Quality by design
Application to chromatography

DCVMN, Bangkok, 2015-10-05

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Imagination at work

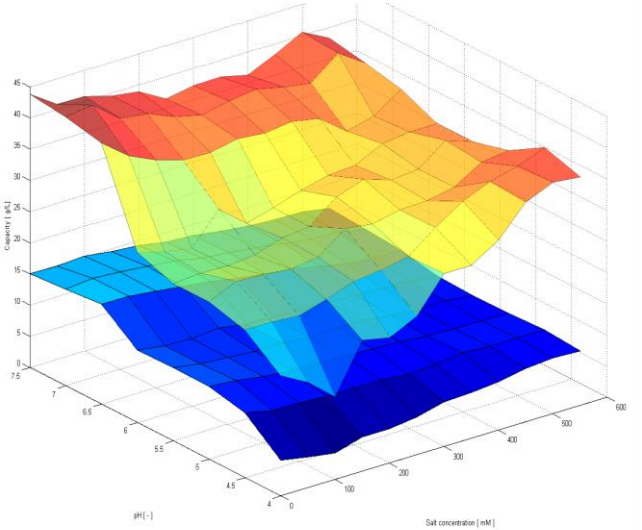
Outline

Introduction, QbD and DoE

Chromatography tools for Design of experiments

Example: - DNA removal Influenza
- Resin screening Insulin

Summary



Quality by Design

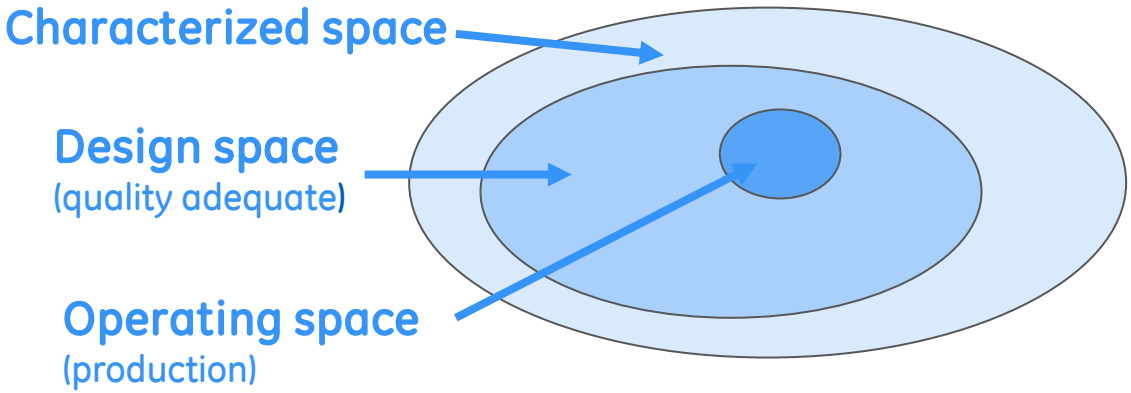
A framework for efficient process development

A systematic approach

QbD, terminology

- Design and conduct studies (DoE) to identify relationships of
  Critical raw Material Attributes (CMA)
  Critical Process Parameters (CPP)
  to
  Critical Quality Attributes (CQA)

- Analyse and assess data to establish appropriate ranges

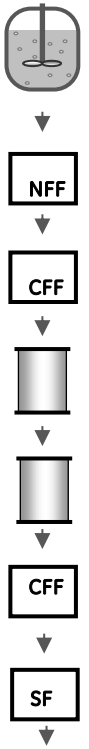


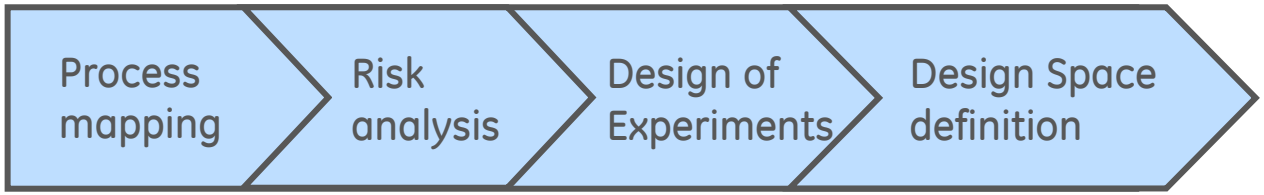
Testing a larger number of process conditions during early process development leads to better process understanding!

QbD workflow:
Defining the process design space

Four key steps

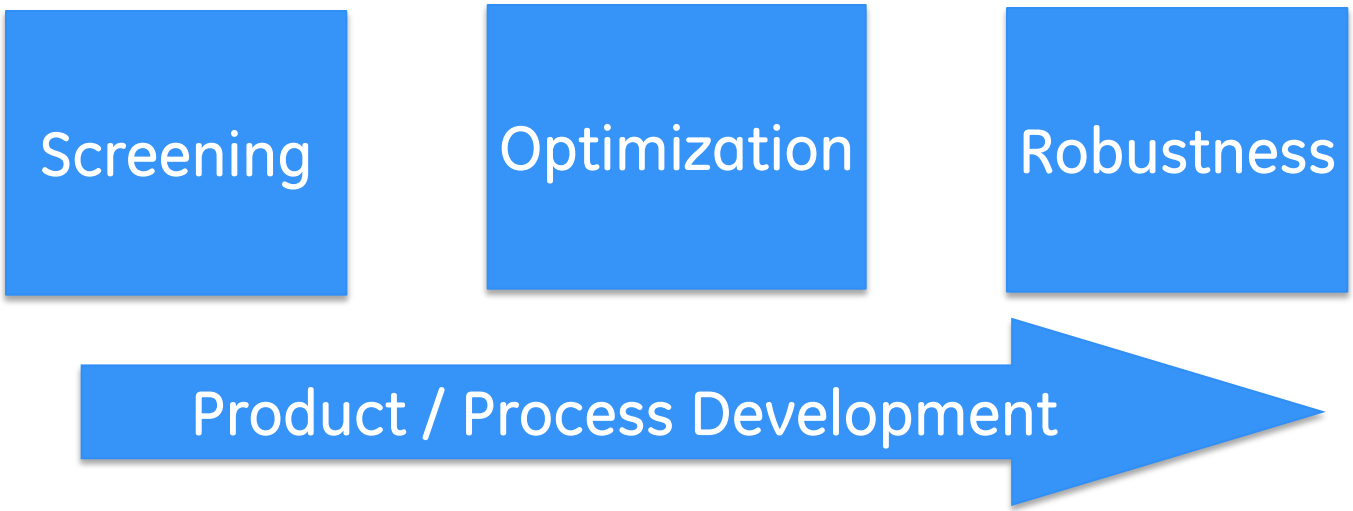
1. Process mapping
2. Risk analysis
3. Design of experiments (DoE)
4. Execution and analysis, definition of design space





