Optimizing biopharmaceutical filtration processes using DoE

Johannes Buyel

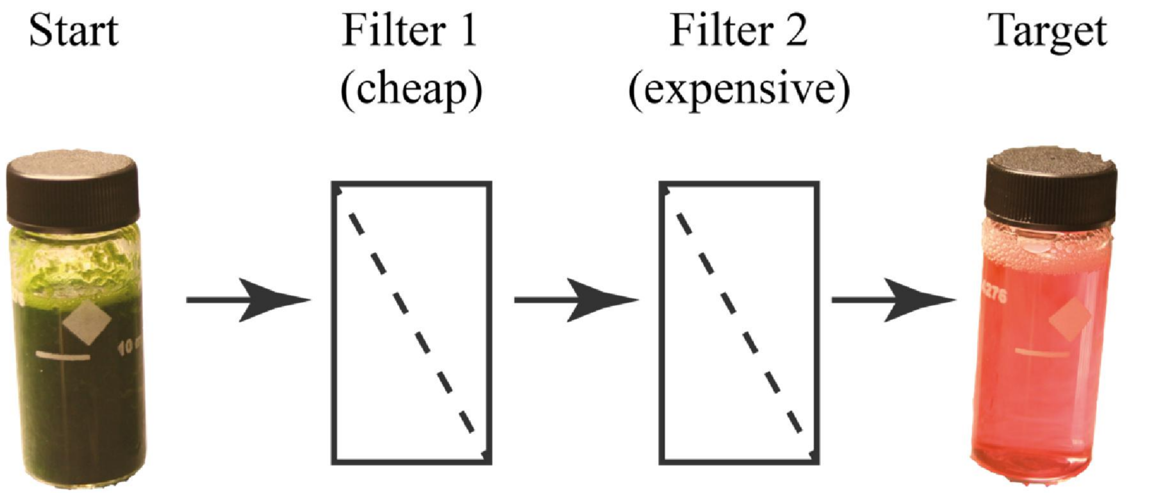
4th European DoE User Meeting – Vienna
28.6.2012

Setting out the problem

- Benefits of plants as a production platfrom for biopharmaceutical proteins
  – Ease of scale up
  – No human phathogens
- Drawback
  – Expensive downstream processing equipment and consumables
- Task
  – Reduce the consumables cost

http://www.freestock.at/wp-content/uploads/euro-muenzen-haufen-720x532.jpg

Starting point



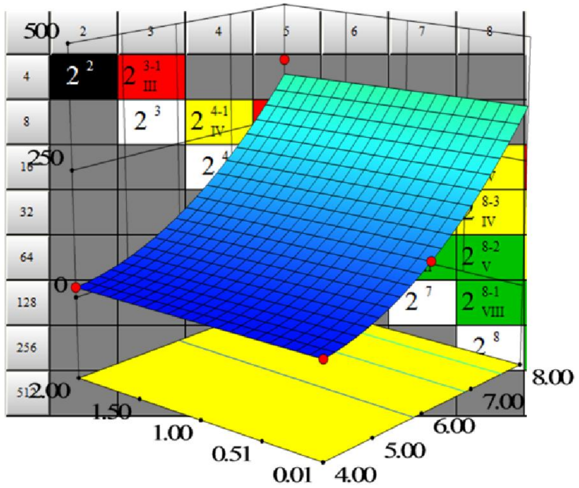
- **Disposable filters are a major cost factor**
  - Filter life time (cost-efficacy) is low compared to other processes improvement possible
- **A broad variety of filter additives is available to improve filter life time**

