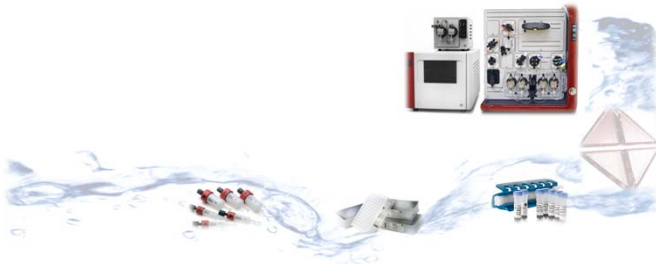
Design of Experiments (DoE) in chromatography
层析中的实验设计(DOE)



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Content内容

- Introduction介绍
- DoE Methodology DoE方法学
- DoE in chromatography 层析中的DoE
- Examples 应用实例

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Introduction

介绍

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What is Design of Experiments (DoE)?
什么是DoE?

Processes depend on multiple factors!

工艺基于多个因子（变量）

- Statistical multifactorial approach
  统计学的多因子实验方法
- Maximize information
  最大的信息量
- Minimize experiments
  最少的实验数量
- Detect interacting factors
  发现交互作用的因子

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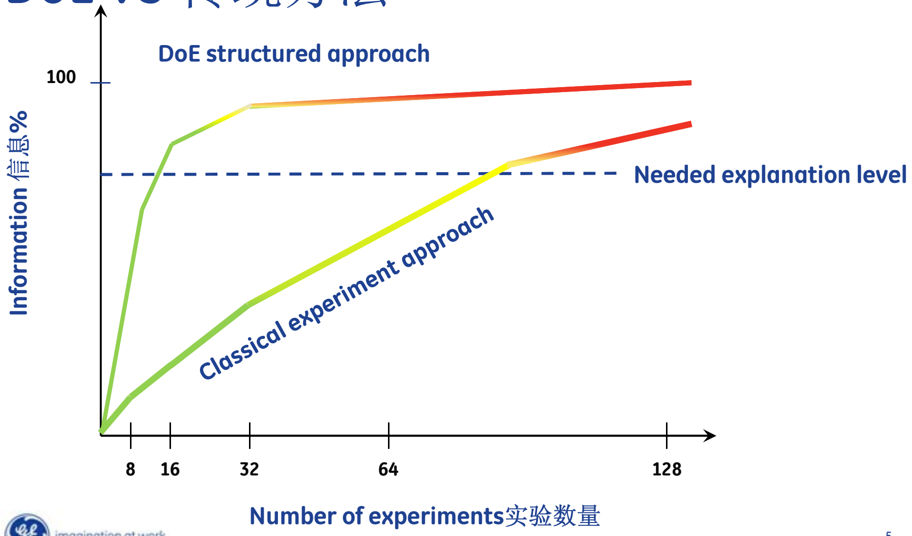
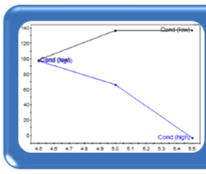
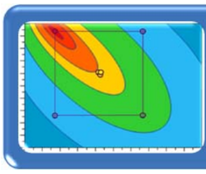
DoE vs. Classical approach
DoE VS 传统方法



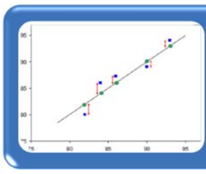
Output输出



Combined effects of input parameters
输入变量的组合效应

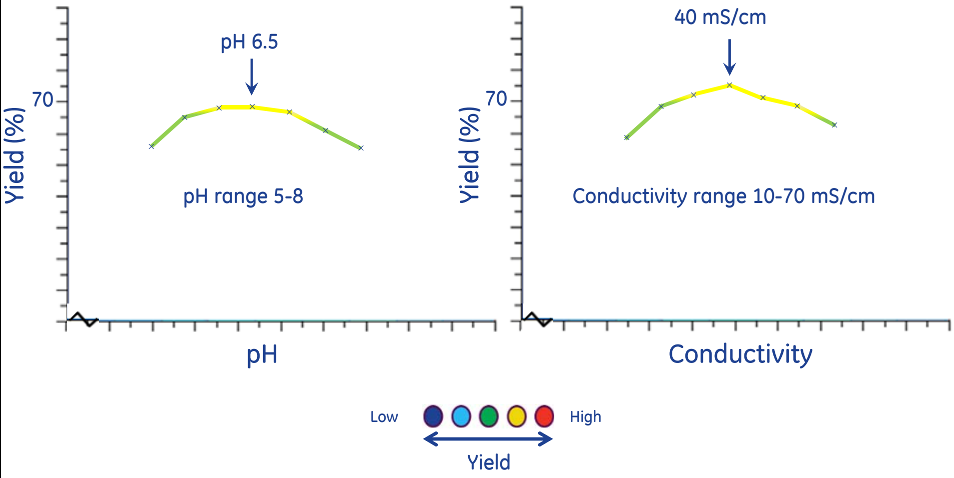


Model of the process
工艺模型



Quantified effects and noise
量化结果和噪音

One Factor At a Time单因子实验



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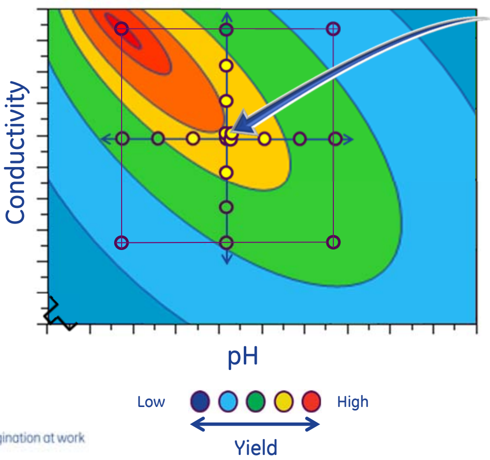
One Factor At a Time vs. DoE
单因子实验 VS DoE



pH 6.5, 40 mS/cm →
Yield 70-75%
Where would you run?

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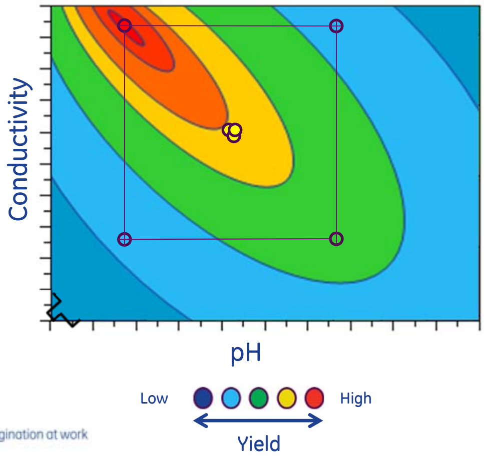
One Factor At a Time vs. DoE
单因子实验 VS DoE

Yield of the reaction is influenced by pH and conductivity! pH和温度共同影响收率



A two-factor interaction effect
两因子的交互相应

- The influence of one factor depends on the setting of another factor
  一个因子的效应取决于另一个因子
- Two-factor interactions are common in chemistry
  两因子交互作用在化学中很常见

One could imagine three-factor interactions but they are rarely (never) significant.
当然也会有三因子交互作用，但是几乎不显著甚至不存在