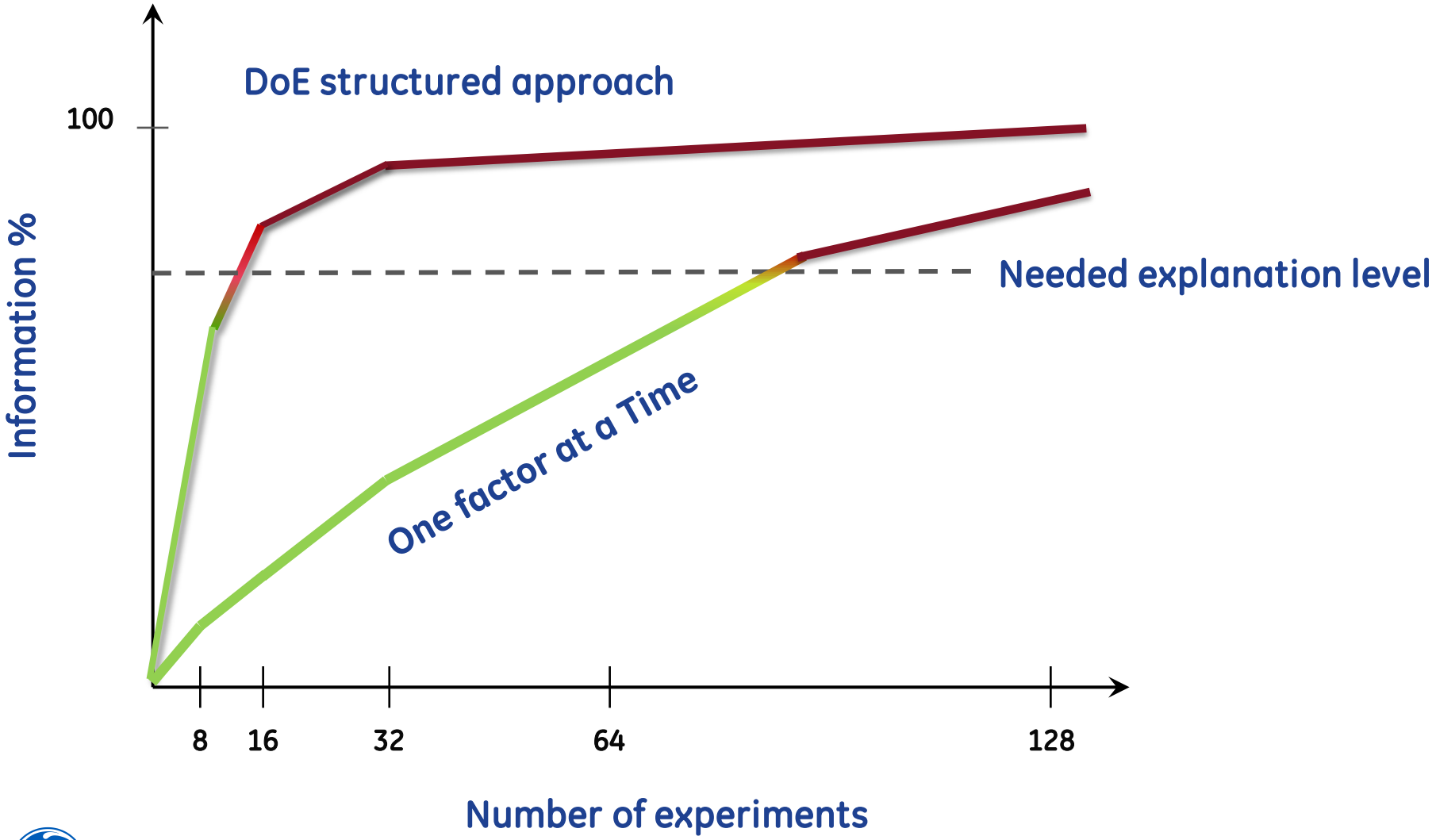
DoE essentials

DoE, three primary objectives



Many factors Find the most important and their appropriate ranges	Find best settings of important factors	Check that small changes do not cause unacceptable change in performance

QbD workflow:
DoE information



DoE Concept

Method/Process

Controlled parameters
i.e. our:
X's,
Conditions
or
Factors

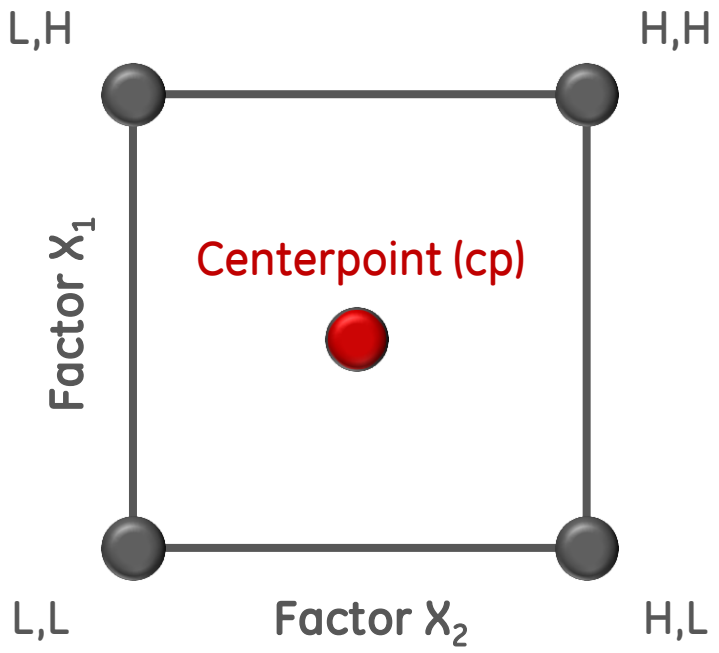
Results
i.e. our:
Y's,
Output
Parameters
or
Responses

We can describe the process using a model!

(Transfer Function)

