance (Y_1) and drug cumulative release (Y_2) were reported as mean ± SD.

ID	Pattern	X_1 (%)	X_2 (%)	X_3 (ml/min)	X_4 (MPa)	X_5 (g)	X_6 (%)	X_7 (%)	X_8 (min)	Y_1 (%)	Y_2 (%)
PB-1	+−+−−++	16	3	0.25	0.02	5	15	60	120	8.78 ± 0.14	75.20 ± 1.02
PB-2	++−+−−−+	16	10	0.15	0.04	5	15	20	120	12.30 ± 0.32	86.60 ± 1.80
PB-3	−++−+−−−	10	10	0.25	0.02	8	15	20	15	11.93 ± 0.28	83.16 ± 1.23
PB-4	+−++−+−−	16	3	0.25	0.04	5	25	20	15	12.14 ± 0.22	85.54 ± 1.45
PB-5	++−++−+−	16	10	0.15	0.04	8	15	60	15	9.07 ± 0.20	79.04 ± 1.66
PB-6	+++−++−+	16	10	0.25	0.02	8	25	20	120	12.47 ± 0.19	90.11 ± 1.57
PB-7	−+++−++−	10	10	0.25	0.04	5	25	60	15	6.79 ± 0.33	78.34 ± 1.48
PB-8	−−+++−++	10	3	0.25	0.04	8	15	60	120	3.37 ± 0.25	72.47 ± 0.97
PB-9	−−−+++−+	10	3	0.15	0.04	8	25	20	120	6.42 ± 0.24	80.95 ± 1.15
PB-10	+−−−+++−	16	3	0.15	0.02	8	25	60	15	5.44 ± 0.22	78.18 ± 1.61
PB-11	−+−−−+++	10	10	0.15	0.02	5	25	60	120	7.08 ± 0.47	79.10 ± 1.48
PB-12	−−−−−−−	10	3	0.15	0.02	5	15	20	15	7.43 ± 0.25	83.57 ± 1.27

+high level, −low level.

Table 2 – Box–Behnken optimization Design of Experiments and their results. Acid resistance (Y_1) and Drug cumulative release (Y_2) were reported as mean $\pm$ SD.

ID	Pattern	Coating weight gain (%)	TEC (%)	GMS (%)	Y1 (%)	Y2 (%)
		X_7	X_1	X_2	Y_1	Y_2
BB-1	+−0	60.00	10.00	6.50	4.51 $\pm$ 0.20	56.17 $\pm$ 1.47
BB-2	0+−	37.50	16.00	3.00	8.29 $\pm$ 0.35	89.04 $\pm$ 1.49
BB-3	0−+	37.50	10.00	10.00	7.59 $\pm$ 0.31	79.87 $\pm$ 1.30
BB-4	000	37.50	13.00	6.50	8.05 $\pm$ 0.25	82.76 $\pm$ 1.15
BB-5	++0	60.00	16.00	6.50	6.47 $\pm$ 0.20	70.22 $\pm$ 1.21
BB-6	0++	37.50	16.00	10.00	10.03 $\pm$ 0.28	93.37 $\pm$ 1.32
BB-7	−−0	15.00	10.00	6.50	11.46 $\pm$ 0.22	95.12 $\pm$ 1.30
BB-8	−+0	15.00	16.00	6.50	13.12 $\pm$ 0.31	100.13 $\pm$ 0.82
BB-9	−0−	15.00	13.00	3.00	12.33 $\pm$ 0.26	97.33 $\pm$ 1.31
BB-10	−0+	15.00	13.00	10.00	12.63 $\pm$ 0.28	98.14 $\pm$ 1.10
BB-11	+0+	60.00	13.00	10.00	6.32 $\pm$ 0.18	65.52 $\pm$ 1.35
BB-12	0−−	37.50	10.00	3.00	6.85 $\pm$ 0.23	75.46 $\pm$ 1.26
BB-13	000	37.50	13.00	6.50	8.14 $\pm$ 0.21	84.21 $\pm$ 1.33
BB-14	000	37.50	13.00	6.50	7.76 $\pm$ 0.23	83.14 $\pm$ 1.20
BB-15	+0−	60.00	13.00	3.00	5.35 $\pm$ 0.20	60.02 $\pm$ 1.24

For Box–Behnken analyses, the regression equation describes the effects of the variables on the responses in terms of linear, interactive and quadratic. The equation followed as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where, b_0 is intercepted, and Y is the measured response associated with the factors (X_1, X_2 and X_3), their interactions (X_1X_2, X_1X_3 and X_2X_3) and quadratic (X_1^2, X_2^2 and X_3^2). The p-values related to the regression coefficients indicated the significance of the factors on the response. ANOVA and the coefficient of determination (R^2) were also applied to determine the suitability of the model [12].