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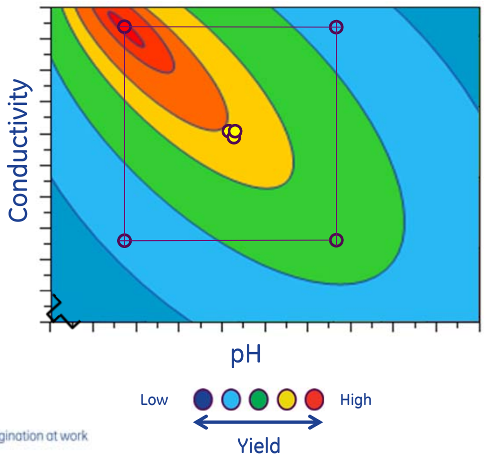
One Factor At a Time vs. DoE
单因子实验 VS DoE

Detection and quantification of interactions i.e. a true optimum can be found !
发现并且量化交互作用 才能找到真正的最优条件



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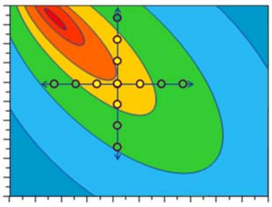
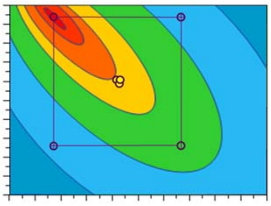
Advantages of DoE over OFAT
DoE对OFAT的优势：



vs.

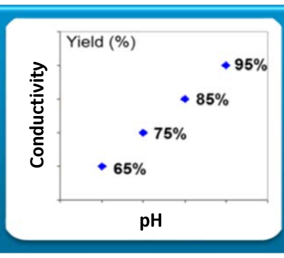


- OFAT → assumes response is a linear combination of the critical factors!
  OFAT: 假定响应是关键因子的线性组合
- OFAT → doesn't explore the whole experimental region!
  OFAT: 没有探索整个实验区间
- OFAT → risk for sub optimization, and/or unpleasant surprises!
  OFAT: 存在局部次优化和不愉快的意外风险!
- DoE → fewer resources for the amount of information obtained
  DoE: 更少的资源获得大量的信息
- DoE → estimates of the effects of each factor are more precise
  DoE: 更精准地评估各个因子的影响
- DoE → systematic estimation of interactions
  DoE: 系统化地评估交互作用
- DoE → experimental information from entire factor space
  DoE: 整个因子空间的实验信息
- DoE → obtain transfer functions to identify the optimum solution
  DoE: 获得传递函数，可以确定最优的结果

Box,Hunter, and Hunter (1978) *Statistics for Experimenters,* New York: Wiley.
Czitrom, Veronica, The American Statistician, May 1999, Vol. 53, No. 2 pp 126-131

Aliasing – something to avoid混淆：需要避免的情况

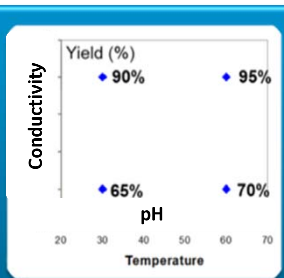
Example: Does Temperature, pH or both affect Yield?
例如：温度, pH 还是两者共同影响收率？



Alternative 1 结果1

pH and Conductivity are said to be **ALIASED** (confounded, correlated)
如果pH和温度被认为是混淆的（困惑的，关联的）

It is impossible to say if the effect we see on yield is due to pH, Conductivity or both!
无法确定对收率的影响是来自于温度, pH还是两者



Alternative 2结果2

pH and Conductivity are **NOT ALIASED** (non-confounded, uncorrelated)
如果pH和温度是不混淆的（不困惑的，不关联的）

We clearly see that both factors affect Yield, and that the effect from Conductivity is largest!
可以清楚地发现两个因子都影响收率，但是pH的影响更加显著

DoE Methodology

DoE方法学

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DoE terminology DoE的术语：

Method/Process

方法/过程

Factors
X's
因子（X）
Conditions
Controlled
Parameters
条件，可控变量

Transfer Function
(Model)
传递函数(模型):
$Y_k = f(X_i)+e$

Responses
Y's
响应（Y）：
Results
Output
Parameters
结果，输出变量

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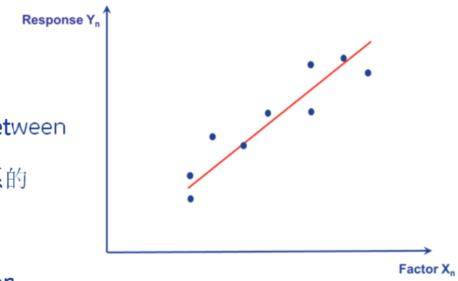
Transfer Function (Model)

传递函数（模型）

$$y = f(x) + e$$

- y — Measured responses
  可检测的响应
- x — Factor (parameter) settings
  设定的因子（参数）
- f(x) — The transfer function/model

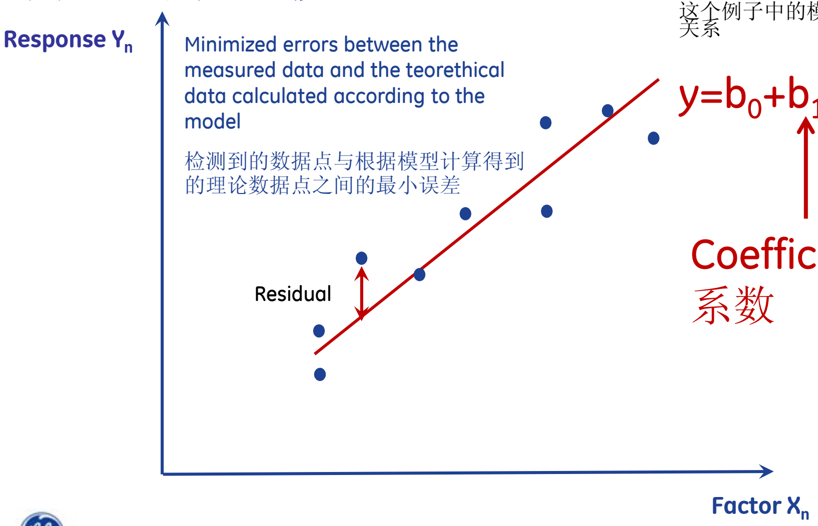
  quantifies the relationship between factors and responses
  定量描述因子和效应之间关系的传递函数（模型）
- e — Residuals= Random experimental variation Quantifiable in a DoE
  残差=可在DoE中量化的随机的实验变量



Transfer Function (Model)

传递函数（模型）

The “model”, in this case is a linear relationship
这个例子中的模型时线性关系



$$y=b_0+b_1x_1+e$$

Coefficients
系数

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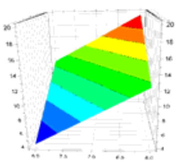
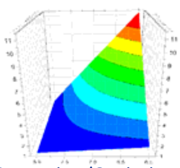
Transfer Function (Model)传递函数(模型)

Linear Terms (main effects) 线性项（主效应） Interaction Term(s) 交互项 Quadratic Term(s) 二次项

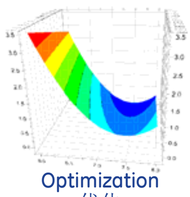
$$y = b_0 + \underbrace{b_1x_1 + b_2x_2}_{} + \underbrace{b_{12}x_1x_2}_{} + \underbrace{b_{11}x_1^2 + b_{22}x_2^2}_{} + e$$



Robustness/Screening
稳健性/筛选



Screening/Optimization
筛选/优化



Optimization
优化

$b_1 - b_2 - b_{12} - b_{11} - b_{12}$ coefficients give the quantified effects for the x's这些为传递函数的系数，是参数X被量化的影响.