$Y_k = f(X_i)+e$

General design construction 2 X's

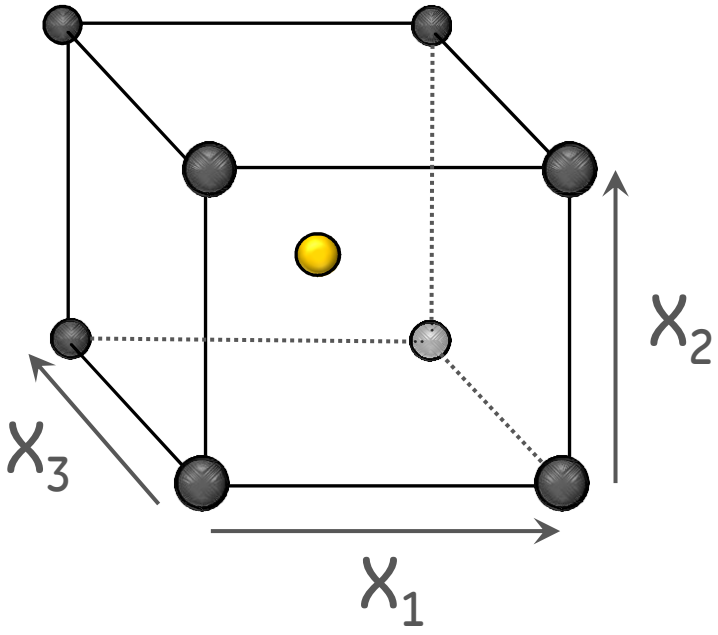


X_1	X_2
L	L
L	H
H	L
H	H
C	C
C	C
C	C

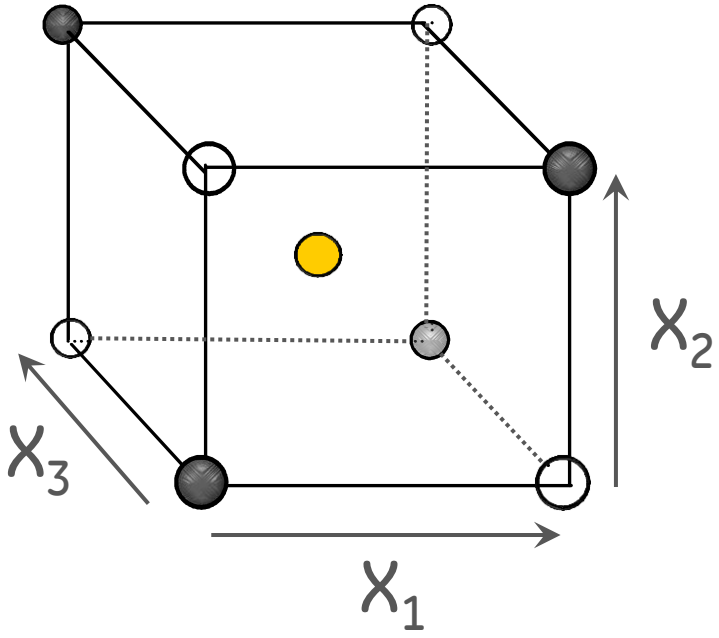
Number of experiments = 2^k + cp's
K = number of factors
H = high
L = low

Centerpoint used for estimation of noise and detection of curvature

Different designs



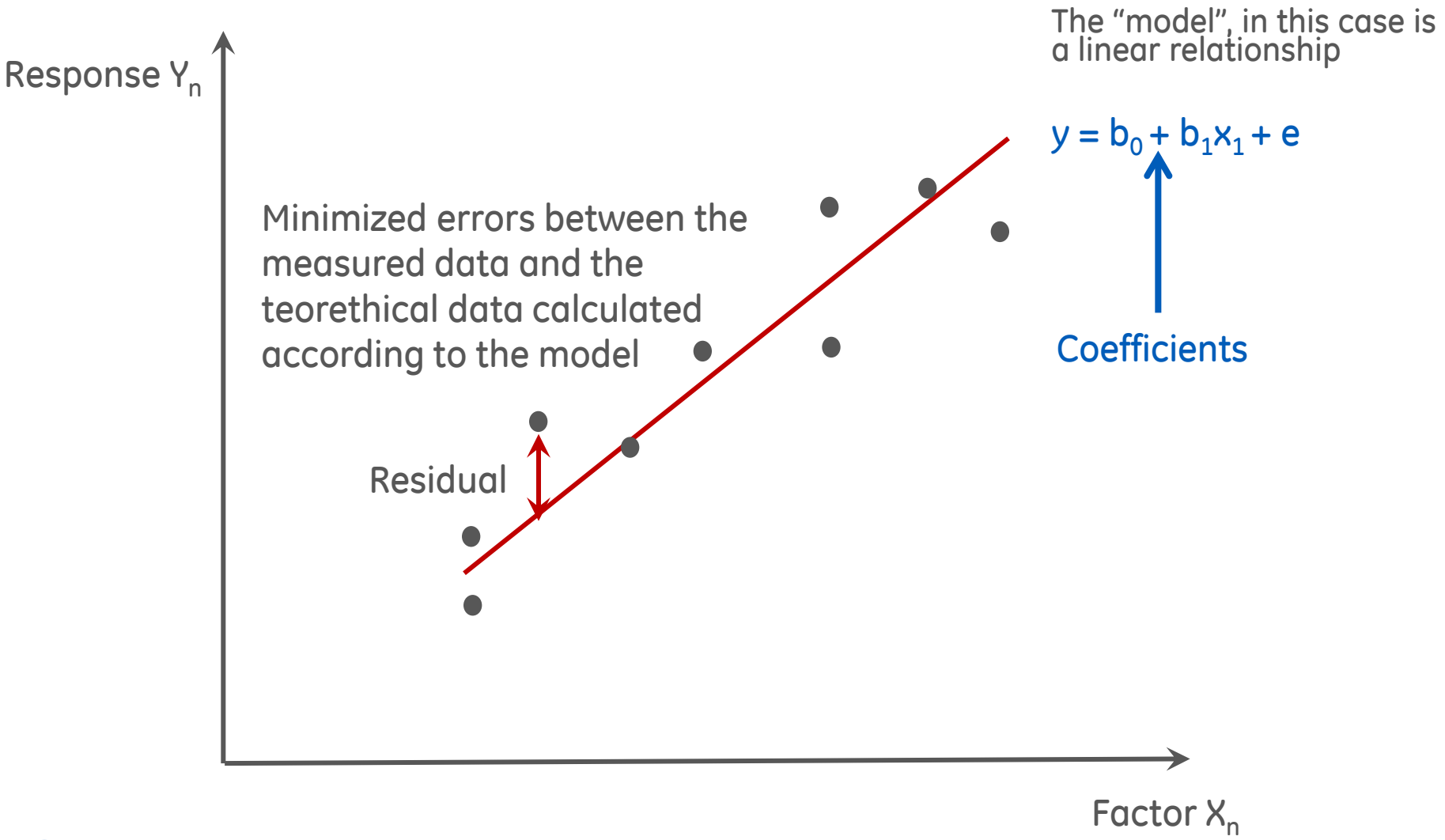
Full factorial design



Fractional factorial design

DoE evaluation

The model graphically



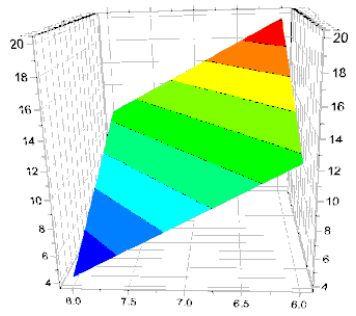
More complex model

Linear terms (main effects)

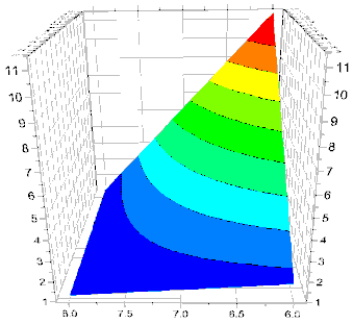
Interaction term(s)

Quadratic term(s)

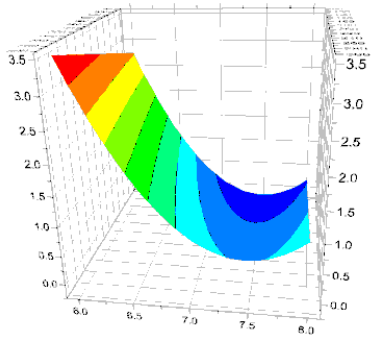
$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 + e$$



