Chapter 2

Establishment of a Design Space for Biopharmaceutical Purification Processes Using DoE

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Abstract

Recent trends in the pharmaceutical sector are changing the way protein purification processes are designed and executed, moving from operating the process in a fixed point to allowing a permissible region in the operating space known as design space. This trend is driving product development to design quality into the manufacturing process (Quality by Design) and not to rely exclusively on testing quality in the product. A typical purification step has numerous operating parameters that can impact its performance. Therefore, optimization and robustness analysis in purification processes can be time-consuming since they are mainly grounded on experimental work. A valuable approach consists in the combination of an adequate risk analysis technique for selecting the relevant factors influencing process performance and the design of experiment methodology. The latter allows for many process variables which can be studied at the same time; thus, the number of tests will be reduced in comparison with the conventional approach based on trial and error. These multivariate studies permit a detailed exploration in the experimental range and lay the foundation of Quality by Design principles application. This article outlines a recommended sequence of activities toward the establishment of an expanded design space for a purification process.

Key words Process optimization, Process robustness, Independent variables, Response, Response surface methodology, Experimental design, Design space, Design of experiment (DoE), Quality by design (QbD)

1 Introduction

In recent years, the pharmaceutical sector has undergone a dramatic change in the way protein pharmaceuticals are being designed and processes operated. These changes are triggered in part by the guidance documents released by the FDA [1, 2], encouraging practitioners to design and validate their processes not only at one fixed processing condition but also in a range of process conditions referred to as the design space. In this context, professionals need to provide evidence that the chosen design space will result in an acceptable product for the patient, keeping safety and efficacy as the foremost priority. This concept is referred to as Quality by Design (QbD). The evidence to be provided is the scientific

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understanding of the driving forces acting upon the complex network of interactions between materials, process, and product [3].

In order to do so, product and process knowledge is required, including the understanding of process variability and the relationship between a process and product's Critical Quality Attributes (CQAs) that could potentially affect its safety and efficacy profile [4, 5]. That product and process knowledge can be achieved through the establishment of a design space for each unit operation. According to ICH Q8 (R2) [6], the design space is "the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality."

This new paradigm is driven by the fact that product design and process operation are both executed in the presence of uncertainty and that the process might be able to compensate its effects onto the product quality. The ultimate reason for a design space is to provide the necessary degrees of freedom for a process control scheme to compensate the presence of disturbances (uncertain environmental conditions) into the manufacturing process [3].

The use of statistical approaches for designing experimental studies and performing data analysis has emerged as a fundamental activity for implementing QbD. A large number of raw materials and process parameters typically have an impact on any given unit operation in a biotechnology process. For example, a process chromatography step can have as many as 30–70 raw materials, 100–200 input parameters, and 50–150 output parameters. Understanding the impact of each raw material and input parameter on each output variable is not practical. A combination of risk assessments using tools such as the Failure Modes and Effects and Analysis (FMEA), and DoE has emerged as the approach of choice to facilitate this task [7].

Statistical techniques have several applications, particularly in situations where several input variables potentially influence some performance measure or quality characteristic of the product or process. DoE has been used in many fields of application besides protein purification [8, 9], such as development of culture media either for prokaryotic or eukaryotic cells [10], recombinant protein and virus productivity optimization [11, 12].

The input variables in DoE, called independent variables or factors, are subject to the researcher's control during the execution of an experiment (e.g., residence time, column load, raw material batches, sample pH, process temperature). On the other hand, the performance measure or quality characteristic is called response [13]. Generally, a process involves more than one response (e.g., yield, purity, or sample quality attributes such as oxidation, deamidation, posttranslational modifications).

Working with statistical design of experiments has several advantages compared with one-factor-at-a-time experiments.

With a small number of experiments, and the consequent saving in costs, a precise estimation of the effects of each individual factor and its interactions is possible. Additionally, there is experimental information in a larger region of the factor space, making process optimization more efficient. Moreover, modern commercial statistical programs are available and help scientists choose a statistical design to investigate the experimental region of interest, and identify and fit an appropriate model from experimental data. These programs require only basic knowledge of statistical experimental design fundamentals, regression modeling techniques and elementary optimization methods and allow for establishing in mathematical form the relationships between factors (critical process parameters) and responses (CQAs).

Here, we describe the steps any researcher could follow in order to develop, optimize, and define the design space for purification processes employing DoE methodology, including guides for definitions of relevant factors to be studied, experiments execution advices, and data analysis guides.