e = prediction error
预测误差
$y_{predicted} - y_{measured}$

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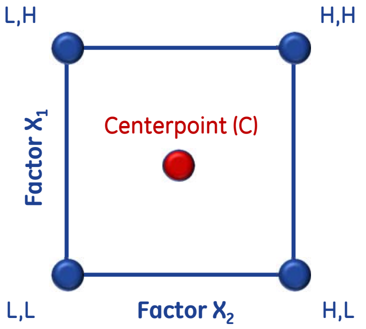
General design construction
一般的设计思路



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

H=high
L=low

Centerpoint used for estimation of noise and detection of curvature
中心点用于评估误差，发现曲率

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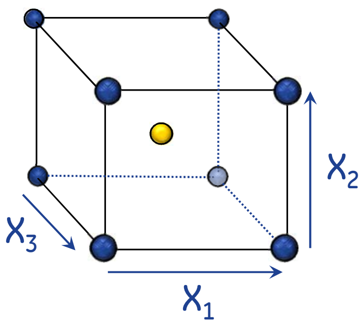
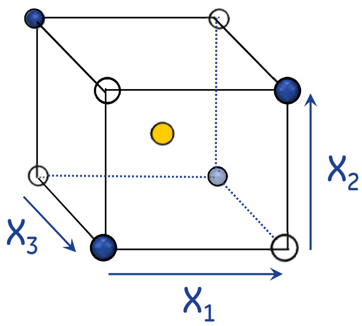
General design construction
一般的设计思路



Full Factorial design
完全析因设计



Fractional Factorial design
部分析因设计

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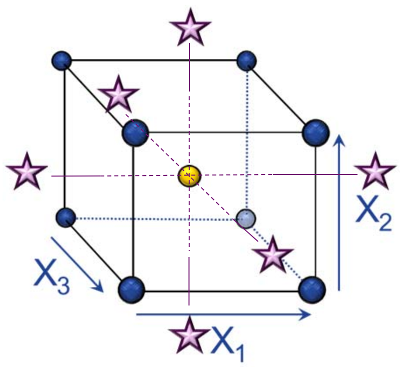
Central Composite Designs (CCD's)
中心复合设计



Corner Points拐点

The factorial part of the design.
For the assessment of linear and 2-way interaction terms.

设计的因子部分，评估线性和2次交互的常数项

Center Points中心点

Used to detect curvature.用于检测曲率

Replicated to estimate pure error
重复点用于估计纯误差

Star Points星点

For the assessment of quadratic terms, quantify curvature.

用于评估二次项的常数项，定量曲率

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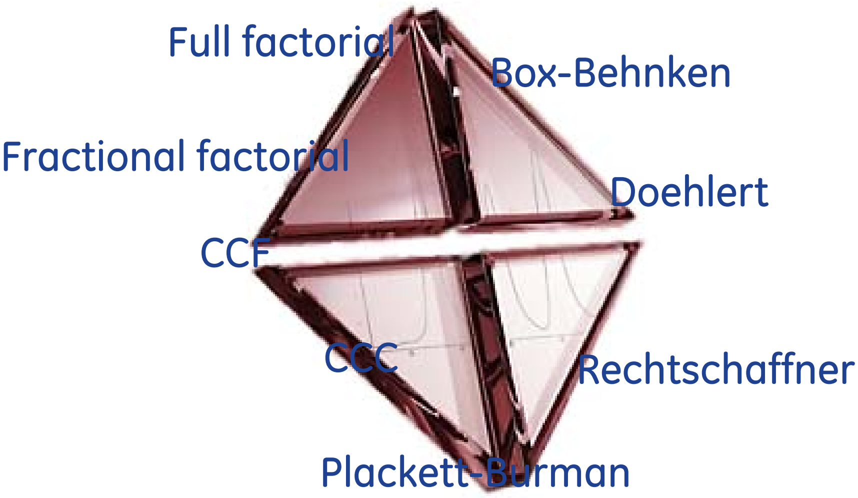
Which design? 选择何种设计方法



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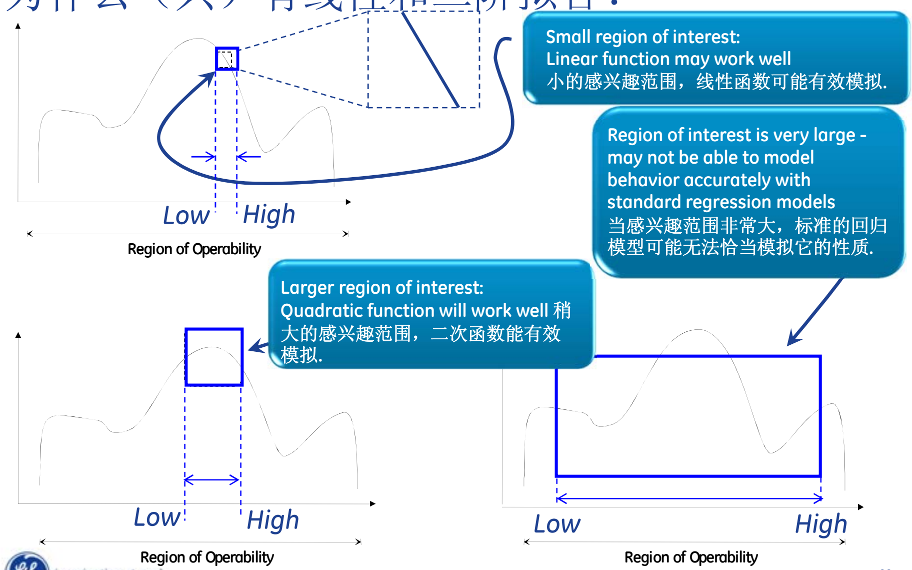
Why (only) linear and quadratic fits?
为什么（只）有线性和二阶拟合？



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How many runs in a design?
一次设计需要多少次实验?

# of runs for some common experimental designs	Number of X parameters: 2	3	4	5	6	7
Fractional factorial res III	--	7	--	11	11	11
Fractional factorial res IV	--	--	11	--	19	19
Fractional factorial res V	--	--	--	19	35	65
Rechtschaffner res V	--	10	14	19	25	32
Full factorial	7	11	19	35	67	131
Central composite RSM	11	17	27	29	47	81
Rechtschaffner RSM	--	13	18	24	31	39
Box-Behnken RSM	--	15	27	43	51	59
Doehlert RSM	9	15	23	33	45	59

- Res III = limited support for linear effects, no support for 2-way interactions and quadratics
- 分辨度为III的设计= 有限支持线性效应（各主效应之间没有混杂），不支持二阶交互效应和二次项
- Res IV = good support for linear effects, limited support for 2-way interactions, no quadratics
- 分辨度为IV的设计= 能很好地支持线性效应，有限地支持二阶交互效应，不支持二次项
- Res V = good support for linear and 2-way interaction effects, no quadratics
- 分辨度为V的设计=能很好地支持线性效应和二阶交互效应，不支持二次项
- Full factorial = good support for linear and all order of interactions, no quadratics
- 全因子=能很好地支持线性效应和全部交互效应，不支持二次项
- RSM = Optimization designs, good support for linear, 2-way interaction and quadratic effects
- 响应面=优化设计，能能很好地支持线性效应，二阶交互效应和二次项

DoE approach DoE的方法

Plan
设计

Screen
筛选

Optimize
优化

Test Robustness
实验稳健性

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In practice....实践中

Plan to find critical factors
计划寻找关键性因子

Set specification limits
设置参数范围

Create an experimental design
创建实验设计方法

Review plan
回顾计划

Perform experiments
执行实验

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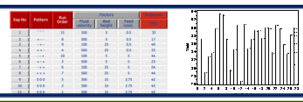
DoE evaluation workflow DoE评估流程