Robustness/screening



Screening/optimization



Optimization

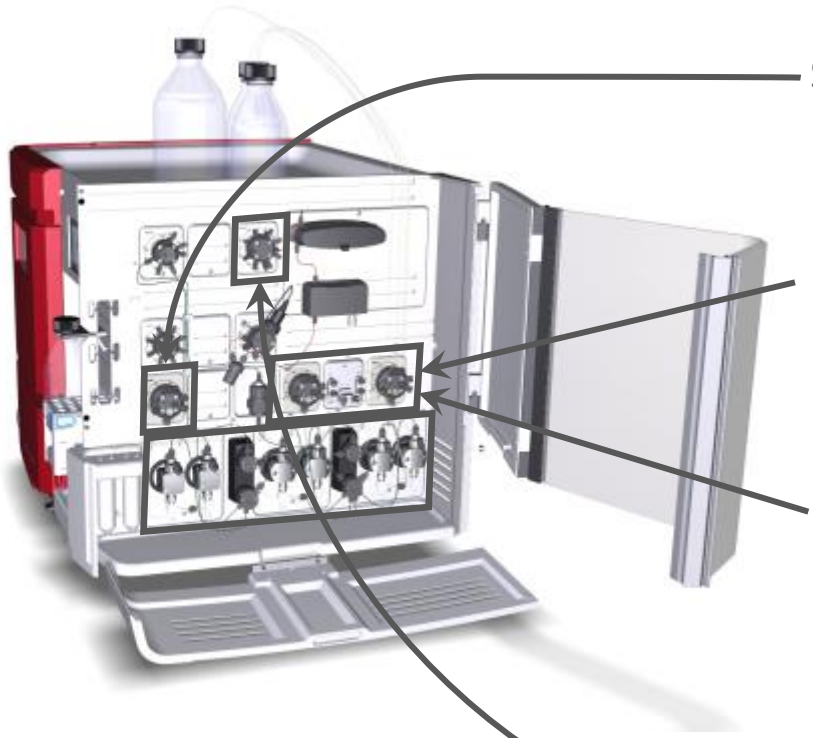
Coefficients (b_1, b_2, b_{12}, b_{11}, b_{22}) give the quantified effects for the x's.

e = prediction error

$y_{predicted} - y_{measured}$

DoE for chromatography

Example of factors and responses in chromatography



Sample conditions

Wash conditions

Elution conditions

Entire process

Factors:

- Load pH
- Load conductivity
- Load concentration
- Mass load
- Wash volume
- Wash pH
- Wash conductivity
- Elution pH
- Gradient elution
- Step elution level
- Cut OD
- Elution Additives
- Resin type
- Resin batch variations
- Bed Height
- Flow rate
- Residence time

Responses:

External data:

- Binding capacity
- Purity/Selectivity
- Activity
- Yield
- HCP
- DNA
- Aggregates

Peak Data:

- Area
- Resolution
- Asymmetry
- Plates per meter

Types of DoE studies in chromatography

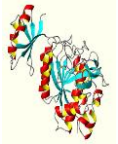
Study	Factors	Comments
Resin screening	Different ligands Ligand conc. Mobile phase composition	
Binding studies	Protein load pH, ionic strength Contact time	Target binds Contaminats in flow thr. Dynamic binding capacity (DBC)
Flow through studies	Load Capacity	Target in flow thr Contaminats binds
Wash studies	Buffer salt and pH Ionic strength Contact time	Wash step(s) can improve purity
Elution studies	- " -	Conditions for step/ gradient elution
Cleaning in place (CIP) Studies	Concentration Additives Time	Comparisons of different CIP solutions Media life time

Analytics in DoE

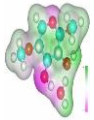
Bacteria based

Virus based

Protein based



Polysaccharide based



DNA based

Challenges in general

- Large number of tests during development
- Sensitivity and precision is critical
- DoE ”creates” variable sample matrices ->effects on analyses methods
- Miniaturization and parallelization puts higher demands on analyze method
  - sensitivity
  - throughput

Experimental formats in chromatography

Formats

Solutions for one factor at a time (slow, more sample)

Standard columns

Volume resin: 1, 5, 20 ml

Solutions for parallel screening (rapid, less sample)

Filter plates

PreDictor™ plates

Volume resin: 2 - 50 µl/well

Minicolumns

PreDictor™RoboColumn™

Volume resin: 0.05 - 0.60 ml

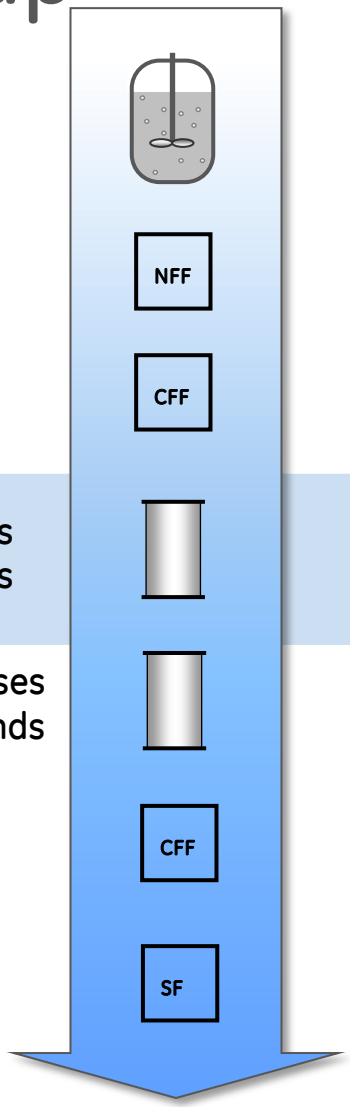
Formats:

	Plates	Mini columns	Std columns
Speed	Very fast	Fast	Fast
Sample use	Low	Low	Larger
Factor screening	Broad	Broad	OFAT[1]
Capacity	Static[2]	Dynamic	Dynamic
Automation	Manual Robot	Robot	Chr. system
Chromatogram	No	After fraction analysis	Yes
Use	One time Screening	Several runs Screening	Several runs Verfication

1) OFAT = one factor at a time
2) Dynamic binding capacity can be predicted from time-dependent batch data

Example:
DNA removal, Influenza

Process map



Cell culture + Influenza virus
MDCK cells, micro carriers, 72 h infection

Clarification
Normal flow filtration

Concentration + sample conditioning
Cross flow filtration

Virus passes
DNA binds

DNA reduction
Capto™ Q chromatography

Virus passes
Proteins binds

Protein reduction
Capto™ Core 700 chromatography

Concentration + buffer exchange
Cross flow filtration

Sterile filtration
Normal flow filtration

Live influenza virus

Experimental - DoE

Material	
Sample	• A/Solomon Island/3/2006 • A/Wisconsin/67/2005 • B/Malaysia/2506/2004
Sample conditioning	Sephadex™ G25 column
Format	Filter plates
Capto Q	50 µl/well
Sample load	400 µl/well
Incubation	10 min/shaker
Supernatant collection (flow through)	Centrifugation, 500 x g

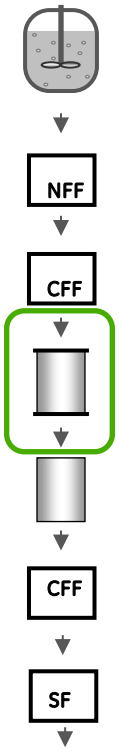
Experimental - DoE

Factors	DoE range
pH (eq, load, wash)	7.0 – 9.0
NaCl (eq, load, wash)	300 – 800 mM