2 Materials

Materials will depend on the purification step to be evaluated. Materials for responses measurement will also depend on the selected responses.

Development and optimization work is generally conducted in small scale and standard equipment and consumables can be used. For example, for a chromatographic purification step: liquid chromatography system ÄKTA Purifier (GE Healthcare, Uppsala, Sweden), XK 16, 26 or 50 columns (GE Healthcare), chromatographic media (GE Healthcare), buffers.

For experiments design and data analysis use Design-Expert® software Version 7.0.0 (Stat-Ease, Minneapolis, USA).

3 Methods

A recommended sequence of activities toward the establishment of an expanded design space is outlined in Fig. 1; some of them can be executed in parallel, and others could be omitted depending on the main goal.

3.1 Select the Process Step to Be Evaluated

Given the number of variables associated with downstream processing of biopharmaceutical products, establishing an expanded design space for all operational parameters and for all unit operations is considered an unnecessary task.

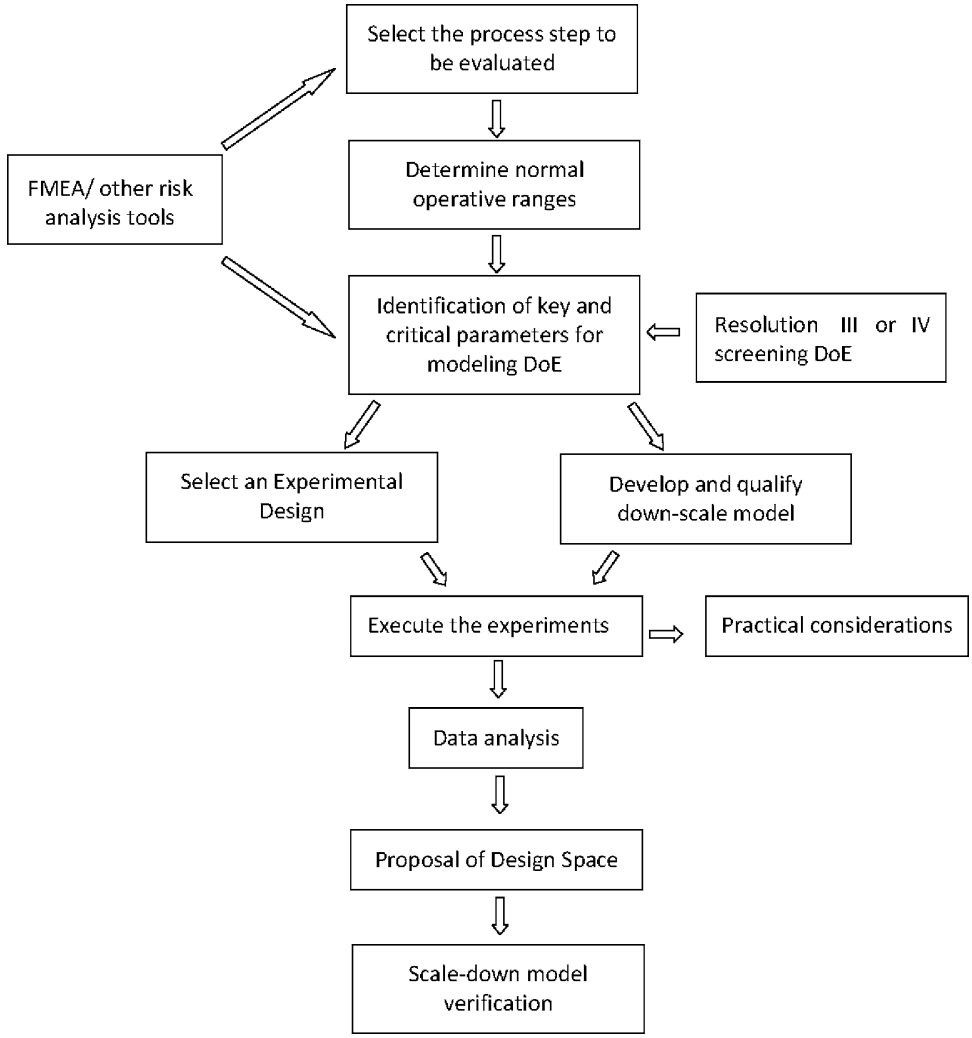

Fig. 1 Sequence of activities toward the establishment of an expanded design space elements design space to applying DoE in a purification process

1. Analyze global downstream process.
2. Perform risk analysis to select the process step most likely to benefit from a design space (*see* **Note 1**).
   The selection criteria should include:
   (a) Impact of a purification step on critical product attributes.
   (b) Potential factors that have an impact on process consistency (e.g., yield).
   (c) Opportunities for manufacturing operational flexibility.
3. Select the step to be evaluated.