Raw data evaluation
原始数据评估



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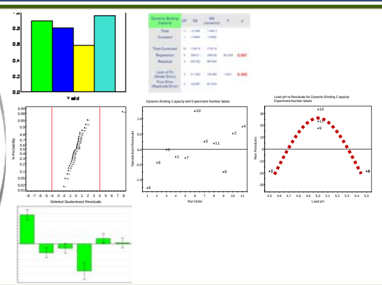
R2, Q2, Model Validity, Rep
R2,Q2,模型合理性，重复性， ANOVA表roducibility, ANOVA table

Residual (e-part) analysis
残差（e）分析

Analyze f(x) part, Coefficient plot, Model refinement
分析f(x)部分，系数图，模型优化



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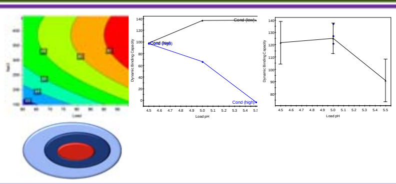
Response surface plot, Interaction plot
相应曲面图，交互效应图

Optimize, define Design space, define Control space
优化，定义设计空间，可控空间



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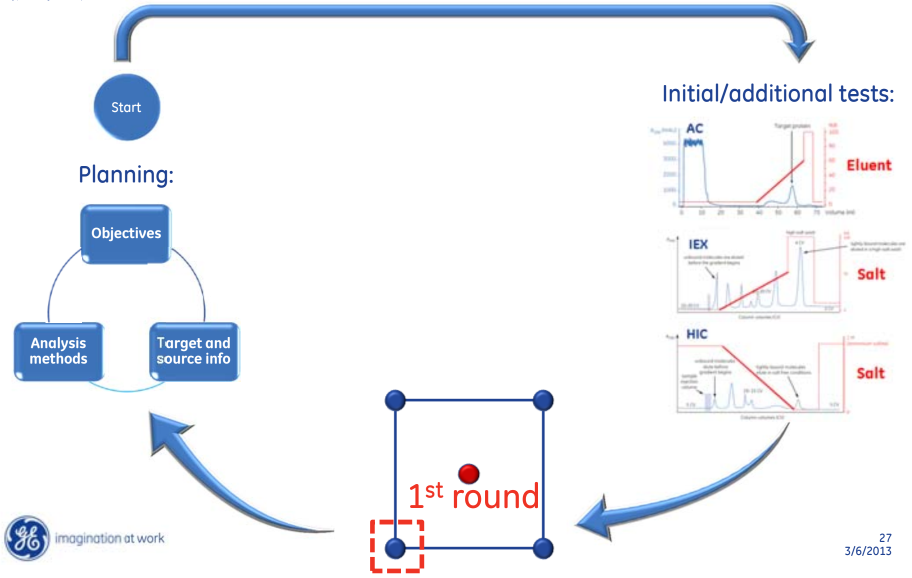
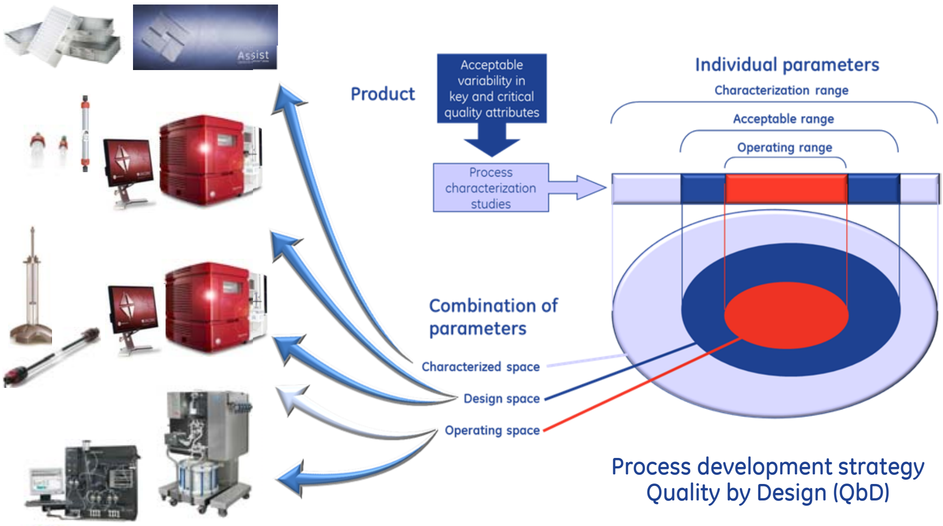
Strategy for performance: Iteration
执行的策略：循环



Another strategy for performance: Scale
执行的其他策略：放大



DoE in chromatography
层析中的DoE

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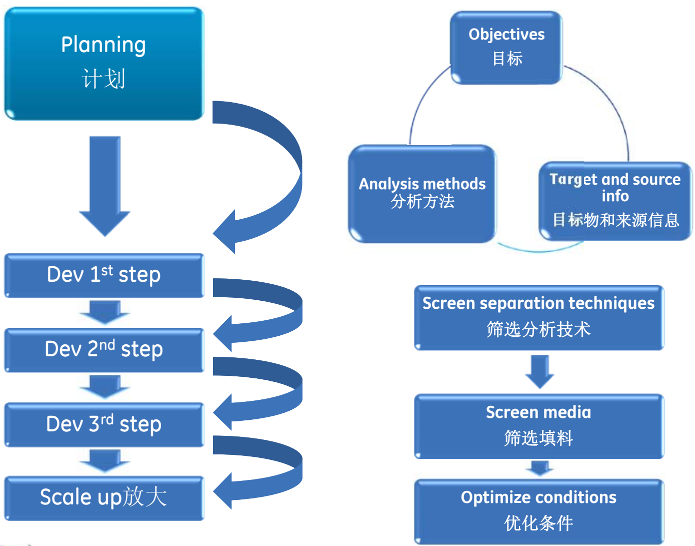
Purification protocol development
纯化方案的开发



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How many purification steps is required需要多少纯化步骤呢?

Protein purification and analysis

蛋白纯化和分析

Protocol development
开发方案

- Confirm correct protein
  确认正确表达蛋白
- Target protein concentration
  目标蛋白浓度
- Activity活性

- Protein solubility蛋白稳定性
- Confirm correct protein
  确认正确表达蛋白
- Target protein concentration
  目标蛋白浓度
- Activity活性
- Purity纯度
- Size homogeneity大小均一性
- Protein stability蛋白稳定性
- Protein modifications蛋白修饰
- Application specific analysis
  特定应用的分析方法

Expression
表达

↓

Sample preparation样品准备

↓

Chromatographic purification
层析纯化

↓

Storage
保存

Routine process
常规步骤

- Target protein concentration
  目标蛋白浓度

- Target protein concentration
  目标蛋白浓度
- Purity纯度

- Size homogeneity 大小均一性
- Confirm correct protein确认正确表达蛋白
- Activity活性
- Application specific analysis
  特定应用的分析方法