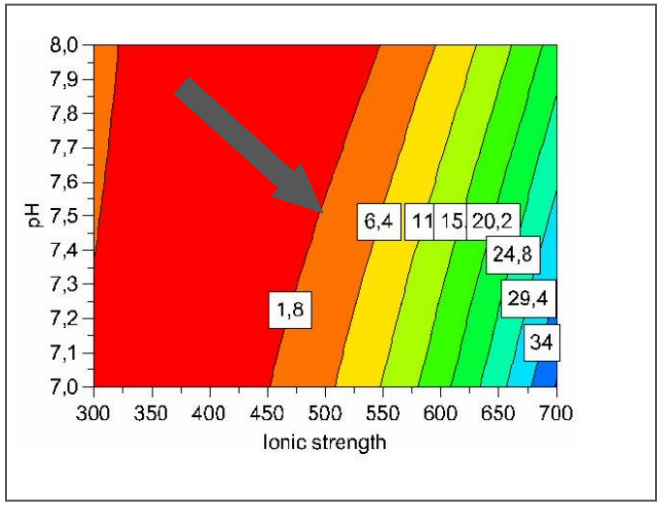
Responses (supernatant)	
MDCK-DNA	qPCR
Influenza, HA	Biacore™

Screening for DNA removal

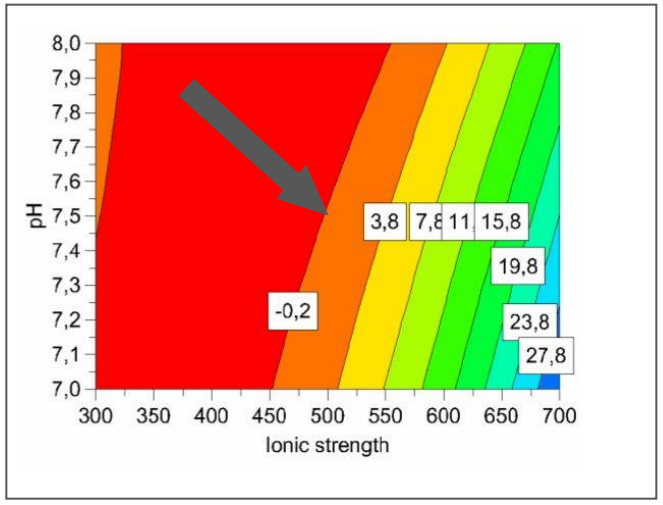
PreDictor plates /Capto Q



A/Solomon Islands/3/2006 (H1N1)



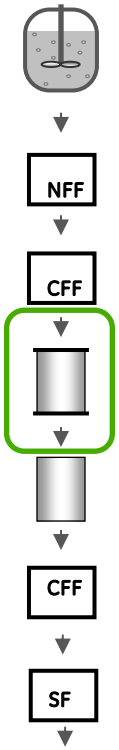
A/Wisconsin/67/2005 (H3N2)

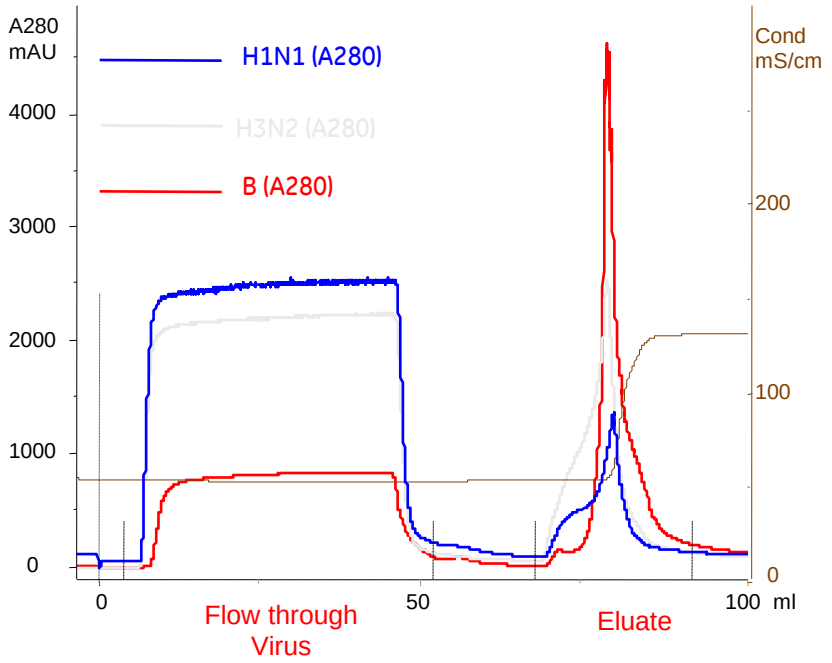


The arrow indicates best conditions

- The level of gDNA (%) in the flow through fraction are shown in the boxes.
- Conditions were chosen in order to achieve complete DNA reduction (red region) and keep the influenza virus in a non-binding mode.

Chromatography on Capto Q





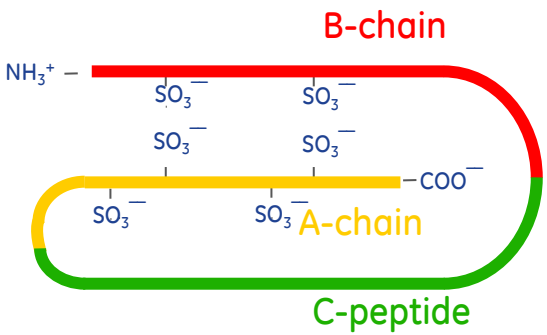
Column:	XK16/20
Volume:	20 ml Capto Q
Flow rate:	2.0 ml/min (60 cm/h)
Equil. buffer:	20 mM Tris, 0.5 M NaCl, pH 7.5
Elution buffer:	20 mM Tris, 1.5 M NaCl, pH 7.5
CIP:	1 M NaOH
Sample load:	40 ml (2 CV)
Flow thr vol.:	1.12x sample volume

Sample	HA yield %	gDNA (ng/ml) before	gDNA (ng/ml) after	DNA log reduction
H1N1	> 90	2010	17	2.1
H3N2	> 90	11300	16	2.9
B	> 90	96800	16	3.8