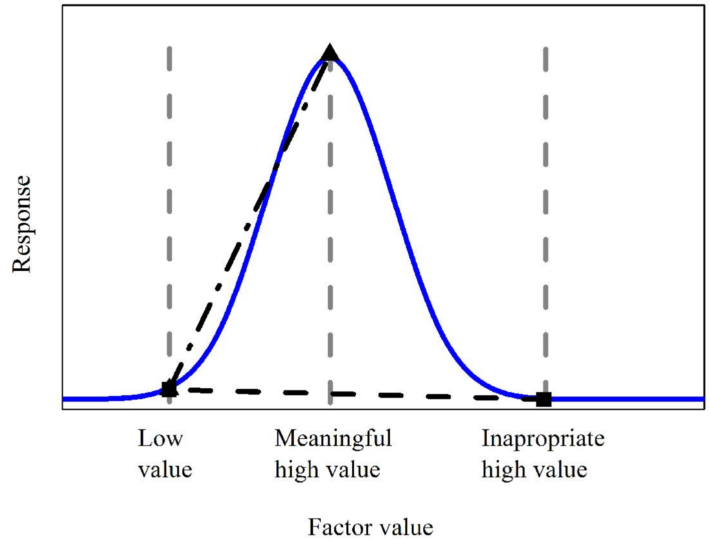
□ Set up a screening plan

Screening of additives

- First thought: 2-Level factorial
- Problems:
  - Meaningful parameter ranges can depend on additive type
  - Additive type is a categoric factor; pH and concentration are numeric

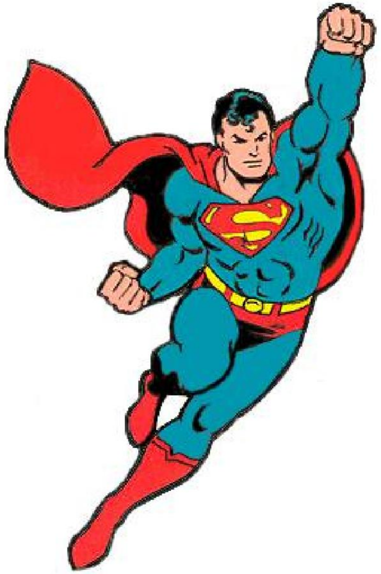
Use an IV-optimal RSM design





Screening plan

- 18 additives and control
- Concentrations spanning three orders of magnitude
- Three pH values: 4, 6 and 8
- Exclude conductivity and incubation time to reduce complexity
- A total of 88 single experiments

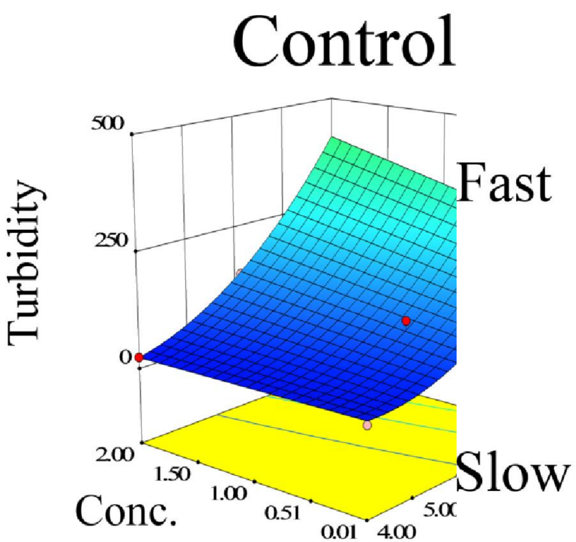


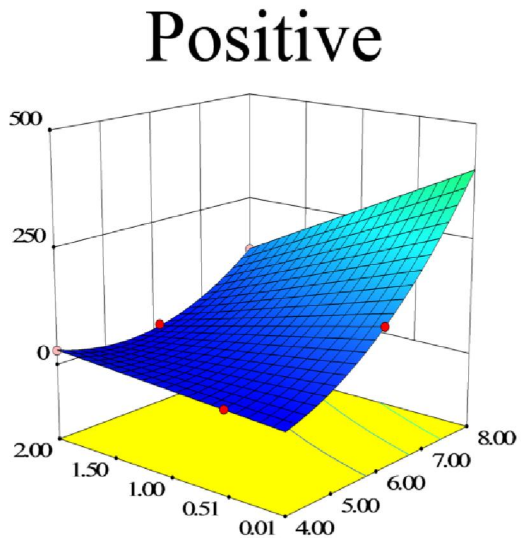
http://www.comicbookmovie.com/fansites/VoicesFromKrypton/news/?a=8934

Identify an additive that reduces turbidity at a wide pH range with a low concentration

