ISSUES IN EXPERIMENTAL DESIGN OF PRE-CLINICAL STUDIES

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Outline

1. QbD and DOE
2. Basic Principles
   a) Randomization
   b) Blocking
   c) Replication
   d) Factorial Structure
   e) Nesting
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QbD and DOE

Design Of Experiments is an essential component in QbD.

It leads to detailed verification of how product and process definition affect critical quality attributes (CQA).

It establishes a basis for defining the design space.
QbD and DOE

Experiments enable us to map the process.

A reliable map lets us drive safely.

A reliable process map permits changes without adverse effects on product performance.

Ergo the benefits of QbD regulatory approval.
QbD and DOE

If you don’t know where you are going, you won’t get there quickly.
Basic Principles of DOE

• Randomization
• Blocking
• Replication
• Factorial Structure
• Nesting (split plotting)

Sir Ronald A. Fisher
Basic Principles: Randomization

Make arbitrary decisions randomly.

- Who gets each treatment?
- Where are materials placed?
- What gets done first?

*Randomization helps to guarantee that the experiment is fair; it is not biased in favor of one of the treatments.*
Basic Principles: Randomization

From the *Horizon* homeopathic dilution experiment, by Martin Bland.

![Graph showing percentage active basophils against sample number with a fitted cubic curve.](image)
Basic Principles: Blocking

Often units can be grouped by factors that are not of direct interest, but will affect results.

- Source of raw materials.
- Intra-subject comparisons (crossover).
- Animals grouped by litter or size.
- Day/time of preparation or measurement.
- Assignment to titer plate or shaker shelf.
Basic Principles: Replication

We need to know something about the variability of outcomes when conditions are not changed. That gives a basis to know if differences between conditions are “just noise”.

Most processes are affected by many factors. An experiment can:

- Modify factors systematically.
- Hold factors constant.
- Ignore factors.
Basic Principles: Factorial Structure

The standard solution:

One-Factor-at-a-Time Experiments.

Modify one factor; hold all others constant.
Basic Principles: Factorial Structure

“No aphorism is more frequently repeated in connection with field trials, than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken. Nature, he suggests, will best respond to a logical and carefully thought out questionnaire.”

Sir R. A. Fisher
Successful pre-clinical experimentation needs:

- Rapid progress and learning.
- Ability to study many factors.
- Solid evidence for decision making.
Clinical trials are different; they usually focus on a small number of comparisons: treatment vs. placebo or SOC.

Dose may be the *only* factor that is studied.
Basic Principles: Nesting

Sometimes there are factors that can be used at an inter-unit level and other factors that can be used at an intra-unit level.

This is known as “nesting” or “split plotting”.

Quality by Design
Tel Aviv 2015

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Basic Principles: Nesting

A nested design with six units and two sub-units. There is one two-level factor at the unit level and a second two-level factor at the sub-unit level.
Basic Principles: Nesting

Example: What factors affect the yield of protein extraction?
Twenty-one days, two process runs per day.
Five factors:
• Inflow feed position – can be set once each day.
• Inflow feed rate.
• Gas flow rate.
• Concentration of protein A.
• Concentration of protein B.
Some examples of QbD experiments.

Optimization of a Refolding Step for a Therapeutic Fusion Protein in the Quality by Design (QbD) Paradigm

Pratap D. Bade, Susmitha P. Kotu, Anurag S. Rathore

Journal of Separation Science 2012
Some examples of QbD experiments.

Goal is to optimize refolding process for a recombinant biotech therapeutic.

A screening experiment with 5 factors and 10 test combinations identified 3 key factors: urea and DTT concentrations and buffer pH.

A detailed experiment explored these factors.

Yield improved from about 60% to 77%.
Some examples of QbD experiments.

A Quality by Design (QbD) Case Study on Liposomes Containing Hydrophilic API: II. Screening of Critical Variables, and Establishment of Design Space at Laboratory Scale

Xiaoming Xu, Mansoor A. Khan, Diane J. Burgess

*International Journal of Pharmaceutics* 2012
Some examples of QbD experiments.

Goal is to optimize encapsulation efficiency, with secondary goals on particle size, zeta potential and shelf life.

A screening experiment with 8 factors and 12 test combinations identified 2 key factors: lipid concentration and drug concentration.

A detailed experiment with these factors led to EE of over 40% with good results for other CQAs.
Some examples of QbD experiments.

Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function.

Ahmed S. Zidan, Omaima A. Sammour, Mohammed A. Hammad, Nagia A. Megrab, Muhammad J. Habib and Mansoor A. Khan

Some examples of QbD experiments.

The present project deals with a case study to understand the effect of formulation variables of nanoemulsified particles of a model drug, cyclosporine A.

This investigation demonstrated the potential of QbD in understanding the effect of the formulation variables on the quality of CyA self-nanoemulsified formulations.
Some examples of QbD experiments.

Quality by design: Optimization of a liquid filled pH-responsive macroparticles using Draper-Lin composite design.

Hasan Rafati, Zahra Talebpour, Laleh Adlnasab, Samad Nejad Ebrahimi

Journal of Pharmaceutical Sciences 2009
Some examples of QbD experiments.

pH responsive macroparticles incorporating peppermint oil (PO) were prepared using a simple emulsification/polymer precipitation technique. [The study] investigated the effect of four independent variables, including the PO to water ratio, the concentration of pH sensitive polymer (hydroxypropyl methylcellulose phthalate), acid and plasticizer concentrations, on the encapsulation efficiency and PO loading. The optimized macroparticles were predicted to yield 93.4% encapsulation efficiency and 72.8% PO loading.
Summary

- Designed experiments provide an organized framework for exploring many factors.
- They provide a solid knowledge base for predicting outcomes and thus lead to the construction of a design space.
- They can ramp up the development process and reduce time to market.
- They require careful planning and discipline.
Thank you for your attention.