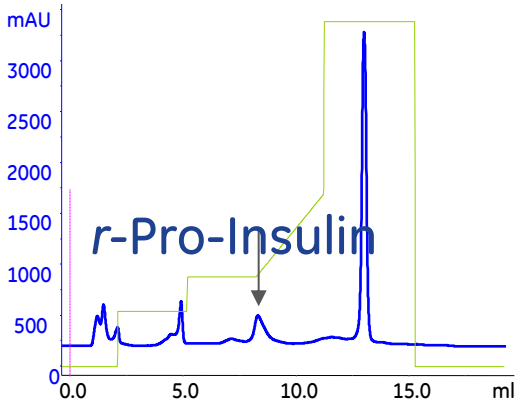
Example:
Resin screening, Insulin

Insulin case study – Capture step, resin screening and optimization

r-Pro-Insulin



Analysis using chromatography



Produced in E.coli

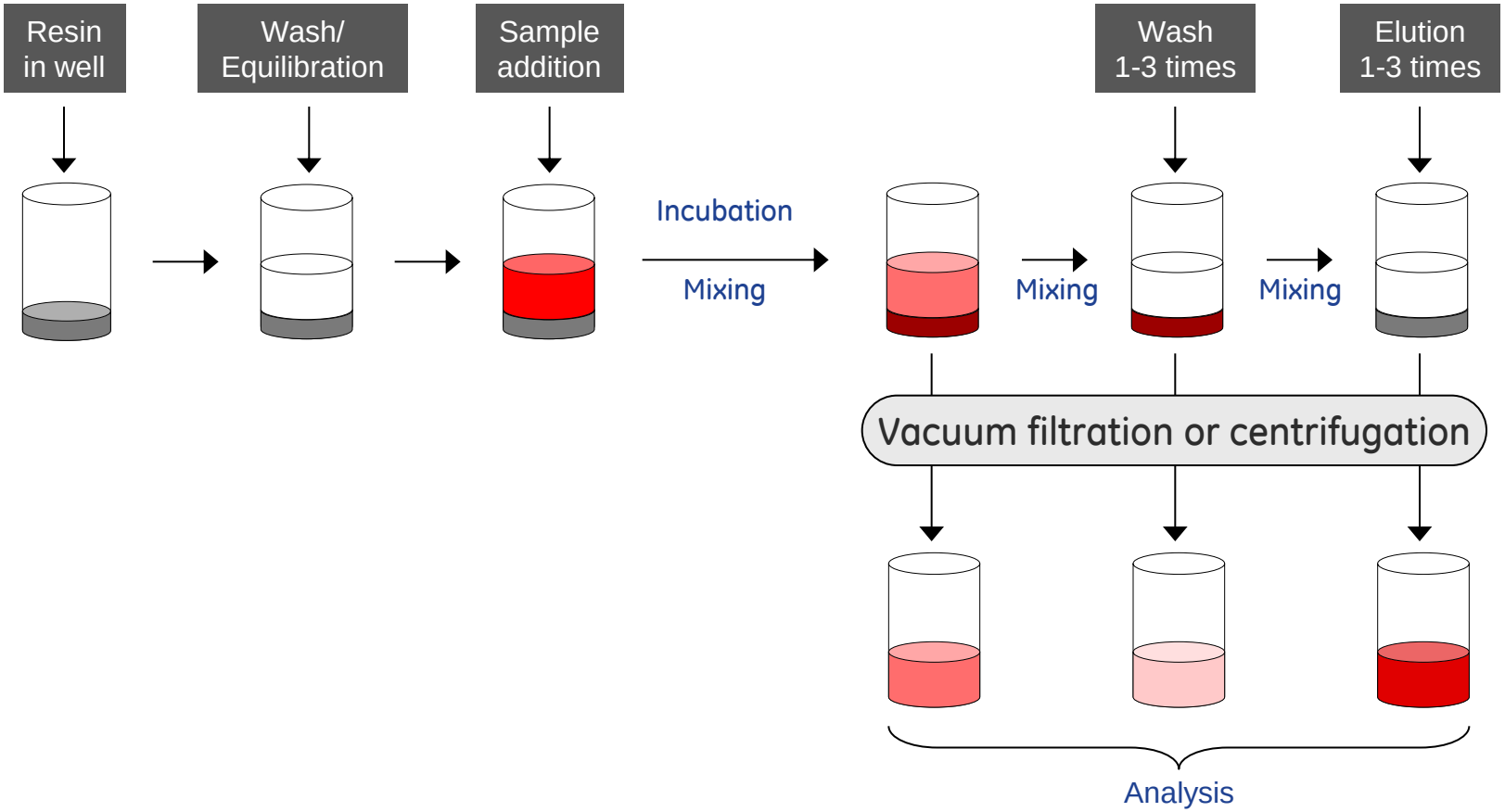
Mw ~11 000, pI ~ 5.6

8 M Urea is needed to dissolve inclusion bodies of *r*-Pro-insulin

Cation and Anion exchangers or Multimodal resins may be suitable

DoE with PreDictor plates

Experimental principle



DoE with PreDictor plates

Screening plates

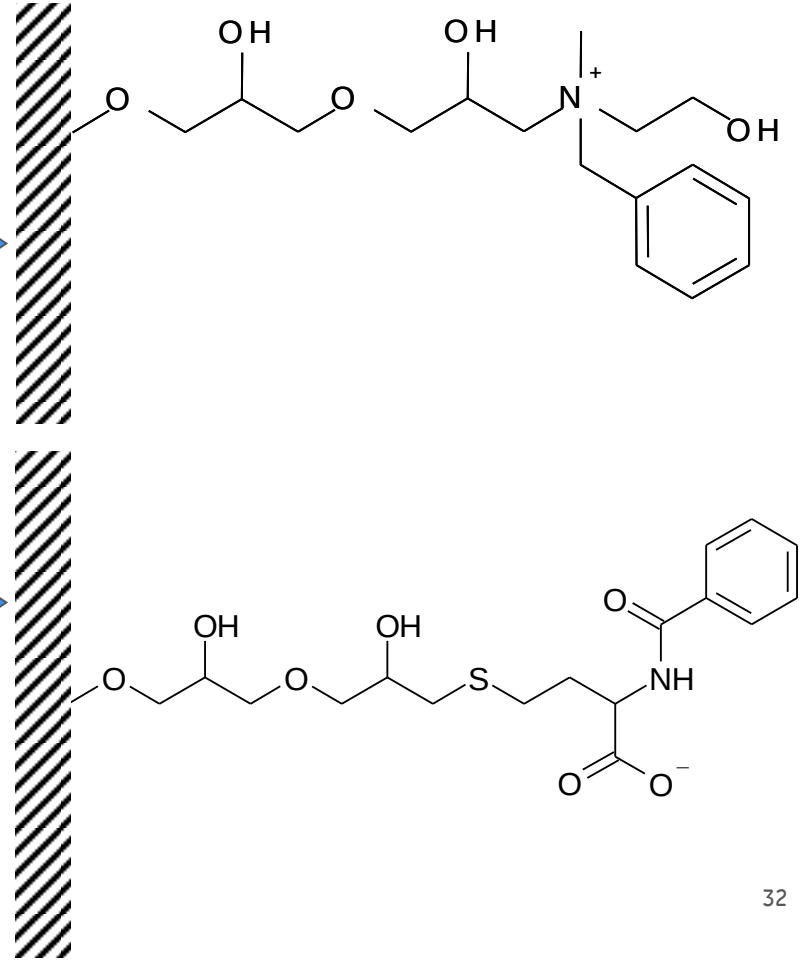
Different resins in same plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto Q			Capto DEAE			Q Sepharose Fast Flow			Capto adhere		
B												
C												
D												
E												
F												
G												
H												

pH: 5.3 – 8.1
NaCl: 0 – 150 mM

	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto S				SP Sepharose Fast Flow				Capto MMC			
B												
C												
D												
E												
F												
G												
H												