Screening results

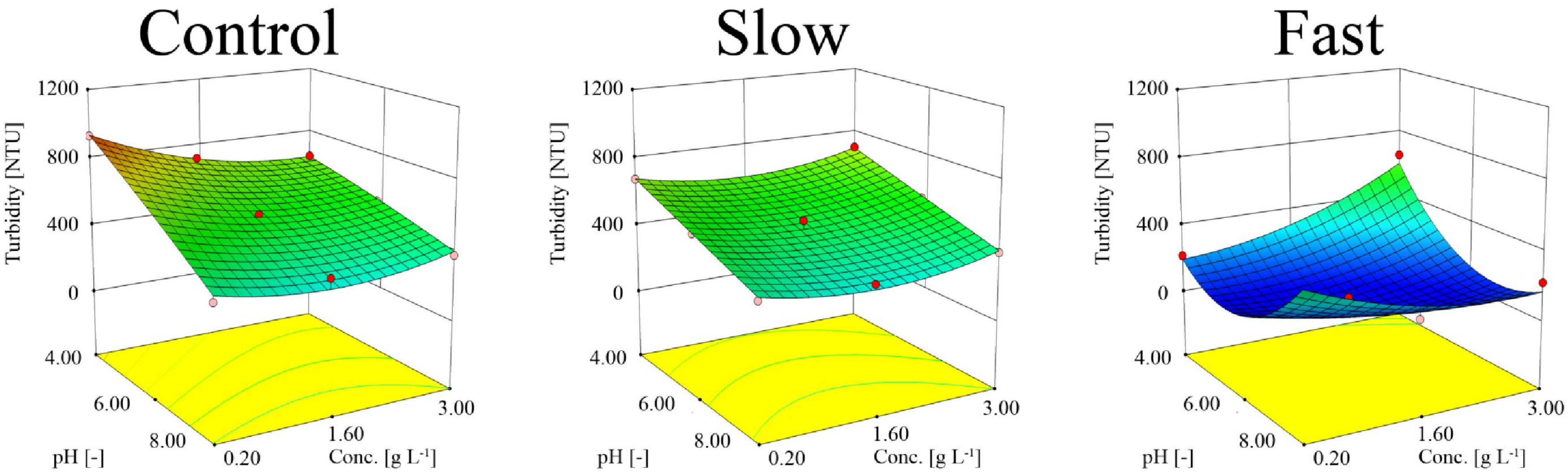
- Identified six out of 18 additives which reduced extract turbidity compared to control
- Useful concentration for all in [g L^{-1}] range
- Measure turbidity at two times after filtration effect vs. velocity





Refinement

- Adjusted concentration range for good additives
- Results:
  - Three additives exhibited an effect on extract turbidity immediately after filtration (without settling time)
  - Confirmed for two additional pH values