3.2 Failure Modes and Effect Analysis

Failure modes and effect analysis (FMEA) is a very powerful risk assessment tool widely used in a variety of manufacturing industries and business practices. Like many risk analysis procedures, FMEA provides a rigorous methodology for identifying, evaluating and documenting potential modes of product of process failure. In contrast to other risk analysis tools, an FMEA results in a numerical ranking of each potential failure, aiding the prioritization of follow-up investigations and the implementation of corrections or controls to mitigate the failure [14].

The risk assessment is based on assigning a ranking from 1 to 10 (low to high), to three critical criteria: (a) the severity of a failure, (b) the expected frequency of occurrence, and (c) the likelihood of detecting the failure. The product of the three scores results in a risk priority number (RPN), which can vary between 1 and 1,000. It is important to evaluate the potential failure with all three criteria because the effects may either multiply or offset one another.

There are 12 steps to the FMEA process:

1. Assembly an FMEA team (*see* **Note 2**).
2. Break down the chromatographic step into single by-steps.
3. Brainstorm potential failure modes.
4. List potential effects of failure.
5. Assign Severity Rankings (S).
6. Assign Occurrence Rankings (O).
7. Assign Detection Rankings (D).
8. Calculate the RPNs ($\mathrm{RPN} = S \times O \times D$) (*see* **Note 3**).
9. Develop the action plan (*see* **Note 4**).
10. Take action and reassign rankings.
11. Calculate the resulting RPNs and display the results in Pareto Chart (*see* **Note 5**).
12. Prioritize the parameters or issues that need to be addressed on a consensus basis (*see* **Note 6**).

3.3 Determine Normal Operative Ranges (NOR)

In early stage development, setting the operative ranges generally starts defining Quality Target Product Profile (QTPP) (*see* **Note 7**). Once the QTPP has been identified, the next step is to identify CQAs and its acceptable variation. Then, it is necessary to establish limits for each CQA using a combination of approaches. When appropriate, certain attribute specifications are based on existing regulatory guidance. For other attributes, relevant limits or ranges are established based on several sources of information that link the attributes to the safety and efficacy of the product, similar to the way this information is used to assess the criticality of these attributes [4].

In summary, identifying the CQAs and setting appropriate specifications and acceptable limits (or validating removal if applicable) are the foundational activities for implementing QbD for biologics. From these settled limits it is possible to find the normal variation in the performance of the process parameter and determine the NOR. There are two possible situations:

If the protein product is being developed, the steps to determine the NOR are as follows:

1. Define QTPP.
2. Define CQAs (*see* **Note 8**).
3. Set protein product specifications (*see* **Note 9**).
4. Establish normal range for operational parameters (*see* **Note 10**).

If the goal of the study is the optimization of a step belonging to an already established and licensed process, the evaluation begins with a thorough examination of what is known about the process step by consulting various sources of information, such as manufacturing procedures, development data, license documents, interview to operators, etc.

3.4 Identification of Critical, Key, and Non-Key Parameters for Modeling DoE

Often there are many possible factors, some of which may be critical and others which may have little or no effect on response. It may be desirable, as a goal by itself, to reduce the number of factors to a relatively small set [2–5] so that attention can be focused on controlling those factors with appropriate specifications [4]. There are two strategies to achieve this goal (Fig. 2).

A brief summary is described:

1. Determine the criticality of each process parameter.
   (a) Performing risk assessment (*see* Subheading 3.2 and/or *see* **Note 11**).
   (b) Performing screening DoE (*see* Subheading 3.6, **step 1**) (*see* **Note 12**).
2. Categorize critical, key, and non-key process parameters from previous studies (*see* **Note 13**).

3.5 Develop a Down-Scale Model

Given the number of experiments required to optimize a chromatographic separation, the majority of these studies will take place at laboratory scale. The scaling down must be done following certain principles that assure that small-scale model is a true representation of what occurs in the manufacturing process. Moreover, the performance of the small-scale model used to develop the design space needs to be representative of the manufacturing-scale process with regard to process performance and product comparability. The development of down-scale model implies its design and subsequent qualification [15].