pH: 3.4 – 5.0
NaCl: 0 – 300 mM



DoE with PreDictor plates

Screening plates

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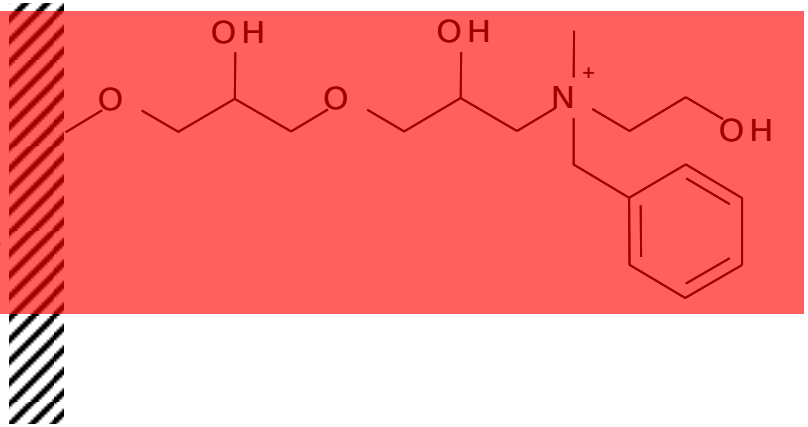
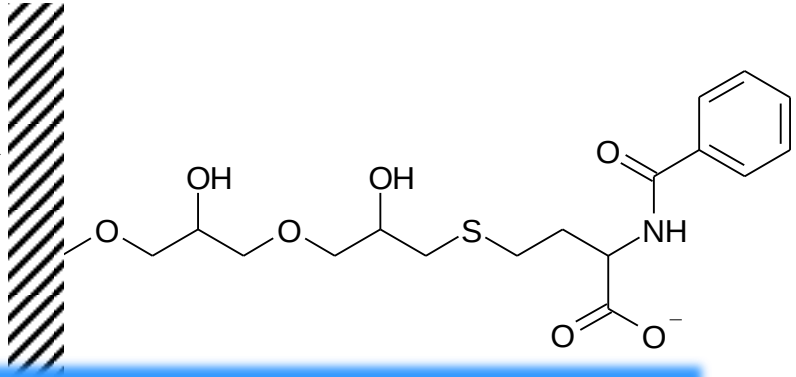
pH: 5.3 – 8.1
NaCl: 0 – 150 mM

	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto S				SP Sepharose Fast Flow				Capto MMC			
B												
C												
D												
E												
F												
G												
H												

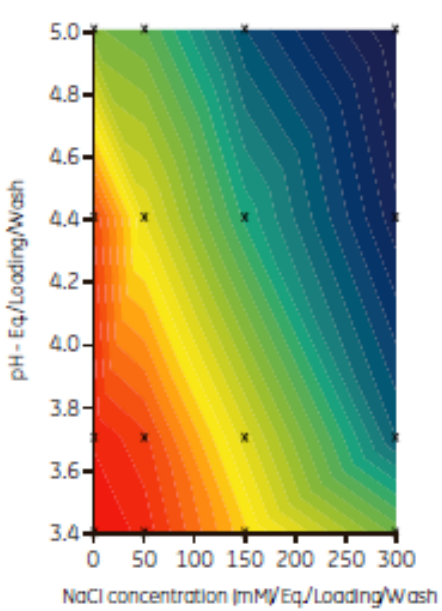
pH: 3.4 – 5.0
NaCl: 0 – 300 mM



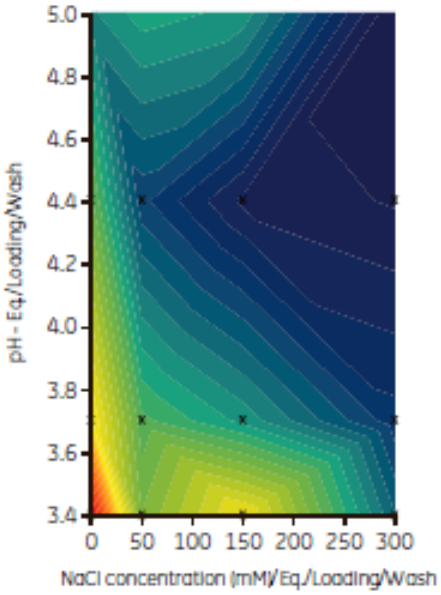


Result:
No binding of *r*-Pro-Insulin to AIEX or Capto™ adhere

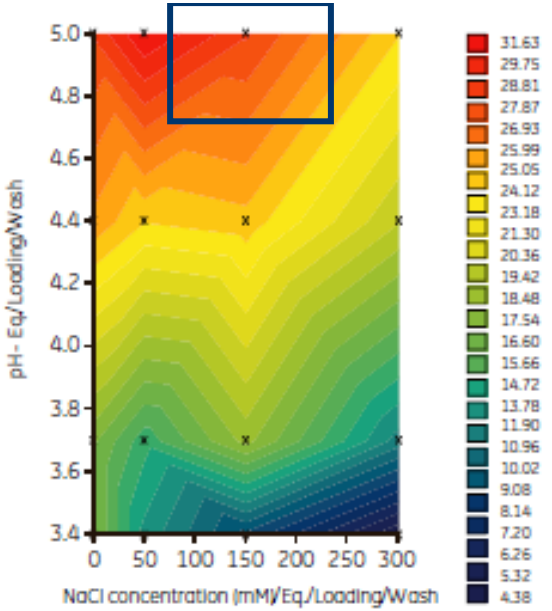
Binding capacity of r-Pro-Insulin on cation- and Capto MMC resins