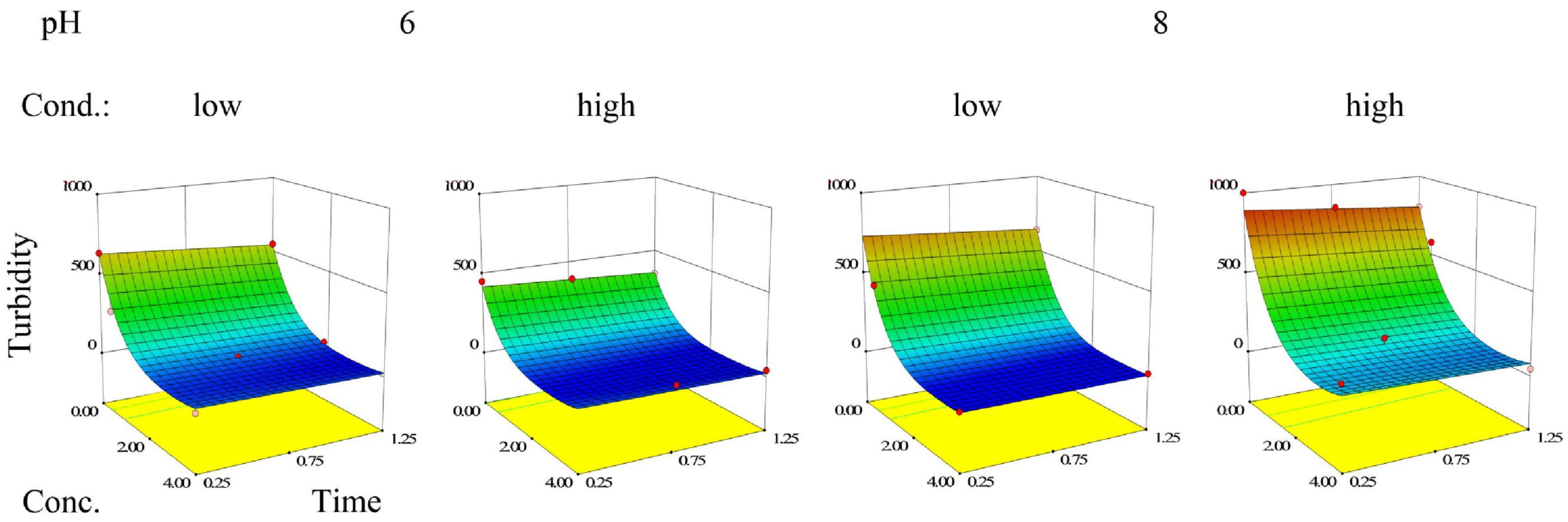
Optimization

- Limit investigation on best performing additive
- Include additional factors to build a more complex model
  - Incubation time
  - Conductivity

http://de.freepik.com/index.php?goto=41&idd=516590&url=aHR0cDovL3d3dy5vcGV uY2xpcGFydC5vcmcvZGV0YWlsL21vdW50YWluLWluLWluay1ieS1zbmVwdHVuZQ==

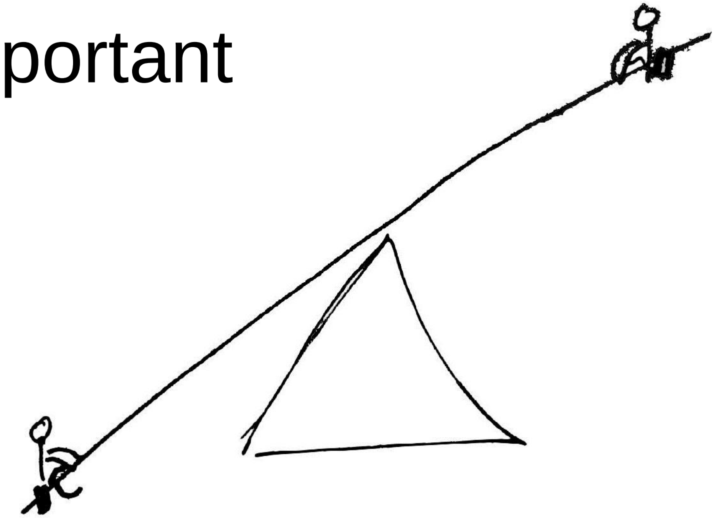
Optimization results

- Incubation times as little as 15 min are sufficient
- A conc. of 2 [g L^{-1}] is optimal balance between turbidity reduction and additive consumption



Robustness test

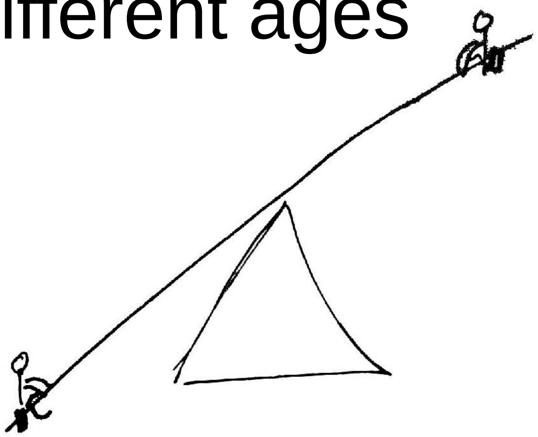
- What to test:
  - Where are “sensitive” spots/regions in the design space? (small factor change, large change in response)
  - Is there a sweet stop/region?
  - Are there other important parameters?