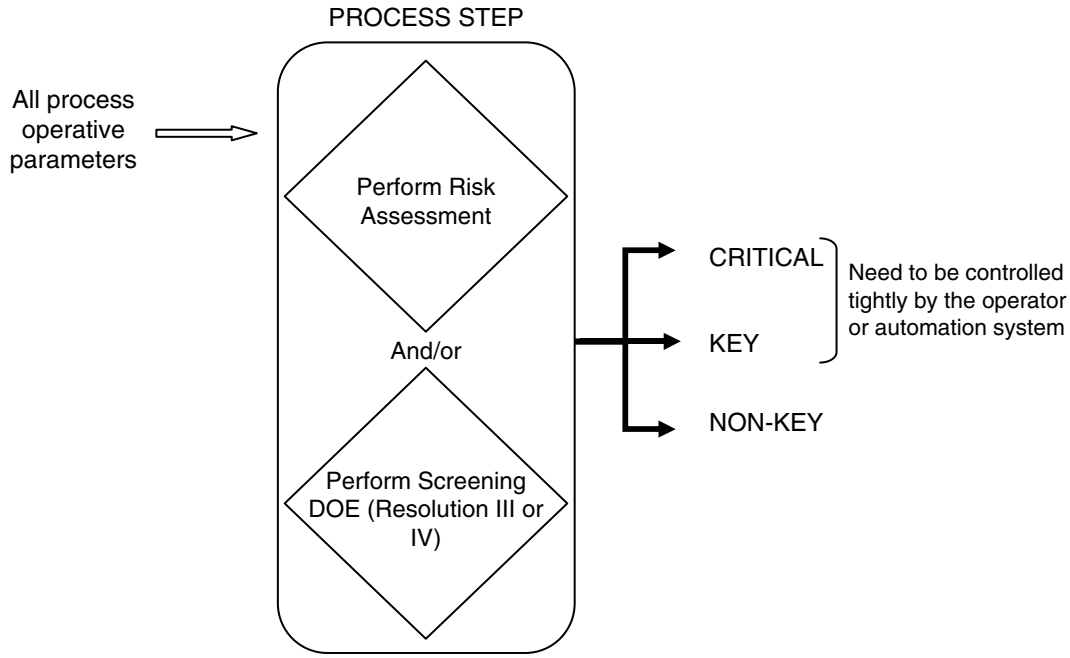

Fig. 2 Identification of critical, key, and non-key operative parameters using risk analysis tools and/or screening DoE methodology

Design down-scale purification model (*see* **Note 14**)

1. Use same chromatography resin than in the manufacturing process (base matrix, functional groups, and ligand densities) (*see* **Note 15**).
2. Use the same fluid distribution system (if possible) (*see* **Note 16**).
3. Use quality of buffers and salts consistent with those used in manufacturing (*see* **Note 17**).
4. Maintain residence time, the bed height and linear velocity (*see* **Note 18**).
5. Decrease the column diameter.
6. Pack the resin into the column and verify quality (HETP and asymmetry factor) (*see* **Note 19**).
7. Normalize all process solution volumes to column volumes (CV). Maintain the CV between scales.
8. Maintain resin loading (g protein/L of resin).
9. Maintain elution pool collection criteria (*see* **Note 20**).
10. Maintain solution pH, conductivity, protein concentration, composition.
11. Maintain temperature (*see* **Note 21**).

Down-scale model qualification

1. Run the down-scale system at least three times observing the scale-down parameters.
2. Evaluate chromatographic profile, product yield (quantitative method), product purity (SDS-PAGE, RP-HPLC).
3. Use appropriate statistical methods (such as Student's *t*-test) to compare with the manufacturing scale (*see* **Note 22**).
4. If discrepancies between the scales were detected identify the potential causes and correct them.

3.6 Select an Experimental Design

Selected critical and key process parameters must be studied as factors in an experimental design beyond those fixed limits in the NOR. This helps to assess the impact of any excursion beyond the usual operating ranges.

Many techniques involving experimental design are available, from methodologies mainly suitable for screening experiments such as full and partial factorials [16] and Plackett–Burman designs [17], to optimization techniques such as response surface methodology (RSM) [18] and central composite designs [19]. For an extensive review of the strategies used in the optimization of chromatographic systems, refer to [20]. Unfortunately, there is not a "multipurpose technique" that applies to all situations, and sometimes it becomes necessary to screen several approaches to find out the one that provides the best result for a particular case.