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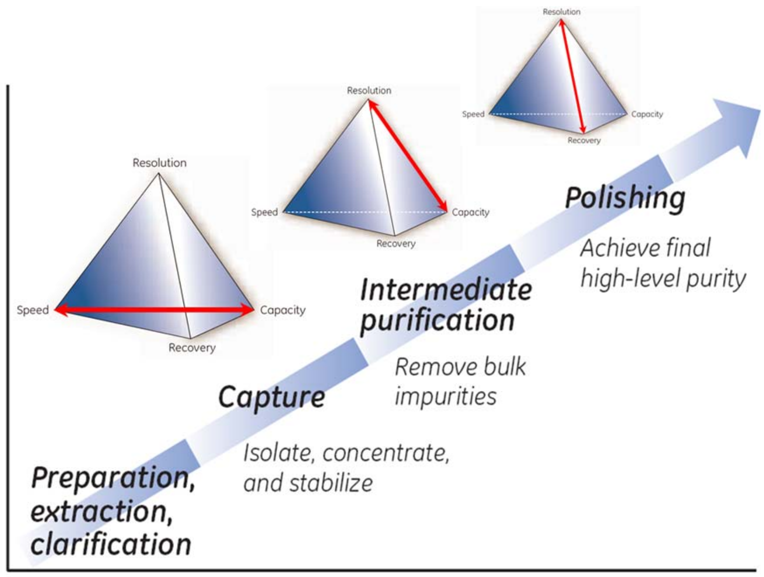
Apply CiPP Strategy!应用CiPP策略！



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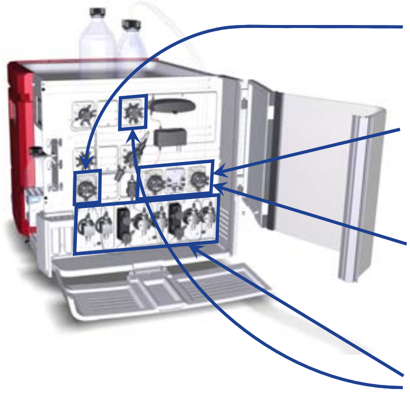
Factors and responses in chromatography 层析中的因子和响应：



Sample conditions
上样条件

Wash conditions
清洗条件

Elution conditions
洗脱条件

Entire process
整个过程

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 Additive
- Media type
- Column size
- Bed Height
- Flow rate
- Residence time

Responses

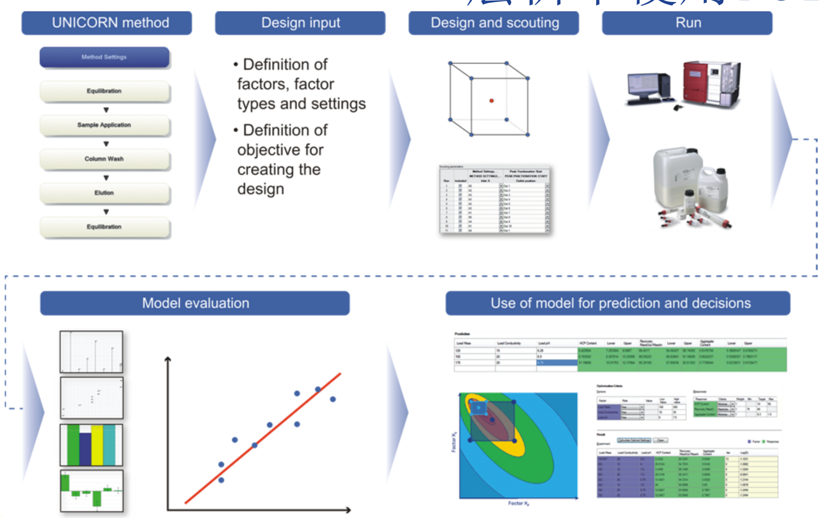
External data:

- Capacity
- DBC (Frontal analysis)
- Yield
- Purity/Selectivity
- Molecular weight
- Activity
- HCP
- DNA
- Aggregates
- Protein A

Peak Data:

- Area
- Concentration
- Amount
- Resolution
- Asymmetry
- Plates per meter

Performing DoE in chromatography 层析中使用DoE



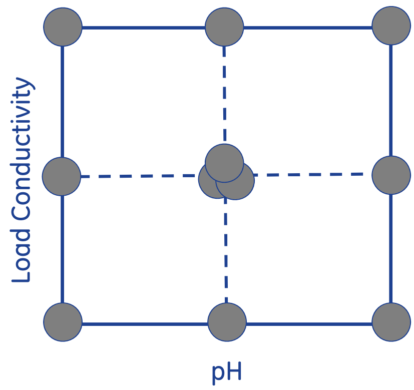
Example: optimization study
实例：优化研究

Optimization of loading conditions
上样条件的优化

•Optimization of MAb sample loading conditions on Capto S

优化Capto S上MAb样品的上样条件

Exp No	Exp Name	Run Order	Load pH	Load Conductivity
1	N1	4	4.5	5
2	N2	10	4.5	15
3	N3	3	5.5	5
4	N4	11	5.5	15
5	N5	7	5	5
6	N6	1	5	15
7	N7	5	4.5	10
8	N8	9	5.5	10
9	N9	2	5	10
10	N10	6	5	10
11	N11	8	5	10

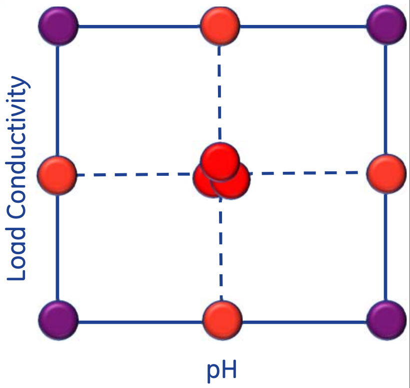


Evaluate CCF design CCF设计的评估：

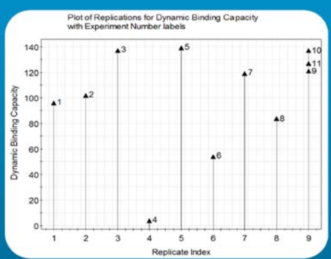
Model: $DBC = k + b_1{*}pH + b_2{*}Cond + \underbrace{b_{11}{*}pH^2 + b_{22}{*}Cond^2 + b_{12}}{*}pH{*}Cond + e$

Our design supports quadratic terms
我们的设计支持二次项

Exp No	Exp Name	Run Order	Load pH	Load Conductivity	Dynamic Binding Capacity
Factorial part			4.5	5	96
			4.5	15	102
			5.5	5	137
			5.5	15	4
Star points			5	5	139
			5	15	54
			4.5	10	119
			5.5	10	84
Replicated Center Points			5	10	121
			5	10	137
			5	10	127



Replicate plot of the raw data
原始数据的重复点图



Replicate variation vs.variation in entire experimental design.
重复变量 VS 整个实验设计中的变量

Replicate variation ok?重复变量OK?

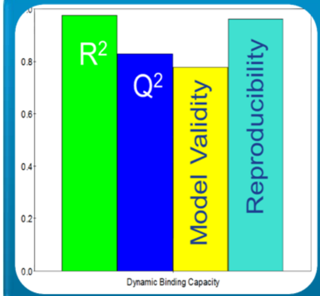
Response target range?响应的目标范围?

Suspicious values?可疑点?

2^{nd} degree curvature?二次曲率？

Summary of Fit拟合的总结

Model模型: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e



Model describes the observed variation in DBC

模型描述了**DBC**实验中所有可检测到的变量

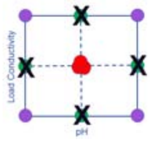
Excellent R^2 & very good Q^2.极好的R^2 & Q^2

Model validity: residual variation < reproducibility of center points, i.e. no Lack of Fit.