http://www.mutengo.co.za/images/clipart/seesaw.JPG

Robustness test

- Test setup:
  - Use conc. in close range to 2 [g L^{-1}]
  - Center incubation time around 15 min
  - Use a wider conductivity range
  - Include incubation temperature as a factor
  - Repeat for plants of different ages



http://www.mutengo.co.za/images/clipart/seesaw.JPG

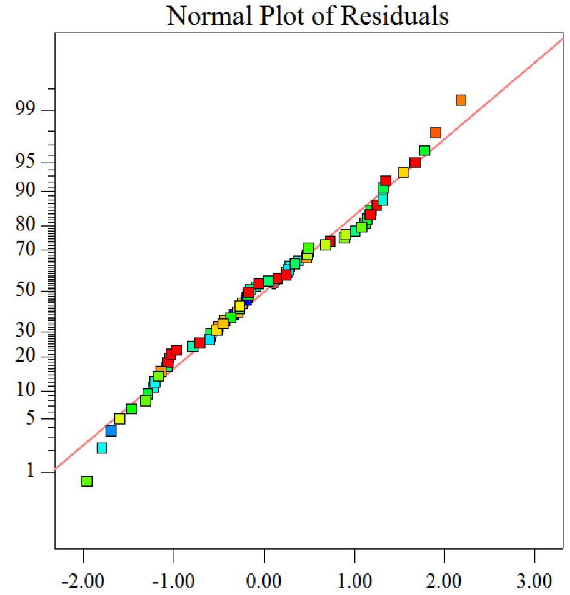
Robustness evaluation (I)

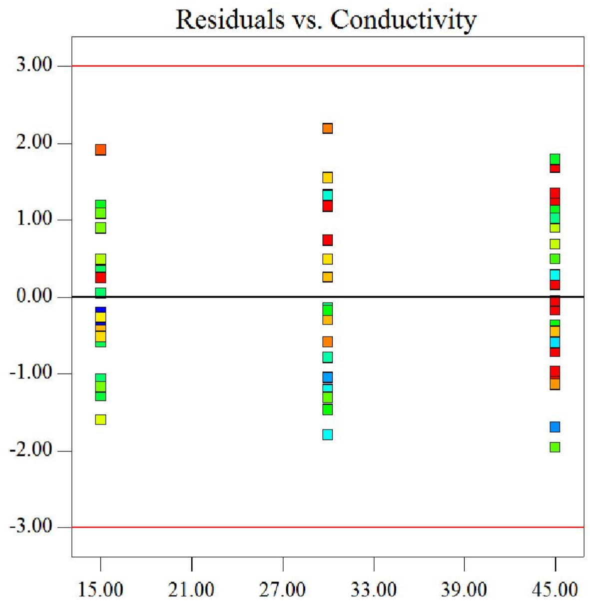
- A “good” model was established:

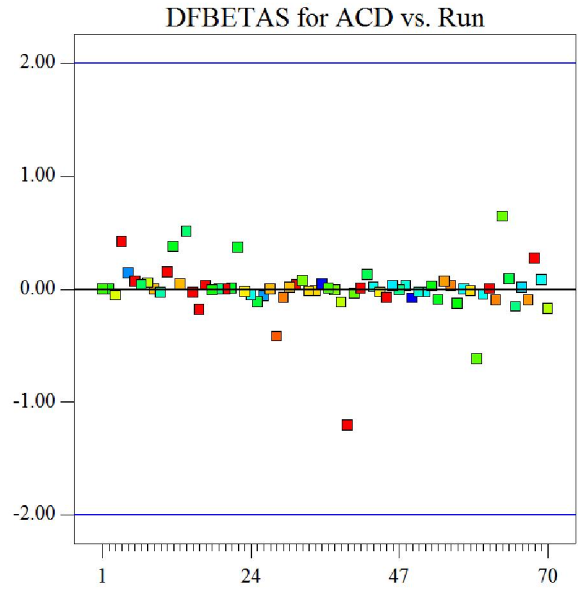
Parameter	Value		Parameter	Value
Std. Dev.	1.669		R^2	0.966
Mean	23.721		Adjusted R^2	0.942
C.V. %	7.038		Predicted R^2	0.887
PRESS	372.509		Adeq. Precision	23.050

Robustness evaluation (II)

- A “good” model was established:

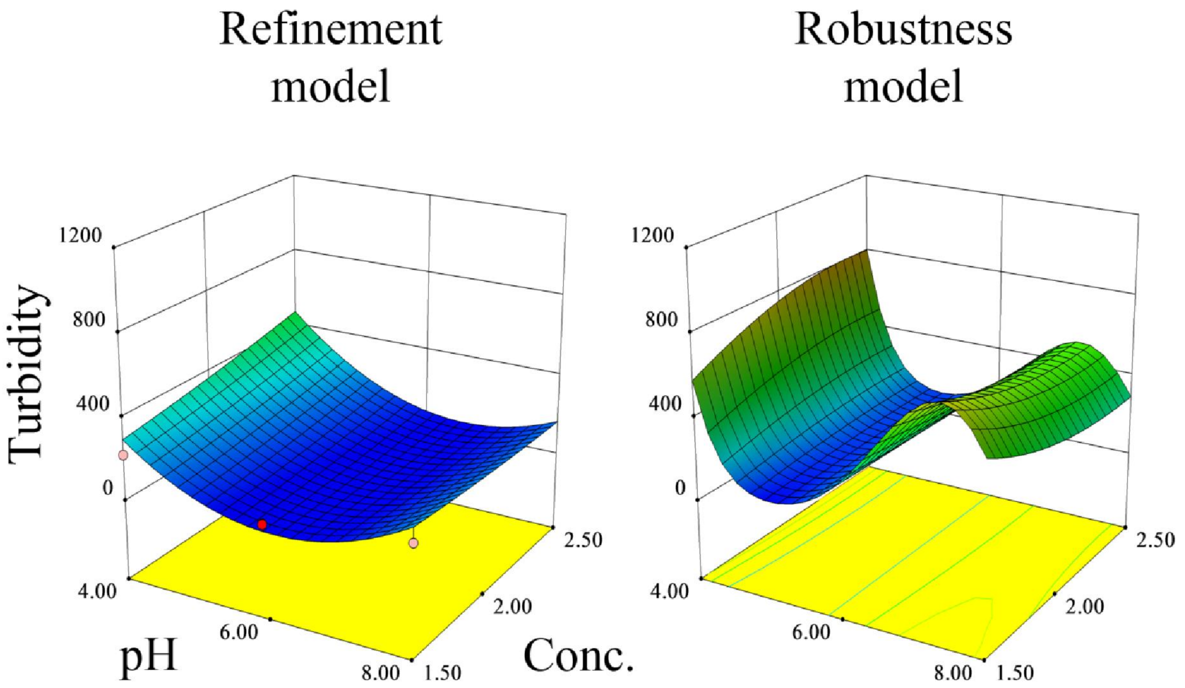






Robustness evaluation (III)

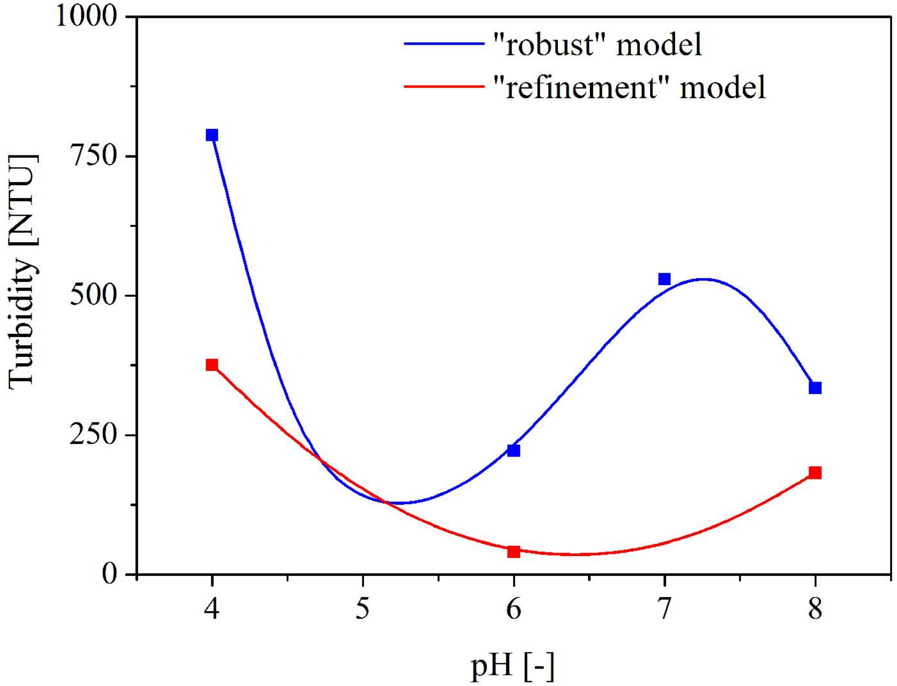
…but:



…no good agreement with previous model!