3. Results and discussion

3.1. Risk assessment

Risk assessment aims to obtain all the potential high impact factors which will be subjected to a DOE study to establish a product or process DS. Just like outlined in the ICH Q9 document, risk identification and risk analysis are two basic components of risk assessment [13]. The first step in the risk assessment was to systematically gather up all the possible factors that could influence product quality. Basing on the literature data, previous study experiences [14,15] and preformulation data, fish-bone diagrams [16] was applied to organize hierarchically these factors (see Fig. 2). The RPN scores using FMEA methodology was depicted Fig. 3. To initiate the FMEA, we broke the failure modes down into those coming from the formulation, process, people, environment, and equipment inputs. Except the formulation and process, other factors leading to the variability in product quality were considered to be lower risk since all the development work was conducted under the conditions which were usually fixed by preliminary experiments and/or prior knowledge. In addition, variables that could affect in vivo performance have generally been scored high.

Eight high-risk factors identified in a risk analysis study have potential impact on Y_1 and Y_2 which were taken as indexes evaluating CQAs. These independent factors included: TEC percentage (X_1, compared to polymer), GMS percentage (X_2, compared to polymer), spray rate (X_3), atomizing pressure (X_4), batch size (X_5), coating aqueous dispersion solid content (X_6, amount of all the solid compared to the amount of the coating aqueous dispersion), coating weight gain (X_7) and curing time (X_8), these eight factors would be used for further screening study to obtain the significant factors influencing selected CQAs by Plackett–Burman design.

3.2. Influence of various factors on Y_1 and Y_2 by Plackett–Burman screening DOE

The goal of this study was to identify the most significant factors affecting the CQAs using Plackett–Burman design. Plackett–Burman design can estimate the significance of the main factor from large numbers of factors with very high efficiency and accuracy [17], thus assuring to quickly reduce the number of high-risk factors needed to be studied in the next step. An eight factors-two levels-12 runs Plackett–Burman screening study was performed using Minitab statistical experiment design software and the responses were Y_1 and Y_2.

As learned from Table 1, Y_1 and Y_2 varied from 3.37% (PB-8) to 12.47% (PB-6), and from 72.47% (PB-8) to 90.11% (PB-6), respectively, for the various factor combinations. Fig. 4 indicated that among all of the factors, coating weight gain, TEC percentage and GMS percentage ($P < 0.05$) strikingly influenced acid resistance, while cumulative drug release was significantly impacted by coating weight gain and GMS percentage ($P < 0.05$). As recorded in Table 3, the relative strength of each factor influencing CQAs was further in detail depicted by the "Effect" value. A positive value indicates an effect that helps to enhance the response value; conversely a negative sign value represents an inverse relationship between the response and the factor. The higher the absolute value the greater the effect of that factor on the responses. Lower Y_1 and
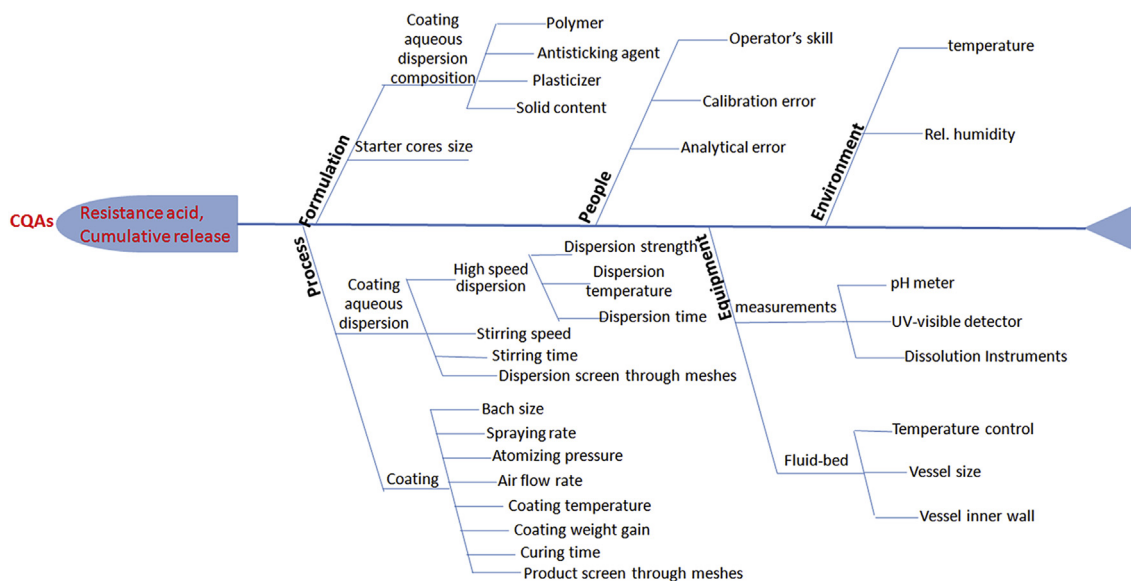
Fig. 2 – An Fish-bone diagram illustrating factors that may have impact on acid resistance and cumulative drug release.