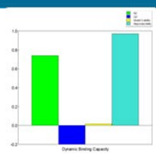
模型的有效性：残差的变化<中心点的重复性

Reproducibility bar: Center point variation low compared to overall variation

重复性：比较总变异和中心重复点间变异计算得到的



Bad model →
差的模型



ANOVA table ANOVA表

Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e

Dynamic Binding Capacity	DF	SS	MS (variance)	F	p
Total	11	131398	11945.3		
Constant	1	114036	114036		
Total Corrected	10	17361.6	1736.16		
Regression	5	16919.1	3383.82	38.2334	0.001
Residual	5	442.522	88.5044		
Lack of Fit (Model Error)	3	311.855	103.952	1.5911	0.408
Pure Error (Replicate Error)	2	130.667	65.3334		

ANOVA statistical testsANOVA统计学检验

Current model OK!现有模型OK！

Variance in model (Regression) > Variance not captured (Residual) → R^2 value is significant

模型（回归方程）方差>剩余方差→ R2显著

Model Error (residuals excluding replicate variance) is NOT significantly greater than the Replicate Error i.e. the model has No Lack of Fit.模型误差（除掉重复试验的剩余方差）不明显大于重复误差，模型不失拟。.

Dynamic Binding Capacity	DF	SS	MS (variance)	F	p
Total	7	87944	12563.4		
Constant	1	74882.3	74882.3		
Total Corrected	6	13061.7	2176.95		
Regression	3	9674.75	3224.92	2.85646	0.206
Residual	3	3386.96	1128.99		
Lack of Fit (Model Error)	1	3256.3	3256.3	49.8413	0.019
Pure Error (Replicate Error)	2	130.667	65.3334		

← Bad model
Significant lack of fit
差的模型
丢失重要的拟合项

Conclusions so far目前的结论

Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e

Statistically significant portion of the observed variation in DBC explained.

DBC中检测变量的统计学显著性得到解释

Variation in model residuals (the model error) is NOT significantly larger than the variation in the replicated center points

模型残差方差（模型误差）不显著大于中心重复点方差.

Our current model is of sufficient complexity to describe the process.

目前的模型有足够的复杂度来描述该工艺。

→ **Next step: Evaluate the residual variation.**

下一步，评估剩余方差

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Analyzing the residuals残差分析

Residuals = observed – predicted for each experiment 残差=观测值-实验预测值

Ideally, the model error (residuals) will consist of normally distributed random variation from the experimental process and measurement systems. IF the residuals are NOT normally distributed, one should try to identify the reason why. 理想情况下，实验过程和分析系统中模型错误（残差）会服从正态分布的随机变化。如果残差不是正态分布，必须分析确定原因

Tool工具:

Normal probability plot of the residuals残差正态分布图

Also, there should be no trends visible when looking at the model error versus either the run order in which the experiments were performed or versus any of the X variables.另外，在残差 vs 实验顺序或 vs 任何X变量时，应该无明显的趋势性

Tools工具:

Residuals vs run order plot残差 vs 实验顺序

Residuals vs individual X variables残差 vs X变量

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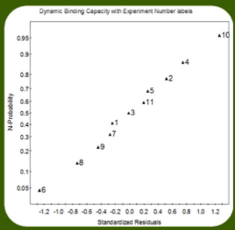
Normal probability plot of residuals
残差正态分布图

Model: $DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*Cond^2 + b_{12}*pH*Cond + e$



Forms a nice straight line with no outliers or groupings in the data
形成一条漂亮的直线，并且没有异常值或者组群的值.

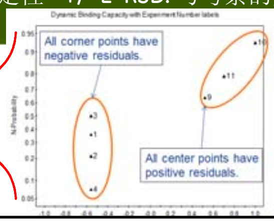
Implies residuals are normally distributed.表明残差是正态分布

Describing the experimental noise描述了实验噪音.

All experiments used to provide information on the random variability of a process.
所有的实验用于为工艺中的随机变量提供信息

Model uncertainty ~ +/-2*RSD. Less near the center of the investigated experimental space, more towards the outer limits of the X parameters.模型的不确定性~ +/- 2*RSD. 与考察的实验区间中心越远，越靠近X的外部极限

Non-normally distributed (random)
非正态分布（随机）
Systematic variation
系统偏差



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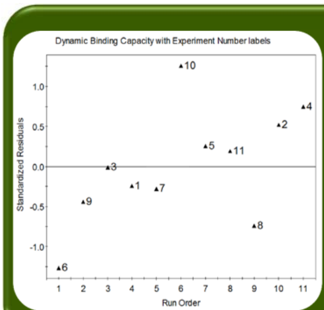
Residuals vs run order残差 vs 实验顺序

Model: $DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*Cond^2 + b_{12}*pH*Cond + e$



There are no strong trends in the residuals when plotted vs the order in which the experiments were performed按照实验顺序作图时，没发现残差有明显

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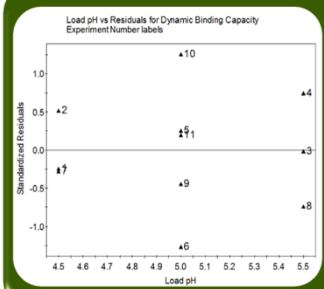
Residuals vs Load pH残差 vs Load pH

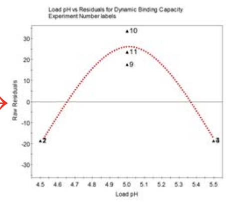
Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e



When plotting the residuals vs Load pH, no curvature or trends are present.

当使用残差Vs上样pH作图时，不存在曲率或趋势

Curvature! →
曲率



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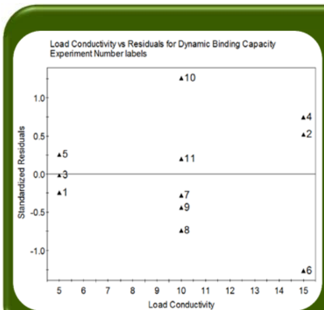
Residuals vs Conductivity残差 vs 电导

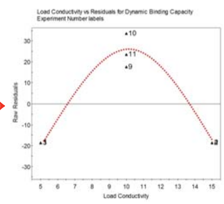
Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e



When plotting the residuals vs Load Conductivity, no curvature or trends are present.

当使用残差Vs上样电导作图时，不存在曲率或趋势

Curvature! →
曲率



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Conclusions so far目前的结论

Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e

The current model describes a statistically significant portion of the observed variation in Dynamic Binding Capacity (DBC)

目前模型具能有统计学意义地描述动态载量中观测到的变化.

The variation in model residuals (the model error) is NOT significantly larger than the variation in the replicated center points.

模型方差中的变化没有比在中心重复点上的变化显著大很多

The residuals are normally distributed with no outliers or groupings in the data, therefore the residual standard deviation (RSD) can be used as a measure of the random variability of the process (including measurement system variation).

残差符合正态分布并且没有异常值或组群，因此剩余标准偏差可以用于估量在过程中的随机变化性（包括测量系统引起的变化）

→ We are now done with the ”e” part of Y = f(X) + e

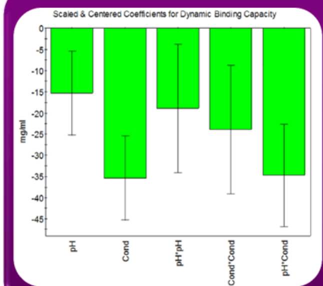
我们现在分析完传递函数的误差部分

→ **Next step: Evaluate the model, i.e. the f(X) part of Y = f(X) + e**

下个阶段：评估模型

Model coefficient plot模型系数图

Model: DBC = k + b_1*pH + b_2*Cond + b_{11}*pH^2 + b_{22}*$Cond^2$ + b_{12}*pH*Cond + e

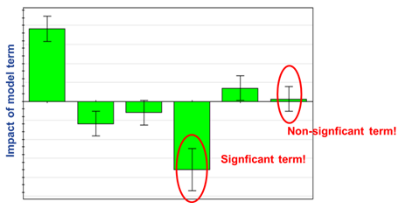


Verifiy that all model terms are statistically significant.