1. Screening experiments: the primary purpose is to select the more important factors that affect the process and require further detailed analysis.
   (a) Choose Plackett–Burman for the estimation of k main effects using $k+1$ runs. In this design, runs are always a multiple of 4.
   (b) Choose a two-level factorial design for estimation of k main effects using 2^k runs.
   (c) Choose a fractional factorial design for estimation of k main effects using 2^{k-1} runs (*see* **Note 23**).
2. Optimization experiments/Robustness testing: the purpose is to estimate not only the main effect but also the interaction and even quadratic effects. The experiments can give information about the shape or curvature of the response surface.
   (a) Choose a Box–Behnken design for the estimation of k main effects and its interactions using $2k(k-1)$ C0 runs, where C0 is the number of center points (*see* Table 1).
   (b) Choose a Central Composite Design for the estimation of k main effects and its interactions using $2k+2k+$C0, where C0 is the number of center points.

Table 1
Box–Behnken design for three factors in three levels and five central points (total 17 runs)

	Coded			Uncoded		
Runs	Factor 1 Sample load	Factor 2 Sample pH	Factor 3 Sample ionic strength	Factor 1 Sample load (mg/mL resin)	Factor 2 Sample pH	Factor 3 Sample ionic strength (mS/cm)
1	1.0	0.0	−1.0	50	8.0	4.0
2	0.0	0.0	0.0	30	8.0	4.0
3	0.0	0.0	0.0	30	8.0	4.0
4	−1.0	0.0	1.0	10	8.0	4.0
5	0.0	1.0	1.0	30	9.0	6.0
6	1.0	0.0	1.0	50	8.0	4.0
7	0.0	0.0	0.0	10	8.0	4.0
8	1.0	1.0	0.0	50	9.0	6.0
9	0.0	0.0	0.0	30	8.0	4.0
10	0.0	−1.0	1.0	30	7.0	2.0
11	−1.0	1.0	0.0	10	9.0	2.0
12	0.0	−1.0	−1.0	30	7.0	2.0
13	−1.0	−1.0	0.0	10	7.0	2.0
14	0.0	1.0	−1.0	30	9.0	6.0
15	−1.0	0.0	−1.0	10	8.0	4.0
16	0.0	0.0	0.0	30	8.0	4.0
17	1.0	−1.0	0.0	50	7.0	2.0

Factors: sample load, sample pH, and sample ionic strength. Levels for sample load are: 10, 30, and 50 mg/mL resin, Sample pH: 7, 8, and 9, Sample ionic strength: 2.0, 4.0, and 6.0 mS/cm. Those levels can be also indicated with codes −1, 0, and +1. The order of experiment execution is completely random

3.7 Execute the Experiments

During the execution of the experiments take into account the following points [21]:

1. Check performance of gauges/pH meter/conductimeter and other measurement devices.
2. A list of experiments that must be done will typically be dictated by software packages. Check that all planned runs are feasible.
3. The experiments must be run at a random way (completely randomized designs). In practice, the randomization is typically performed by a computer program.

4. Nuisance factors are those that may affect the measured result, but are not of primary interest. For example, in chromatography step, they might be the specific operator, the room temperature at which the experiment was run, a different starting sample or resin age. In some cases nuisance factors can be controlled with a technique known as "blocking" that may be used to reduce or eliminate the contribution to experimental error provided by those factors (*see* **Note 24**).
5. Watch out for process drifts and shifts during the runs.
6. Avoid unplanned changes (e.g., swap operators during experiments execution).
7. Allow some time (and backup material) for unexpected events.
8. Preserve all the raw data.
9. Record everything that happens.

3.8 Data Analysis

It is always necessary to examine the fitted model to ensure that it provides an adequate approximation to the true system. If the model does not provides an adequate fit, exploration and optimization of a fitted response surface will give poor or misleading results. For checking model adequacy several methods are available, such as residual analysis, test for outliers or testing for lack of fit. All of them are included in several software programs such as Design-Expert® version 7.0.0, whereas mathematical development of those methods is available in literature [13].

1. Create a statistical model.
2. Construct DoE plots, such as main effects mean plots and interaction plots (Fig. 3).
3. Perform model adequacy checking. Verify normal probability distribution of residuals through normal probability plot of residuals, test for Lack of Fit and test for outliers.
4. If none of the model assumptions were violated, examine the ANOVA ($\alpha = 0.05$ is generally employed). If model assumptions were violated, try to find a cause. A transformation could help. If a transformation is used return to point 2.
5. Use the results to conclude about experimental objectives.