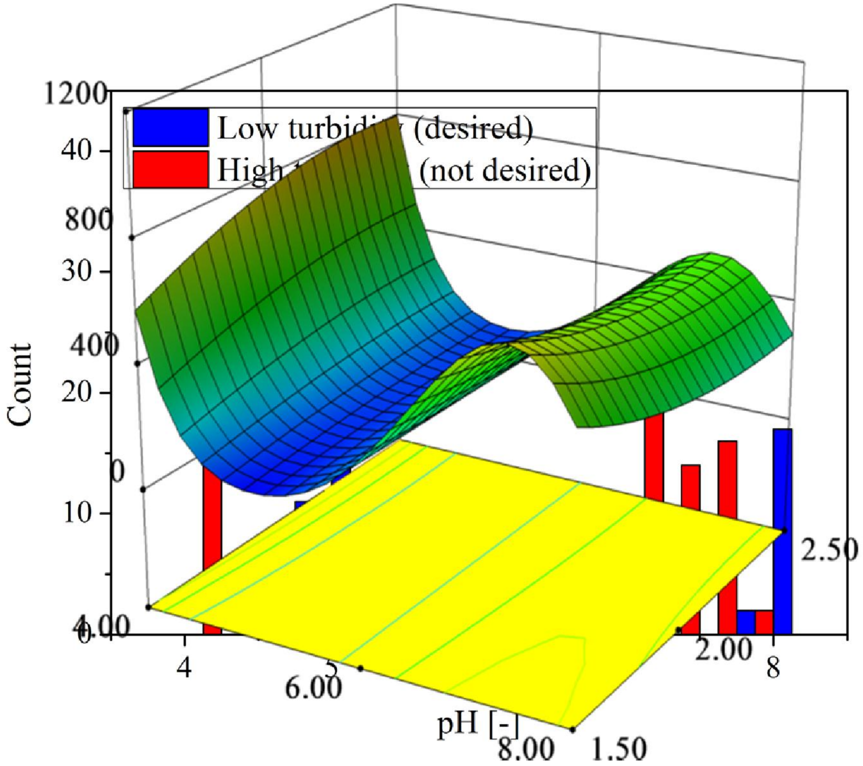
Robustness evaluation (IV)

- What causes the differences between the models?
  - "Real" effect between pH 7 and 8?
  - Inability of the model to depict bio-chemical reality a plateau?



Robustness evaluation (V)

- Even if the model is correct:
  – Graphical identification of preferred or “dangerous” regions is difficult
  – Instead: use point prediction tool and analyze “all solutions” for factor level abundance



Robustness evaluation (VI)

- However, histograms have limitations:
  - Inconvenient data export and manual analysis
  - Loss of information on interaction
- Will a more detailed analysis of model output be possible in DesignExpert 9?
  - “region” prediction (bubbles in the design space)
  - RSM slope

http://ahfdchief.files.wordpress.com/2011/09/bubbles.jpg

http://www.minibild.de/images/data/media/3/Weg_zum_Hoher_Goell.jpg

Next steps

- Test model at “suspicious” pH values
- Add pH value of 5 to the design
- Validate model in bench-top scale

Conclusion

- DesignExpert was successfully used to identify a filter additive
- A series of IV-optimal RSM designs was used for screening instead of a 2-level factorial design
- Robustness and data evaluation was difficult with the tools currently available in DesignExpert 8.0.7.1

Thanks a lot for your attention!

Please feel free to ask and/or comment!

Refinement model data

Parameter	Value		Parameter	Value
Std. Dev.	71.351		R^2	0.955
Mean	493.774		Adjusted R^2	0.895
C.V. %	14.450		Predicted R^2	0.558
PRESS	1312211		Adeq. Precision	18.452