higher Y_2 were desired. In this study, the results demonstrated that decreased TEC/GMS percentage and increased coating weight gain would contribute to lower Y_1. While both increasing the GMS and decreasing the coating weight gain would resulted in the increase of Y_2. Eudragit L30D-55 was enteric-coated material, which dissolves merely in pH 5.5 or above medium. The larger the coating weight gains the thicker of coating film. An attempt to decrease the coating film excessively would result in discontinuous coating film so that leading to too much drug release in the gastric region. In contrary, exorbitant thick film might handicap drug releasing from formulation and Y_2 turned out to be too low. TEC was plasticizer which can decrease the film forming temperature to favor coating. Besides, TEC was also hydrophilic material, which was easily dissolved in water thus playing a pore-forming agent role in the enteric-coated film. For this reason, too high levels of TEC will result in too much drug release in gastric environment, while the low value TEC can't decrease the film forming temperature during coating process in efficiency. GMS was an antisticking agent, which can
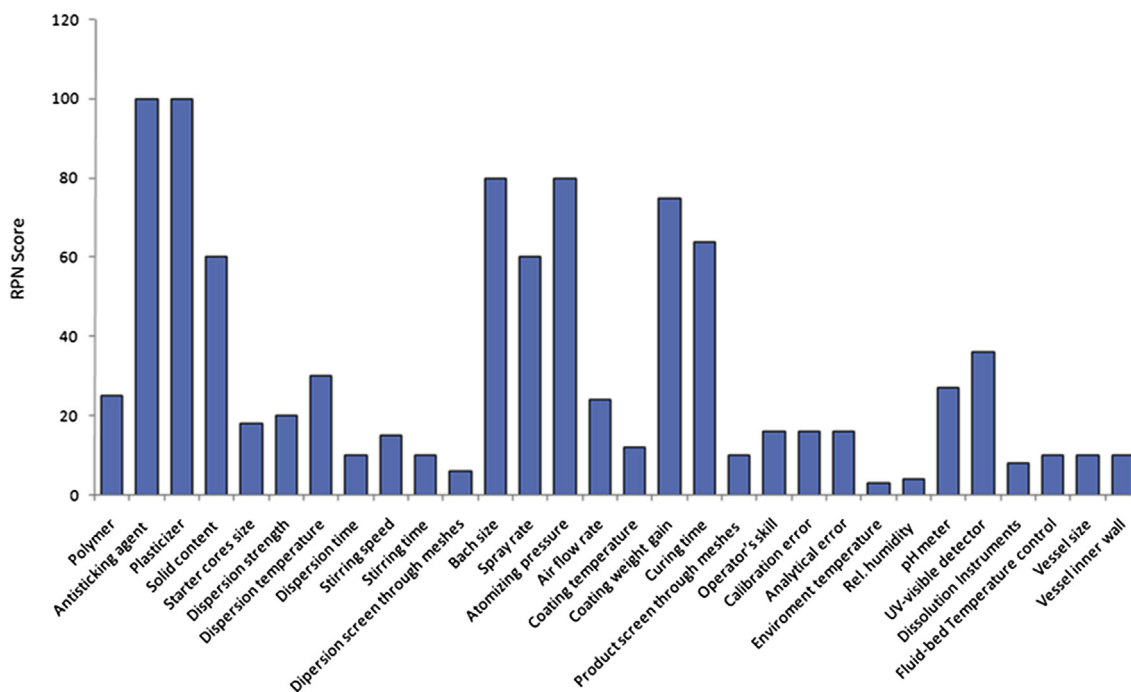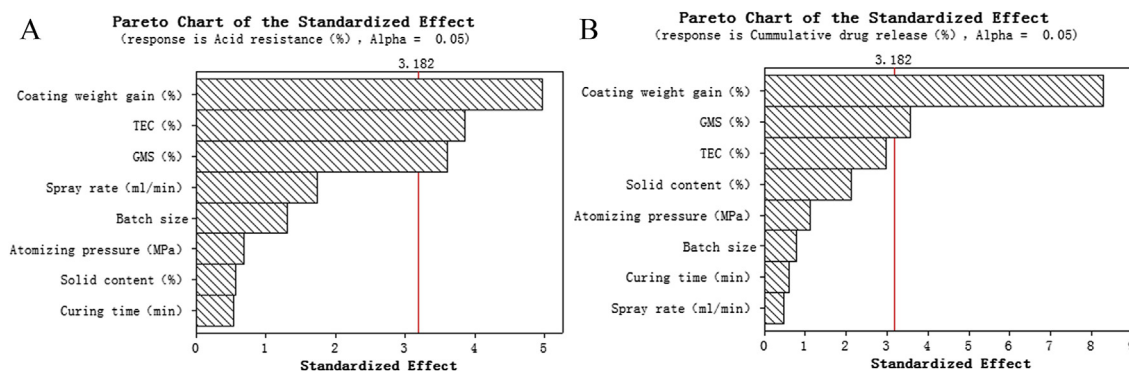