3.9 Proposal of Design Space

Optimization and design space definition in the case of process with multiple responses can be efficiently performed by modeling each elementary response separately and then applying a multicriteria decision-making procedure such as the Derringer and Suich desirability function [22]. This method proposes a desirability function, which includes the researcher priorities and desires on building the optimization procedure. Such procedure involves creating a function for each individual response d_i and finally obtaining a global function D that should be maximized choosing the

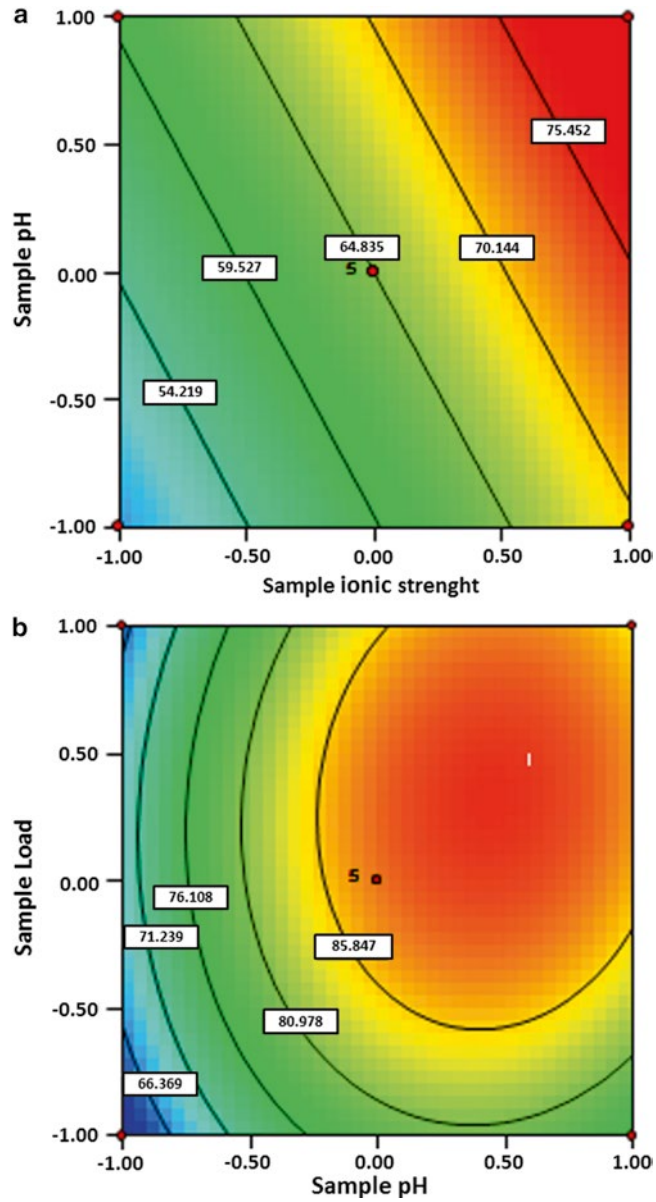

Fig. 3 Contour plots showing the effect of the mutual interactions between two independent variables (the other variable is held at its center level) on every response. (**a**) sample pH and sample ionic strength effect on purity (**b**) sample load and sample pH effect on yield

best conditions of the designed variables. The function D varies from 0 (value totally undesirable) to 1 (all responses are in a desirable range simultaneously), and it is defined by Eq. 1:

$$D = \left(\prod_{i=1}^{n} d_i^{r_i} \right)^{1/\sum r_i} \quad r_i = 1,2,3,4,5 \tag{1}$$

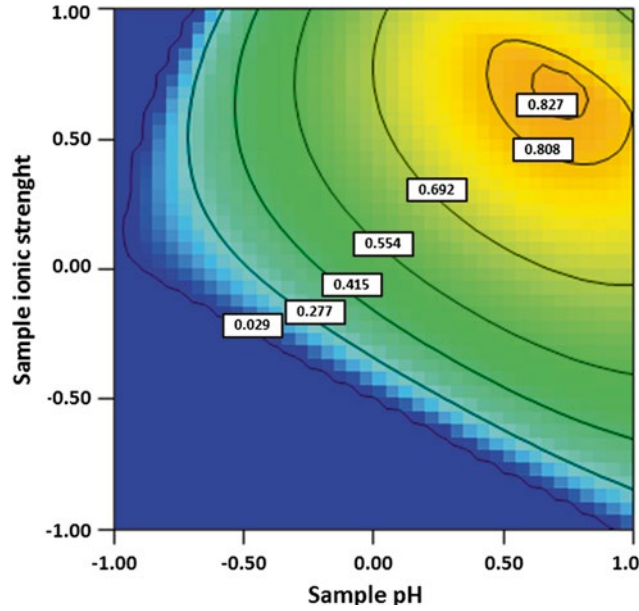

Fig. 4 Contour plot exhibiting the interactive effects of sample ionic strength and sample pH while the sample load is maintained at 2.76 mS/cm. *Blue* areas correspond to combination of variables in which $D = 0$ (Color figure online)