Statistically significant model terms (95% confidence level)

验证了所有的模型系数都是统计学显著(95%置信)

Model refinement: Remove non-significant model terms!
模型修饰：去除不显著的模型条件



Interpreting the model解释模型

Now that we have successfully evaluated and verified that the model is sound from a statistical perspective, we can look at interpreting the quantified cause and effect relationships for our investigated process.

现在我们已经成功从统计学观点评估并验证了模型的可行性，接下来可以解读量化的因果关系

Remember that statistical significance, while important, always should be considered from a domain expertise perspective. In other words, the DoE model should make sense.

记住，统计学意义虽然重要，但是总应该从一个专业领域的视角考虑。换句话说，DoE模型应该能被解释。

For the purpose of interpreting the model, the following tools are frequently used in DoE:为了解模型，下面的工具常在DoE中用到：

Interaction plots交互作用图

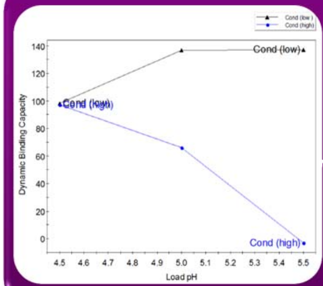
Main effect plots主效应图

Response surface plots响应曲面图

Interaction plots 1(2)交互作用图

How the effect from pH on DBC depends on conductivity

pH对DBC的影响取决于电导



Effect from Load pH on DBC depends on the Load conductivity.

上样pH对DBC的影响依赖于上样时的电导

Low conductivity (5 mS/cm) → increasing the pH results in an increased DBC, but at high conductivity cm) the effect from increasing the pH is a drastic DBC.

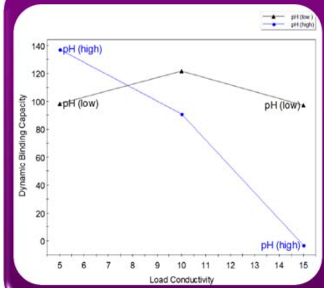
低电导(5ms/cm) → 增加pH会增加DBC；高电导时增加pH会使DBC急剧下降。

Note: this plot should only be interpreted for significant interaction effects.

注意：这条曲线仅仅解释有重要交互关系的影响因素

Interaction plots 2(2)交互作用图

How the effect from conductivity on DBC depends on pH
电导对DBC的影响取决于pH



This plot shows how the effect from Load conductivity on DBC depends on the Load pH.

这张图显示了上样电导对DBC的影响依赖于上样pH

At low pH (4.5), increasing the conductivity results in a slightly increased DBC followed by a slight drop, but at high pH (5.5) the effect from increasing the conductivity is a drastic drop in DBC.

低pH时（4.5）：增加电导会使DBC先小幅增加，再小幅降低；高pH时，增加电导会使DBC急剧下降。

Note: this plot should only be interpreted for significant interaction effects.
注意：这条曲线仅仅解释有重要交互关系的影响因素

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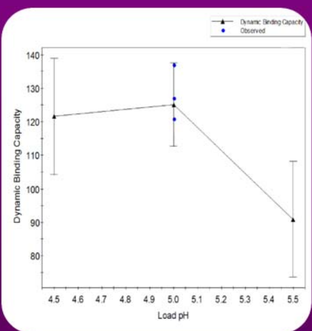
Main effect plots 1(2)主效应图

How pH affects DBC around the center point
在中心点pH影响DBC



This plot shows the effect from Load pH on DBC at center point setting for Load conductivity.

这张图显示了在上样电导中心点时上样pH对于DBC的影响

The error bars indicate the width of the model prediction confidence intervals.

误差棒显示了模型预测值的置信区间的宽度。

The replicated center points are shown in blue.

重复点用蓝色显示

Note: this plot should mainly be used for X parameters involved in no or relatively small interaction effects, or in direct combination with the interaction plot(s).
注意：这个图主要用于该X变量没有或较小的参与到交互作用中

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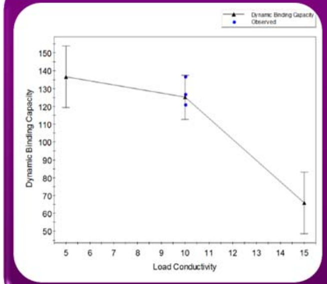
Main effect plots 2(2)主效应图

How conductivity affects DBC around the center point



Similar to the previous slide, this plot shows the effect from Load conductivity on DBC at center point setting for Load pH.

与上张slide相似，这个图显示的是在中心点电导影响DBC。

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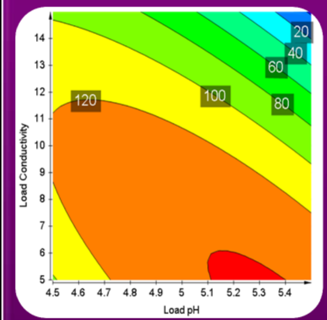
2D Response surface plot
2D 相应曲面图

How DBC depends on both Load pH and Load Conductivity
pH和电导对DBC的影响



This response surface shows the quantified effects, i.e. the f(X) part, from Load pH and Load Conductivity on DBC.
显示的是pH和电导对DBC定量的效应

Great tool for visualizing interaction and curvature effects.可视化交互效应和曲率的工具

The residual standard deviation (RSD) should always be considered when looking at these plots.
当观察这些图时，剩余的标准偏差（RSD）要一直铭记于心

For this model, RSD = 9.4, ie the model uncertainty is roughly +/- 18.8 mg/ml.
从这个模型看出，RSD=9.4, 模型的不确定性大概是+/- 18.8 mg/ml.

Example:Robustness testing

实例：稳健性实验

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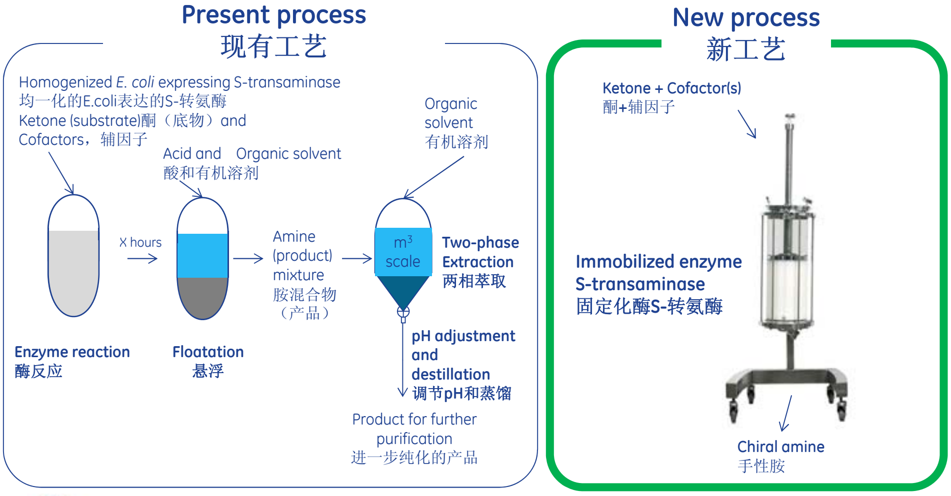
Robust NHS coupling?