Fig. 3 – Pareto chart showing RPN scores for the operating parameters for ECPs coating process. Parameters that had RPN scores higher than the threshold (RPN = 60) were considered for further experimentation.

Fig. 4 – Standard Pareto charts showing the effects of independent variables on acid resistance and on cumulative drug release.

decrease the viscosity of the coating aqueous dispersion to avoid the adhesion among pellets and it also benefits for coating process. As emulsifier Tween-80 was always accompany with GMS, which was 40% of the amount of GMS. It is well known that Tween-80 is well hydrophilic surfactant, when GMS was in the high content meaning Tween-80 was also in the high levels, for the same reason like TEC which would result in poor product quality. Too low GMS content could not decrease the viscosity of coating aqueous dispersion, but would lead to the failure of the adhesion among pellets during development and coating. A good correlation was obtained between the observed and predicted values as indicated by the R^2 value of 0.9510 and 0.9701for Y_1 and Y_2.

Basing on the result of the screening study, it is concluded that the responses were impacted significantly by coating weight gain, TEC percentage and GMS percentage. These three parameters were further examined for their interactions and their effects on product quality attributes via the Box–Behnken DOE. From the Coef. of Table 3, it inferred that spray rate has an inverse impact on the Y_1 and Y_2, and which was at a low value favoring to improve product quality; all of the residual factors of atomizing pressure, batch size, coating aqueous dispersion solid content and curing time were found to be less significant and hence kept at 0.02 MPa, 5 g, 20%, and 15 min, respectively, considering cost and time saving in the next phase.

3.3. The responses surface for both Y_1 and Y_2 were obtained by Box–Behnken DOE

This study aimed at understanding the effects and interactions between coating weight gain, TEC percentage and GMS percentage on Y_1 and Y_2. The levels used for selected parameters and the experimental results are listed in Table 2. As shown in Table 2, Y_1 and Y_2 varied from 4.51% to 13.12% and from 56.17% to 100.13%, respectively. Table 4 illustrated the statistical analysis results using Design Expert software; the values of the regression coefficients (coded) of the variables are associated with the influence on the CQAs. The largest part of the absolute values for the coefficients (coded) meant the variables had the most potential effect on the response. Analysis of variance (ANOVA) was performed to evaluate the model significance. A model will be considered statistically significant if the P-value represented by "Prob > F" is 0.05 or less. F-ratio is the "mean square between" divided by the "mean square within". The low value of the F-ratio's means more errors in the model. The adequacy of the developed models were estimated by "lack of fit", R^2, adjust R^2 [R^2 (adj)] and predicted R^2 [R^2 (pred)]. The "lack of fit" estimates the error variance independently of the model. A significant "Lack of Fit" ($P > 0.05$) indicates that the variability measured by the replicates does not explain the gap between predicted and experimental data points. The R^2 value is the maximum

Term	Effect		Coef		Std Err Coef		T		P	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Constant			8.602	81.022	0.3719	0.4784	23.13	169.37	0	0
TEC	2.863	2.847	1.432	1.423	0.3719	0.4784	3.85	2.98	0.031*	0.059
GMS	2.677	3.407	1.338	1.703	0.3719	0.4784	3.6	3.56	0.037*	0.038*
Spray rate	1.29	−0.437	0.645	−0.218	0.3719	0.4784	1.73	−0.46	0.181	0.679
Atomizing pressure	−0.507	−1.063	−0.253	−0.532	0.3719	0.4784	−0.68	−1.11	0.545	0.347
Batch size	−0.97	−0.74	−0.485	−0.37	0.3719	0.4784	−1.3	−0.77	0.283	0.496
Solid content	−0.423	2.03	−0.212	1.015	0.3719	0.4784	−0.57	2.12	0.609	0.124
Coating weight gain	−3.693	−7.933	−1.847	−3.967	0.3719	0.4784	−4.96	−8.29	0.016*	0.004*
Curing time	−0.397	−0.567	−0.198	−0.283	0.3719	0.4784	−0.53	−0.59	0.631	0.595

Table 3 – Estimated effects and coefficients for acid resistance (Y_1) and for cumulative drug release (Y_2) (coded units).