d_i is the partial desirability function for each response i and r_i is the relative importance assigned to this response. Relative importance r_i is a comparative scale for allotting emphasis to each d_i in the expression of the function D.

1. For multiple response processes, use the Design-Expert® Software in order to perform data analysis for every response (Subheading 3.8, **steps 1–5**).
2. For design space definition as well as process optimization set the d_i function for every response, through maximization or minimization.
3. Assign a relative importance for every response, setting r.
4. Combine all individual functions d_i, and maximize D.
5. The region where D reaches a maximum constitutes the optimum operating conditions for that purification process. At the same time, where $D = 0$, it is possible to find the edge of failure, which is the process conditions where one or more responses do not fit specifications (Fig. 4).

3.10 Scale-Down Model Verification

A comparison of the manufacturing-scale process data to the predictive models developed during process studies will provide evidence for the validity of the scale-down models. Two scenarios are possible [4]:

When manufacturing data do not exist, the comparison will need to be done prospectively:

1. Scale the process following scaling up principles.
2. Perform the process at full scale.
3. Use appropriate statistical methods to compare with the results obtained at small-scale.

When manufacturing-scale data exist, there are two approaches to comparison:

1. The validity may be demonstrated showing that the small-scale data are within the full-scale historical range.
2. Alternatively, for a true side-by-side comparison, the validity of models can be demonstrated with a scaled-down "satellite" process that is run in parallel with the full-scale manufacturing process and starting with sample material taken from the last one.

4 Notes

1. In line with standard risk assessment practices, these elements should be evaluated in terms of probability of occurrence, detectability, and severity of the consequences and prioritized appropriately as described in Subheading 3.2. The timing of such an analysis would depend on the availability of sufficient process and product understanding, but would best be performed with an established purification process prior to extensive characterization.
2. FMEA should always be conducted by teams. The best size for a FMEA team is four to six people, carefully selected, based on the contribution they can make to the specific FMEA. FMEA team members do not necessarily need to have extensive knowledge of the process being targeted. In fact, sometimes it helps to get an outsider's fresh perspective.
3. It should be taken into account that RPN is a result of subjective opinion and it is quite likely that the composition of the team has an influence on the rankings. Indeed, FMEA is not an "absolute" method.
4. FMEA is an iterative process; whenever a potential failure mode is identified, an FMEA corrective action can be implemented and doing so, lower RPN values will be obtained.
5. Pareto charts are useful for visualizing high RPN scores. Typically, the RPN values fall into clusters of very high, moderate, and very low.

6. Based on the RPNs, as visualized in Pareto chart, the team should agree on a cutoff value for studying the "high" RPNs first. This cutoff can also be made prospectively (although for a biological process, this may be difficult and is not necessary) or it can be retrospectively based on the results. The cutoff can be based on criteria such as obvious clustering (the top 25 %, 50 %, etc.).
7. In case a biopharmaceutical is being developed, a QTPP must be established first. QTPP has been defined as a "prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product." QTPP should be established as soon as a product has been identified as a viable candidate for commercialization and should be revised at key stages of product development, with any changes approved by the appropriate governance [7].
8. CQAs have been defined as "a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" [7].
   Because biotechnology products usually have numerous quality attributes that can potentially have an impact on safety and efficacy, CQA identification is best accomplished by using risk-based analysis, in accordance with the ICH Q9 guidance [7]. This assessment should consider, for example, previous clinical background of the product, nonclinical (animal) studies, in vitro biological activity assays, level of impurities, and manufacturing capability.
9. Drug substance and drug product specifications are two components of the control strategy for achievement of the QTPP and represent the ranges within which the product is considered to meet the desired product quality. It should be pointed out that there is not a one-to-one correspondence between a list of CQAs and specifications.
10. The NOR is the range at which the parameter is typically controlled during routine operations and is usually the range found in the manufacturing instructions. It takes into account the minimum and maximum values tested during initial development and a review of process history, which shows the capability of the operators, facility, equipment, and utilities.
11. Much process knowledge may be available from earlier process development and from previous experience with similar processes. In this sense, a risk assessment may be useful to identify the importance of operating parameters on CQAs.
12. Screening experiments with low-resolution DoE (resolution III or IV) are an efficient way, with a minimal number of runs,

of determining the main effects. They may also be used as a first step when the ultimate goal is to model a response with a response surface methodology. Parameters showing minor effects on process or product across wide ranges (i.e., non-key parameters) are less pertinent to process control and can be excluded from further process characterization studies.