NHS偶联的稳定性实验？



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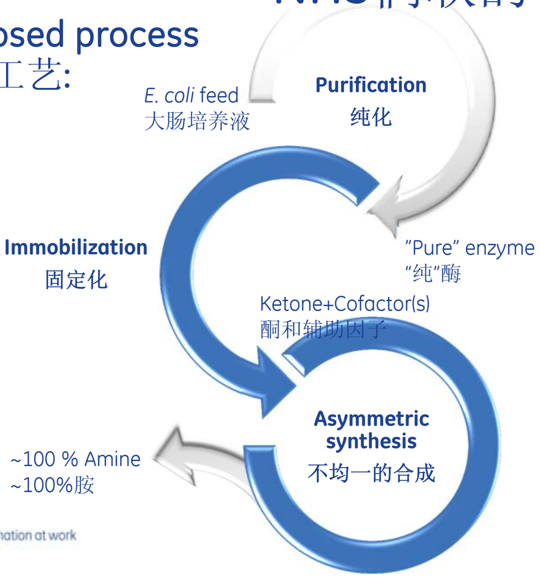
Robust NHS coupling?
NHS偶联的稳定性实验？

Proposed process
建议工艺:



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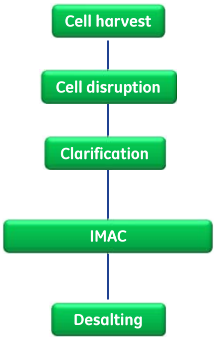
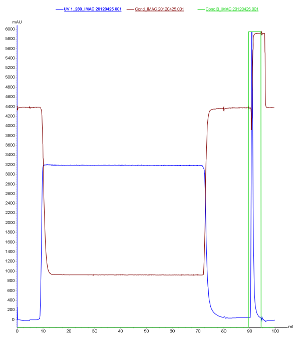
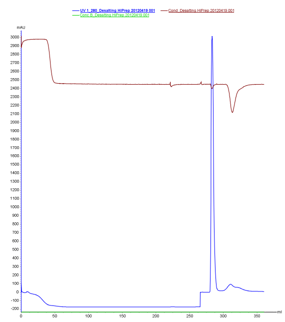
IMAC purification and desalting
IMAC纯化和脱盐







Columns: HisTrap™ 5 ml
Injection method: System pump via Mixer valve V9-M
Sample volume: 60 ml
Sample: Clarified homogenate from recombinant *E. coli* expressing transminase
Eluent A: 20 mM sodium phosphate buffer, 0.5 mM pyridoxal phosphate (PLP), 500 mM NaCl, 20 mM imidazole, pH 7.65
Buffer B: 20 mM sodium phosphate buffer, 0.5 mM PLP, 500 mM NaCl, 500 mM imidazole, pH 7.65
Flow: 5 ml/min
UV cell: 2 mm
System: ÄKTA™ pure

System configuration: 0.5-mm tubing, Column valve V9-C, UV-monitor U9-M, pH/restrictor valve V9-pH, Outlet valve V9-O

Columns: HiPrep™ 26/10 Desalting
Injection method: Superloop™
Sample volume: 4.5 ml
Sample: Eluate from IMAC purification
Eluent: 200 mm $NaHCO_3$, 0.5 mM PLP, 500 mM NaCl, pH 8
Flow: 5 ml/min
UV cell: 2 mm
System: ÄKTA pure

System configuration: 0.5-mm tubing, Column valve V9-C, UV-monitor U9-M, pH/restrictor valve V9-pH, Outlet valve V9-O

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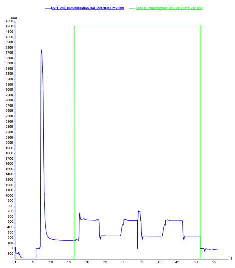
Robust NHS coupling?NHS偶联稳定性试验？(Full Factorial完全析因)

Factors因子
Load上样量: 3-5 mg
Time时间: 30-120 min
pH: 8-9

Response响应
Coupling efficiency偶联效率

Exp no	Run No	pH	Incubation time (min)	Load (mg/ml)
1	9	8	30	3
2	11	8	120	3
3	2	8	30	5
4	1	8	120	5
5	3	9	30	3
6	7	9	120	3
7	8	9	30	5
8	10	9	120	5
9	6	8.5	75	4
10	4	8.5	75	4
11	5	8.5	75	4



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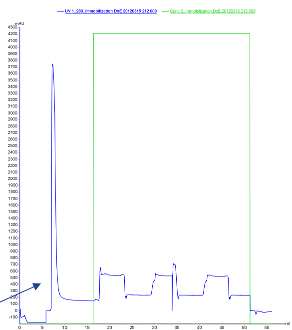
Scouting of NHS coupling conditions 优化NHS偶联的条件

Columns: HiTrap™ NHS-activated HP, 1 ml
Injection method: Loop valve, V9-L, with 1-ml loops
Sample volume: up to 0.7 ml
Sample: Purified transminase in carbonate buffer, pH 8.0, pH 8.5, pH 9.0
Eluent A: 100 mM sodium phosphate buffer, 0.5 mM PLP, pH 7.5 (coupling buffer)
Buffer B: 100 mM Tris-HCl, 0.5 mM PLP, pH 8.5
Buffer C: 100 mM Acetate buffer with 0.5 mM PLP, 0.5 M NaCl, pH 4
Buffer C: 1 mM HCl
Flow: 1 ml/min
UV cell: 2 mm
System: ÄKTA™ pure

System configuration: 0.5-mm tubing, Column valve V9-C, UV-monitor U9-M, Loop valve V9-L



N-hydroxysuccinimide group, which is released during the coupling reaction, shows strong absorbtion at 280 nm (pH above 6). Clearly this peak can also be caused by protein that was not coupled. See column instructions for description of evaluation of binding efficiency.
偶联过程中释放的N- 琥珀酰亚胺基团，在280nm有强吸收值(pH>6)，可以清晰地看到这个峰是由于没有偶联的蛋白造成的。利用这个峰来计算偶联率

Method outline

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Robustness check of NHS-coupling conditions NHS偶联的条件的稳定性试验检验

Scouting runs using ÄKTA™ pure
使用ÄKTA™ pure进行Scouting

Factors

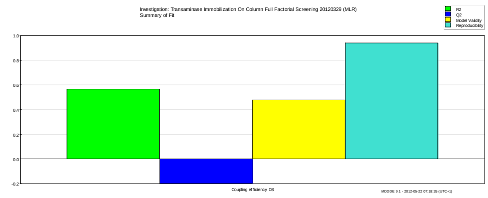
Load: 3-5 mg

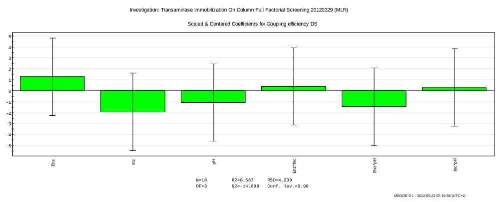
Time: 30-120 min

pH: 8-9

Response

Coupling efficiency: 87.5-98.3 %





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Summary总结

Organized approach which connects experiments in a rational manner

有组织的方法，使得实验理性科学地进行

More information by investigating the influence of all factors together

通过同时考察所有因子的影响来获得更多的信息

More precise information is acquired in fewer experiments

更少的实验得到的是更加准确的信息

Results are evaluated in the light of variability

根据可变性来评估结果

Support for decision-making

决策强有力的支持

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Thank you!

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Handbooks from GEHC

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