*Means P-value is less than the a priori value of 0.05 and is statistically significant.

Table 4 – Estimated regression coefficients for acid resistance and for cumulative drug release (Quadratic).

Term	Coefficient (coded)		SE Coef (coded)		Coefficient (uncoded)		F-ratio		P	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Constant	7.98	83.37	0.14	0.82	14.57395	106.71152	202.57	153.64	< 0.0001*	< 0.0001*
X_7	−3.36	−17.35	0.084	0.5	−0.31649	−0.77402	1600.61	1197.7	< 0.0001*	< 0.0001*
X_1	0.94	5.77	0.084	0.5	0.20394	−1.08651	124.52	132.37	0.0001*	< 0.0001*
X_2	0.47	1.88	0.084	0.5	−0.50696	−0.4776	31.13	14.08	0.0025*	0.0133*
$X_7{}^*X_1$	0.075	2.26	0.12	0.71	1.11E−03	0.033481	0.4	10.16	0.5556	0.0243*
$X_7{}^*X_2$	0.17	1.17	0.12	0.71	2.13E−03	0.014889	1.99	2.74	0.2177	0.1591
$X_1{}^*X_2$	0.25	−0.02	0.12	0.71	0.02381	−1.90E−03	4.43	7.96E−04	0.0893	0.9786
X_7^2	0.94	−3.57	0.12	0.74	1.85E−03	−7.05E−03	57.42	23.42	0.0006*	0.0047*
X_1^2	−0.03	0.61	0.12	0.74	−3.38E−03	0.067917	0.06	0.69	0.8155	0.4454
X_2^2	0.24	0.45	0.12	0.74	0.019354	0.037041	3.68	0.38	0.1133	0.5655

*Means P-value is less than the a priori value of 0.05 and is statistically significant.

squared regression coefficient that can be achieved by a model using only the variables in it, which is an indication of how well the model fits the experimental data, and the closer the value is to 1, the better the model is. The R^2 (adj) is a modified form of R^2 considering the number of terms used within the model and the R^2 (pred) is an estimation of how well the model predicts a response value. Table 4 demonstrated the suggested most suitable model were quadratic for both responses of Y_1 and Y_2.

In Table 4, it was observed that Y_1 was significantly affected by coating weight gain, TEC percentage and GMS percentage ($P < 0.05$). Analysis of variance (ANOVA) in Table 5 manifested that the model was statistically significant in its prediction of Y_1, as depicted by Prob > F of 0.0032, F-ratio of 20.02 and the Prob > F for "lack of fit" of 0.3882. The model was good with R^2 of 0.9973, and can well depict independent factors on the response (Y_1) with good the R^2 (adj) and the R^2 (pred). Table 4 implied no coating weight gain-TEC percentage-GMS percentage interaction on Y_1 for all of the P-value > 0.05.

The three studied factors were also investigated in Y_2. As learned from regression analysis, coating weight gain was inversely correlated with increasing Y_2 ($P < 0.05$) (Table 4). The increase in TEC and GMS percentage was also observed to significantly increase Y_2 ($P < 0.05$). There was a striking interaction between the coating weight gain and the TEC percentage with respect to an increase in the Y_2 ($P < 0.05$). ANOVA statistics elucidated that a "Prob > F" of 0.0205, F-ratio of 8.57 and the Prob > F for "lack of fit" of 0.1639, indicating that the factors in the model were significant. In addition, the model was speculated to well predict the response with R^2, R^2 (adj) and the R^2 (pred) were 0.9964, 0.9899 and 0.9479, respectively.

Counter and response surface plots were also analyzed to visualize the effects of the parameters and their interactions on the responses. Fig. 5 showed the effects of coating weight gain, TEC percentage and GMS percentage on Y_1 and Y_2.

### 3.4. Establishment and evaluation of the DS

DS was defined by the ICH Q8 as "the multidimensional combination and interaction of input variables (material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change; however the movement out of the design space is considered a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to the regulatory assessment and approval" [5]. The DS makes QbD a reality and the wider the DS, the more robust and flexible the process is to accommodate variations. In this study, RSM in conjunction with optimization was applied to establish DS.

The quadratic response surface of CQAs as a function of selected variables was given in Fig. 5. The objective of optimization is to optimize input variables range for meeting a goal. A vital step of optimization is to achieve appropriate response functions for both dependences and independences. In Design Expert, the desirability response values were set $Y_1 \leq 10\%$ and $Y_2 \geq 80\%$. When GMS was at low and high limits set in experiment, Fig. 6A and B showed the proposed DS, comprised of the yellow overlap region of ranges for the two CQAs. As depicted in Fig. 6C. the overlay part of the yellow region in Fig. 6A with B satisfied both $Y_1 \leq 10\%$ and $Y_2 \geq 80\%$, in which GMS was from 3% to 10%. However, coating weight gain and TEC percentage were variables and it was difficult to determine the exact value in real operation during development. Hereby, In order to determine the range of TEC percentage and coating weight gain and to achieve the most

Table 5 – Summary of ANOVA and lack of fit for testing model.