13. "Key" and "critical" operating parameters are terms adopted from a PDA technical report [23]. Critical operating parameters affect critical product quality attributes when varied outside of a narrow (or difficult to control) operating range. Key operational parameters also have a narrow (or difficult to control) operating range. However, they affect process performance (e.g., yield, duration), but not product quality. The remaining "non-key" parameters can affect process or product but are easily controlled within wide acceptable limits [4].
14. Residence time is a critical parameter that must be maintained while scaling down the process step. Typical scale-down column diameters range from 0.5 to 1.6 cm, while the maximum diameter for manufacturing scale columns may be as large as 2.0 m or higher. Therefore, the scale-down factors for chromatography steps may range from 1:100 to 1:100,000.
15. Resins with the same functional group but differences in base matrices or porosities may yield different levels of virus and DNA removal, respectively.
16. The fluid distribution system of a column (tubing, net, column hardware, etc.) plays a crucial role in separation. These elements, though difficult to maintain with identical geometry and materials of construction between large and small scale, should be kept as similar as possible.
17. Procedures for preparation of buffers and solutions for scale-down studies should be according to established protocols used in large-scale manufacturing, since subtle changes in ionic strength or pH could lead to altered elution and purity profiles.
18. In some cases, residence time has been maintained by changing both the bed height and linear velocity.
19. Very often, column packing can play an important role in chromatographic separation. Differences in column packing at the two scales may have an impact on the separation and be visualized as differences in the chromatograms. It is therefore important to evaluate the quality of column packing as a tool for comparison at different scales. Height-equivalent-to-a-theoretical-plate (HETP) and asymmetry factor (A_s) are typically used to evaluate quality of column packing.
20. Elution pool volumes are determined by the pooling criteria for the elution. The method of pooling will have an impact on

pool volumes and possibly on product purity. Collection of the product pool is typically initiated and controlled by using a UV absorbance detector. The pool collection is usually initiated when the UV absorbance increases above baseline or attains a set absolute absorbance as the product starts to come off. The end of pool collection could be defined in different ways: (a) fixed number of CVs, (b) when the UV absorbance returns to a certain predetermined level, or (c) when the UV absorbance has returned to a certain proportion of the UV peak maximum. Each of these methods has particular advantages and disadvantages. The third method has a greater likelihood of maintaining consistent composition of the elution pool through varying load levels but will allow relatively greater variation in elution pool volume. However, it requires absorbance to be in the linear range of detection throughout the elution.

21. Fluctuations in temperature could lead to changes in pH and conductivity of certain buffers, which could affect retention of proteins. Among the various chromatographic techniques, hydrophobic interaction chromatography has been reported to be especially prone to changes in performance due to temperature variations, which could give rise to large changes in product retention or selectivity [15].
22. One should be careful in applying the *t*-test in scale-down processes when a single lot of a load material is applied to the column. Replicates at the small scale give a good estimate of the variability of the small-scale system, but manufacturing processes may vary more due to changing feed streams. Often the scale-down data displays less variability than the process data set, as the runs are typically conducted with identical equipment over a short time span, using the same lots of raw materials, and the product peaks are typically analyzed together [15].
23. With these designs two or more effects are confounded (or aliased). That is, results can only be attributed to their combined influence rather than their individual influence.
24. The basic concept is to create homogeneous blocks in which the nuisance factors are held constant and the factor of interest is allowed to vary. Within blocks, it is possible to assess the effect of different levels of the factor of interest without having to worry about variations due to changes of the block factors, which are accounted in the analysis. Thus, the analysis of the experiment will focus on the effect of varying levels of the primary factor within each block of the experiment.

    In case a full factorial design has to be blocked, the price to pay is not to longer distinguish the high-order interaction(s) from the blocking effect—they have been "confounded" or "aliased."

In fact, the blocking effect is now the sum of the blocking effect and the high-order interaction effects. This is fine as long as the assumption about negligible high-order interactions holds true, which it usually does.

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