BOOSTING A PROCESS WITH DIGITAL TOOLS

Purification of oligonucleotides by ion exchange

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INTRODUCTION

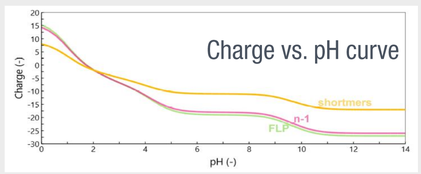
- Purification of oligonucleotides by ion exchange (IEX) is complex and many operating parameters need to be selected. There is a high interest in developing digital tools allowing to speed up process development, securing scale-up and decreasing waste.
- We present an innovative mechanistic model for oligonucleotide purification by IEX.
- We provide a step-by-step methodology to use the model and illustrate it with data from the literature and from AstraZeneca and GSK, leading pharma companies in the field.

MODEL

The model accounts for: YPSO Ionic®

Solution equilibria

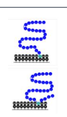
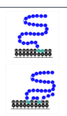
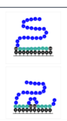
- The variable charge of the oligonucleotide with pH



Solid-liquid equilibria

- The possibility that not all of the oligo's charges interact with the resin
- The possibility that the oligo can have a various number of charge interactions
- The competition between the oligo and the other species in solution (e.g., OH-, phosphate) at the surface of the resin

Example of an oligonucleotide with 8 interacting charges and a maximum exponent of 4

Parameter	Monodentate	Bidentate	…	Octadentate
Interacting charges (z_i)	1	2	…	8
Selectivity coefficient (K_{z_i})	K_1	K_2	…	K_8
Exponent in mass action law (z_e^{max})	1	2	…	4
Configuration examples			…	

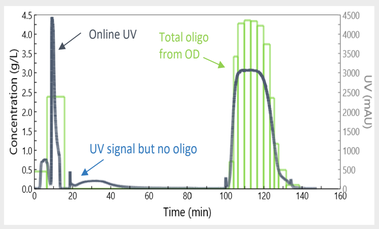
METHODOLOGY

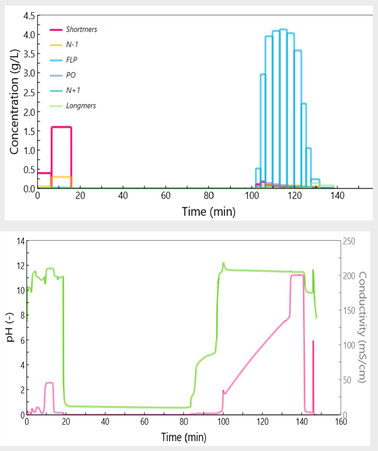
1. Establish analytical methods
2. Establish the list of species of interest
3. Characterize the solutions
4. Characterize the resin
5. Characterize the interactions between the solution and the resin
6. Vary key operating parameters

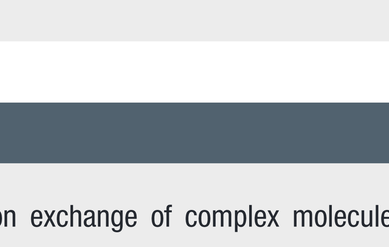
- Building a mechanistic model requires suitable analytical methods, a species list and a few well-targeted experiments.
- In particular, buffer-resin interactions are evaluated based on experiments performed in the absence of oligonucleotide.

- Model parameters to describe oligo-resin interactions are found from:
  - ✓ A breakthrough curve (not shown)
  - ✓ Few pulse experiments (not shown)
  - ✓ A full purification experiment (see below)

Typical data from full purification experiments:







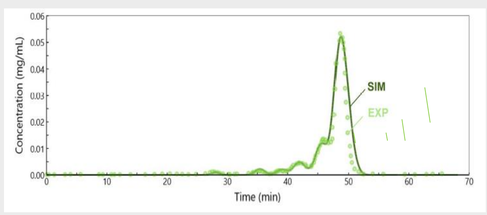
RESULTS

In the following, we partially illustrate steps 3, 5 and 6 of the proposed methodology. A complete application of the method will be available in [2].

Characterize the solutions

- The behavior of the oligonucleotide in solution can be described by a titration curve (see right).
- The calculated pH is calculated based on the charge vs. pH curve.

Characterize interactions between the solution and the resin



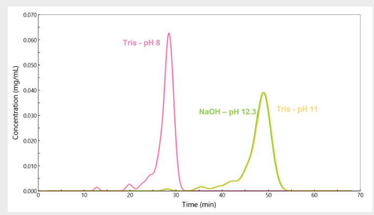
- The figure on the left shows an example of a reference purification experiment.
- The simulation (line) describes well the experimental data (points).

Vary key operating parameters

The model was then used to predict other experimental conditions [1]. Observed trends are well in line with those reported experimentally.

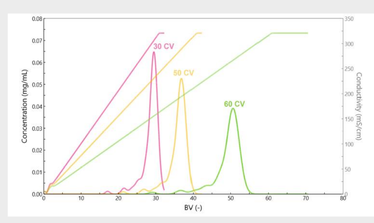
Impact of buffer pH

- The model correctly predicts an increase in the retention time when increasing the pH from 8 to 11 or 12.
- This is due to the higher number of negative charges at higher pH.



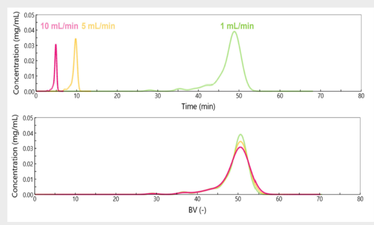
Impact of gradient slope

- The model correctly predicts an increase in peak retention time, peak width and peak resolution when decreasing the gradient slope.



Impact of flow rate

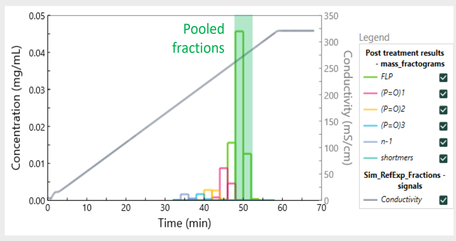
- The model correctly predicts a decrease in retention time and an increase in peak width when increasing flowrate.



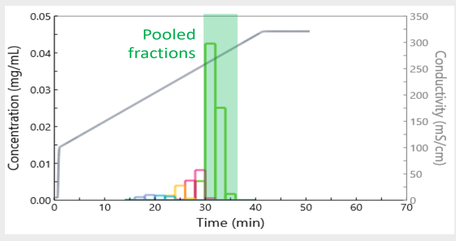
In-silico process optimization

- After applying the methodology, the model could be used for optimization.
- The gradient starting salt concentration and duration were adjusted (see below).

Reference process



Optimized process



- Yield and productivity were greatly improved, while solvent consumption and run duration decreased (see table).
- No additional experiment was needed for this investigation.

Criterion	Reference process	Optimized process	Change
Purity (%)	99.6	99.2	-0.6%
Yield (%)	78.2	93.0	+19%
Productivity (L/g/s)	3.0E-5	4.7E-5	+57%
Eluent consumption L/g product	584	367	-37%
Total run duration (min)	68.11	50.75	-25%

CONCLUSION

- We have developed a new predictive model for ion exchange of complex molecules like oligonucleotides.
- After applying the methodology, the model can be used to predict the influence of key operating parameters and to perform process optimization.
- We provided a clear methodology to use the model. This methodology has been shown to provide significant process insights with minimal experimental effort.

REFERENCES

[1] Deshmukh et al., Organic Process Research & Development 2002, 4, 205-213
[2] Kobl et al., article in preparation

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