Source	ANOVA parameters							Prob > F
	DF	SS	MS	Prob > F	F-ratio	R2	R2 (adj)	R2 (pred)	(Lack of fit)
Q_{2h}	3	3.39	1.13	0.0032	20.02	0.9973	0.9923	0.9667	0.3882
$Q_{cumulative}$	3	51.66	17.22	0.0205	8.57	0.9964	0.9899	0.9479	0.1639
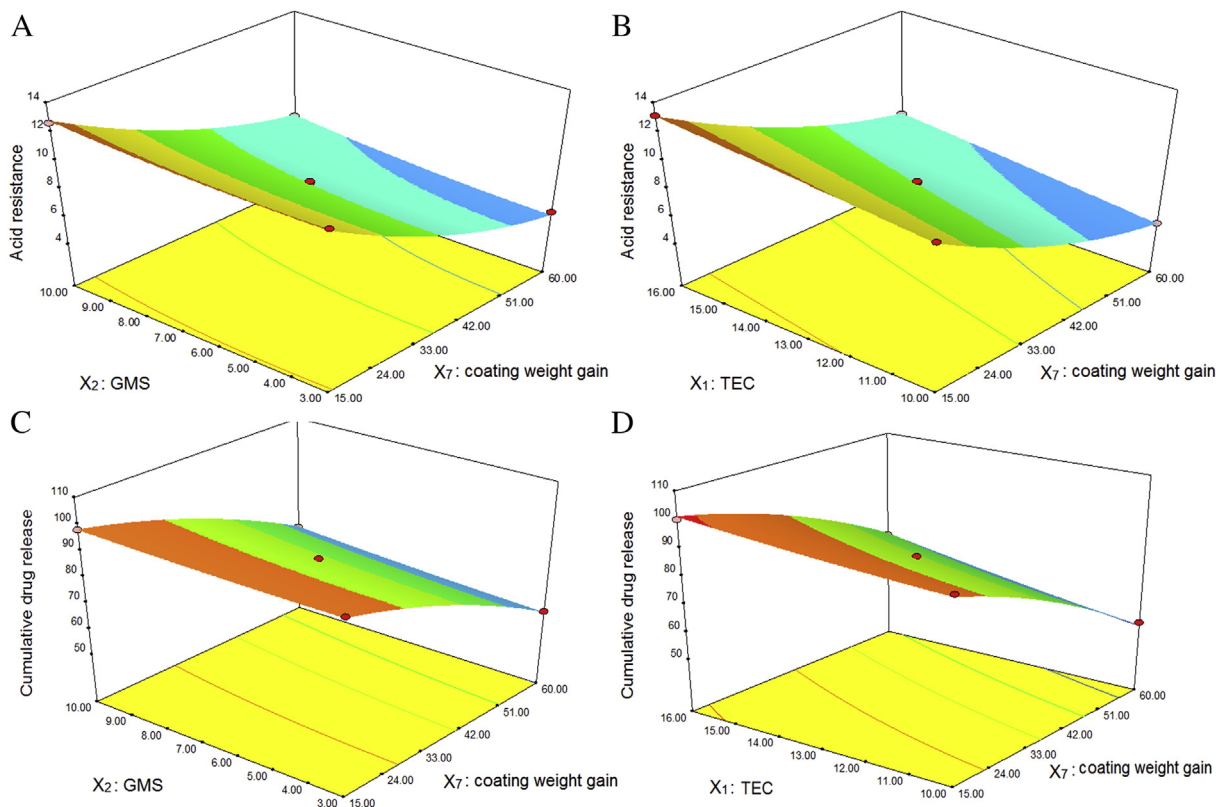
Fig. 5 − Response surface (3D) plot of the effects of variables on the acid resistance and on the cumulative drug release of prepared ECPs. (A) coating weight gain/GMS and (B) coating weight gain/TEC were on the acid resistance; (C) coating weight gain/GMS and (D) coating weight gain/TEC were on cumulative drug release.

robust and flexible product, mathematics knowledge was applied here to develop the relationship between coating weight gain and TEC percentage. Specifically, a biggest inscribed parallelogram was plotted in the orange overlay region shown in Fig. 6D, whose abscissa of X_7 and ordinate of X_1, the four coordinates were (23%, 10%), (33%, 10%), (36.5%, 16%), and (46.5%, 16%), respectively. It was easy to obtain the exact value of X_1 and X_7 inside the parallelogram with math knowledge. The DS was established which was delineated in the orange region in Fig. 6D, the range of the independent was, GMS percentage of 3%−10%, coating weight gain and TEC percentage of the point inside the orange parallelogram region. Known from the variables range of DS, the range of TEC percentage and GMS percentage were thoroughly same with the Box−Behnken DOE. Based on the preformulation experience, levels of TEC and GMS must be in the certain range to ensure that the production was performed favorably. Such results don't mean that the fixed range of variables was too small, but illustrated that the available operation range was wide in manufacture ensuring product quality.