SP Sepharose™ FF



Capto S

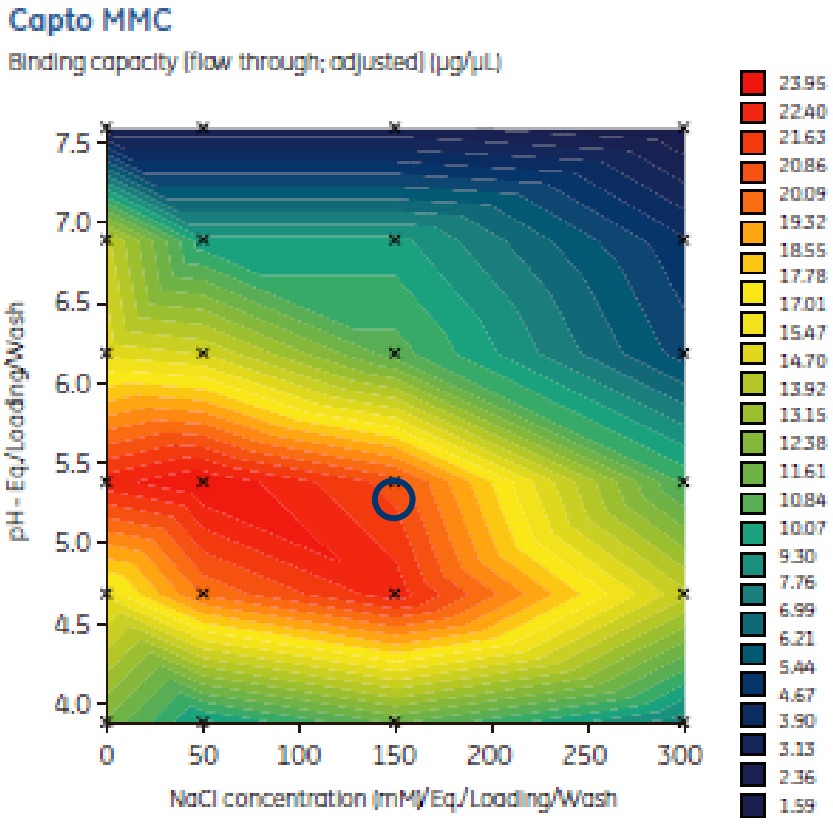


Capto MMC

Conclusion: Best resin at 150 mM salt → Capto MMC

Evaluation done in Assist SW – interpolation, no modeling

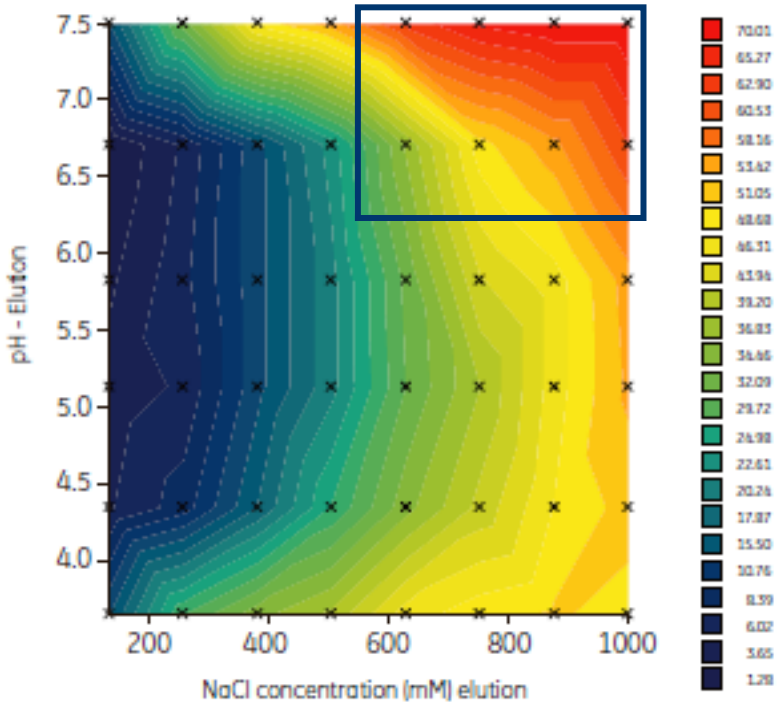
Expanded pH study on Capto MMC: pH 4-8



Conclusion:

Highest binding capacity = ~25 mg/ml at pH ~5.2 & 0-150 mM NaCl

Elution study in Capto MMC



Design:	
Sample load:	180 µl
total protein conc.:	8 mg/ml
pro-insulin conc.:	5 mg/ml i.e. ≈ 70% of est. capacity
Resin volume:	50 µl
pH range:	3.7 – 7.6 (6 levels)
NaCl range:	150 – 1000 mM (8 levels)

Conclusion:
Best elution conditions gave 70% Yield

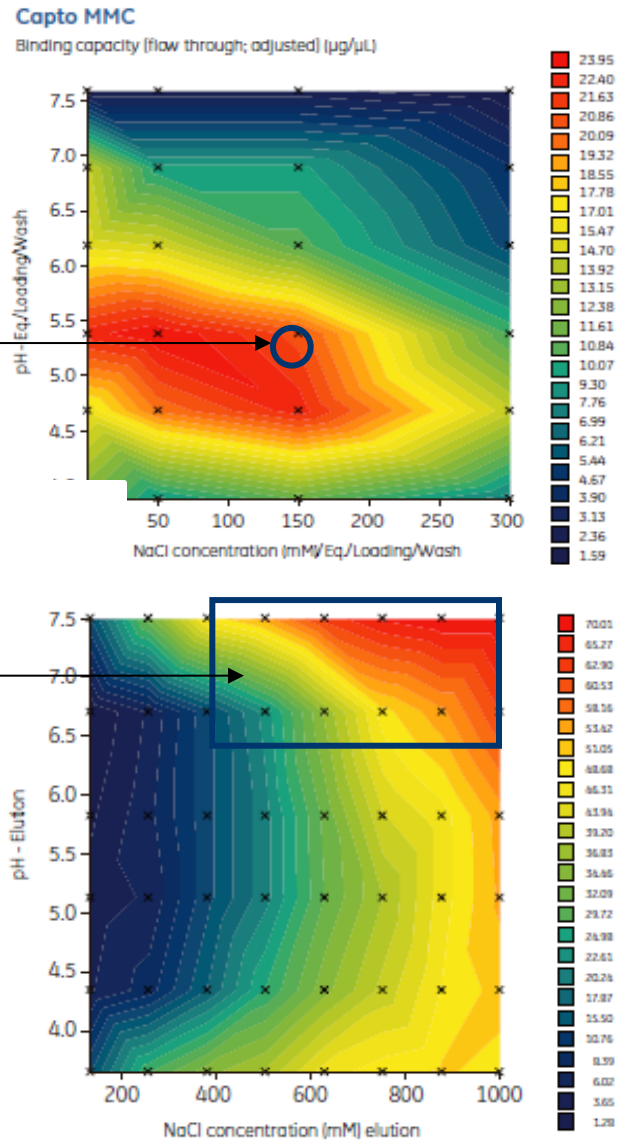
Conclusions from DoE studies

Binding conditions:

no desalting needed before chromatography,
pH in feed should be around 5.2

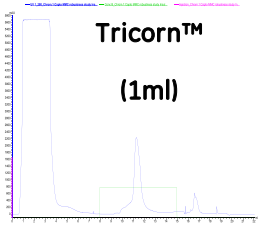
Elution conditions:

Conditions to be further optimized,
pH > 6.2, NaCl > 400 mM

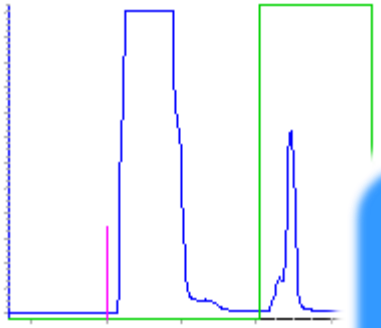


Workflow: HTPD Screening -> Column Optimization -> Scale Up

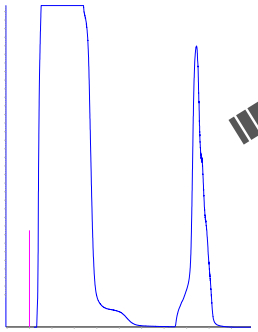
Screening



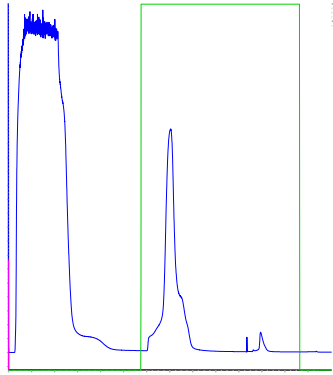
Optimization



HiScreen™
(2x5ml)



HiScale™
(40ml)



AxiChrom™
(400 ml)

→ Consistent scale up with results of
> 80% purity and > 95% yield

Summary

Summary

Quality by Design corner stones

- Systematic approach based on good science
- Increases product and process understanding

4 key steps in setting up the Process Design Space

- Process mapping
- Risk analysis and mitigation (FMEA*)
- Design of Experiments (relation of CMA, CPP to CQA)
- Design space description

DoE, more information by investigating the influence of several factors together

More precise information is acquired in fewer experiments

- Parallel formats enables coverage of larger experimental regions faster

* Failure Mode Effect Analysis

Thank you !

www.gelifesciences.com/bioprocess

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