### 3.5. Determination of control strategy of the prepared NAP-ECPs

For ensuring a product of required quality of robustness and consistency during producing, ICH Q10 defines the control strategy as "a planned set of controls, derived from the understanding of current product and process that assures

process performance and product quality" [18]. The normal operating ranges is CS which is defined as the upper and/or lower limits for the critical material attributes and CPP. In the CS, the parameters were routinely controlled during production in order to assure the reproducibility [1]. The acceptable range of both material attributes and process parameters were determined basing on the knowledge space from screening design and DS, the detail information was following as Fig. 7.

3.6. Confirmation tests

To evaluate the accuracy and robustness of the obtained model, a confirmation test was carried out with low, medium and high value of all the eight factors within CS. The model confirmation experiments design and results were shown in Table 6. The results showed that the predicted and observed responses values of the formulations with the different variables combinations were nearly similar. A good agreement was obtained between the model prediction and experimental observation. Thus, the validity of the model was established and the formulation variables and process parameters were robust within the control space.

In QbD, robustness estimation is moved into method optimization for the definition of DS to ensure the CQAs values which were deduced from any working inside the DS are acceptable [19]. Once the DS is established, the validation becomes an exercise to demonstrate that the process will deliver a product of acceptable quality when operating within
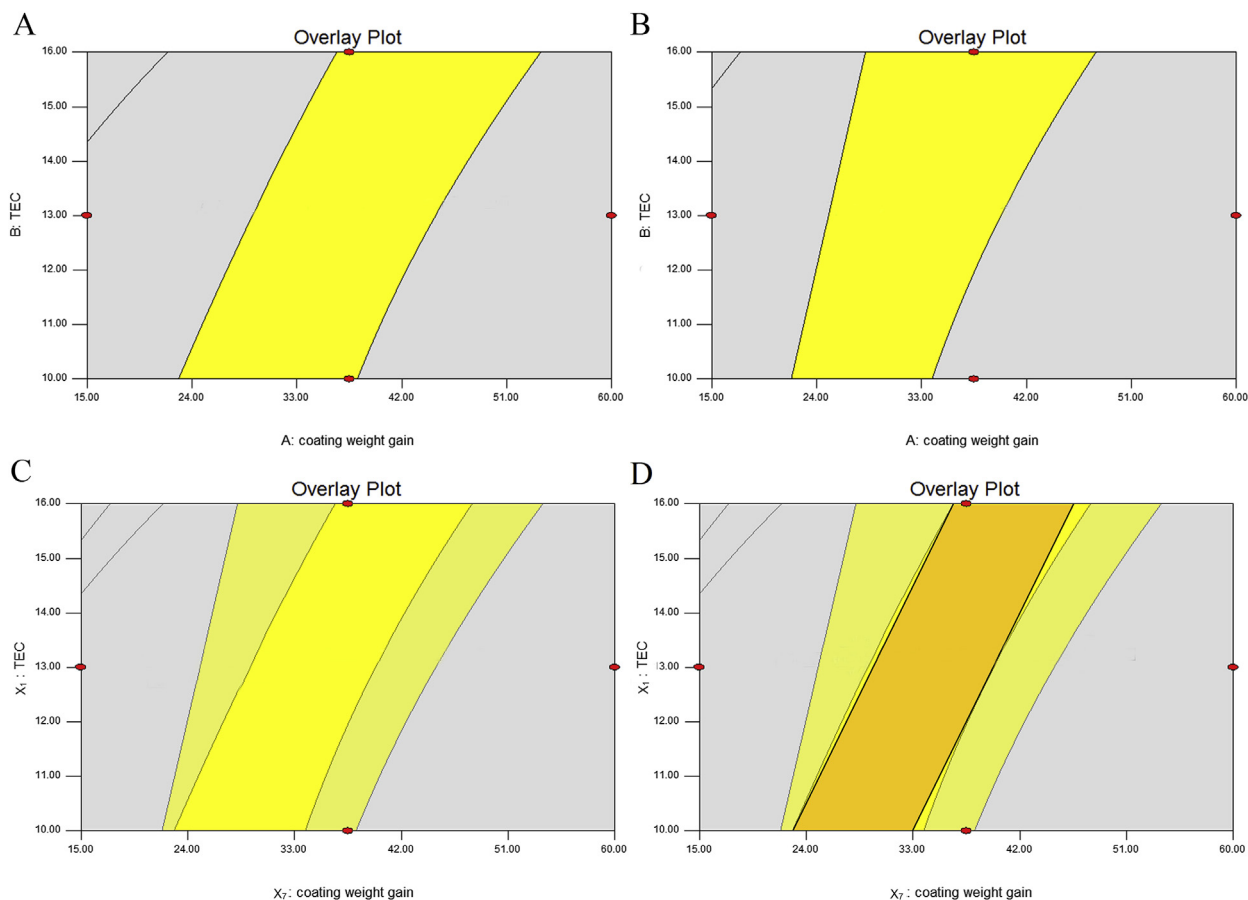
Fig. 6 – Design space of prepared ECPs comprised of the overlap region of ranges for the three CQAs using GMS percentage of (A) 3% and (B) 10%; (C) the theory region and (D) the operating region.
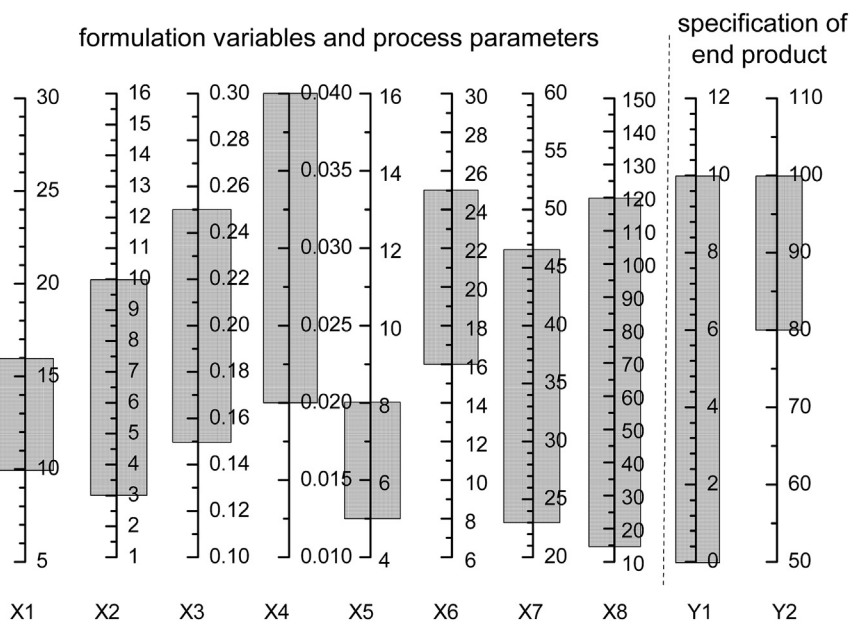
Fig. 7 – The control space of the prepared NAP-ECPs.

Table 6 – Model confirmation experiments design table. X_1: TEC percentage (%w/w); X_2: GMS percentage (%w/w); X_3: spray rate (ml/min); X_4: atomizing pressure (MPa); X_5: batch size (g); X_6: coating aqueous dispersion solid content (%w/w); X_7: coating weight gain (%w/w); X_8: curing time (min). The response acid resistance (Y_1, %) and drug cumulative release (Y_2, %) were reported as mean ± SD for experimental value.

Level	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Y_1		Y_2	
									Predicted	Experimental	Predicted	Experimental
Low	10	3	0.15	0.02	5	15	23	15	9.70	9.62 ± 0.14	88.68	89.36 ± 1.36
Medium	13	6.5	0.2	0.03	6.5	20	34.75	67.5	8.34	8.77 ± 9.15	85.44	84.64 ± 1.28
High	16	10	0.25	0.04	8	25	46.5	120	8.64	8.66 ± 0.17	85.93	85.05 ± 1.34

the DS [20]. No current regulatory document provides guide-lines on how to estimate the DS level [19] and no new concept exists to implement a control strategy in the pharmaceutical industry [21]. Within our approach, basing on the initial risk assessment and DS, a CS created with all the eight factors was involved. The confirmation tests illustrated that both the un-important factors had little effect on the selected CQAs and the robustness of the model.

4. Conclusion

This current case study demonstrated how QbD approach can be applied toward the development of the ECPs preparation. Fish-bone paragraph and FMEA analysis favors to identify critical formulation and process parameters that affect ECPs product quality. And next, the Plackett–Burman and Box–Behnken design were used for screening the significant factors and optimizing the variables range, respectively. The final aim of this approach is to achieve a process model of the ECPs preparation, thus a DS can be established based on it, and a CS could be further obtained. Confirmation tests were carried out at three levels of low, medium and high of the variables and the results manifested that the prediction and experimental observation were in a good agreement, which confirmed the accuracy and robustness of the model.

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