COMPARISON OF SINGLE COLUMN AND MCSGP PROCESSES

APPLICATION TO OLIGONUCLEOTIDE PURIFICATION BY IEX

PROCESS: ION EXCHANGE, SINGLE COLUMN, MCC (MCSGP)
PRODUCT: OLIGO

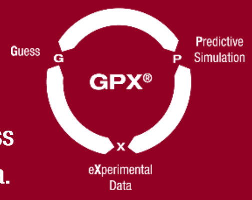
CHALLENGE

The **Multicolumn Countercurrent Solvent Gradient Purification (MCSGP)** process is a strong possible alternative to single-column chromatography for improving chromatographic process performances. **Mechanistic simulations** can provide an independent opinion as to what criteria can be improved, and a platform of which to make informed decisions prior to investing.

Here is an illustration of a **rational comparison** of the single-column and MCSGP processes, with mechanistic simulation applied to the example of oligonucleotide purification by ion exchange chromatography.

METHODOLOGY

Based on the <u>GPX® Concept</u> : in order to address your challenges, we capitalize on the **Guess ability** of different experts, the possibility of **Predictive simulation** and the use of **eXperimental data.**



❑ Experimental observations

This case study was inspired by the experimental data from *Deshmukh et al. OPRD (2002) 4, 205-213*, which reports the separation of Full Length Product (FLP) from $(P{=}O)_1$, $(P{=}O)_2$, $(P{=}O)_3$, n-1 and shortmers impurities *(Fig. 1)*.

The experiment was performed as follows:

- Process: single-column
- Column volume: 1 mL
- Resin: strong anion exchange
- Flowrate: 1 mL/min
- Eluent: 20 mM NaOH with linear gradient of NaCl

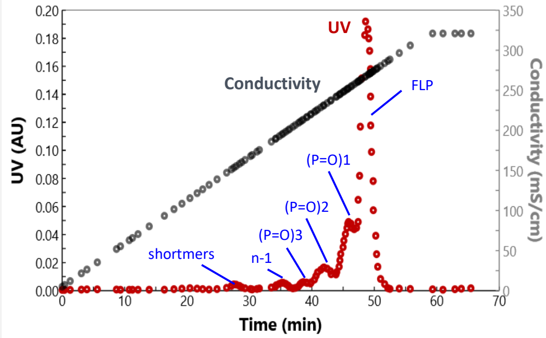


Fig. 1

❑ Presentation of the model

The model embedded in Ionic accounts for a certain number of physical phenomena:

- The acid-base equilibria in solution (only the dissociation of water in this specific example, but potentially phosphate, tris, etc)
- The variable charge of the oligonucleotide with pH *(Fig. 2)*
- The possibility for the oligonucleotide to interact with the resin with a number of charges lower than the charge in solution (e.g., the oligonucleotide may bear 30 charges in solution but interact only with 10 of them)
- The competition between the oligonucleotide and the other species in solution at the surface of the resin (Cl- and OH- in this example)

Only one experiment was used to fit model parameters *(Fig.1)*. A satisfactory agreement was observed between the simulation and experimental data *(Fig. 3)*.

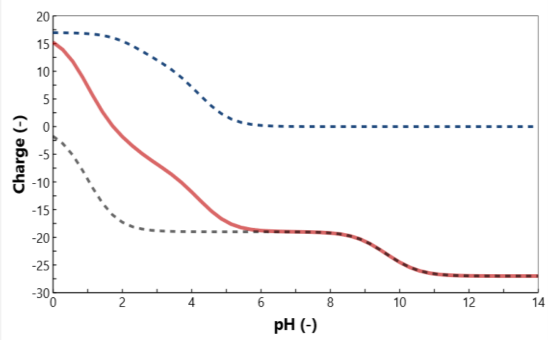


Fig. 2

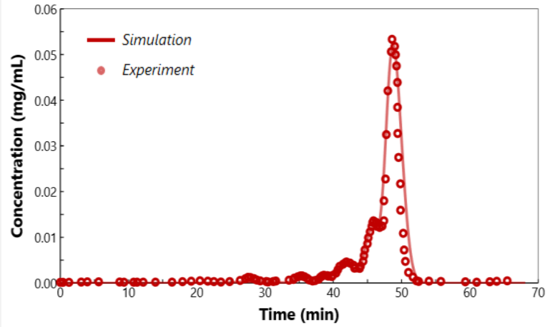
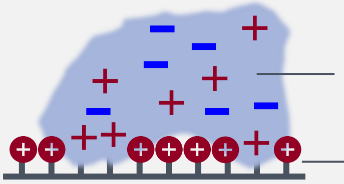


Fig. 3



Results

❑ Design and simulation of the MCSGP

The MCSGP can isolate a product eluting between weakly retained and strongly retained impurities. In the case under consideration, a hypothetical strongly retained impurity (n+1) was included in order to illustrate the capabilities of the process *(Fig. 4)*.

The MCSGP operating parameters were determined based on the experimental design approach described in *Steinebach et al. J. Chrom. A (2017) 1492, 19-26* with a target purity of 96%.
Two identical columns of 1 mL were used with the following time cuts *(Fig. 4):*

- For collection: 47.5 to 50 min (shaded in grey)
- For recycling: 45 to 47.5 min and 50 to 52 min (shaded in light grey)

A maximum flowrate of 2 mL/min was considered. The MCSGP was simulated over 20 cycles *(Fig. 5 and Fig. 6)*.

Note that the experimental design approach of the MCSGP by no means provides an optimized process, but rather a starting point that can then be further fine tuned.

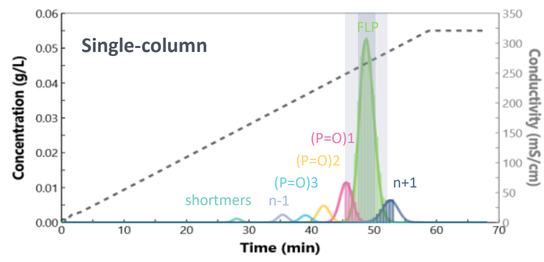


Fig. 4

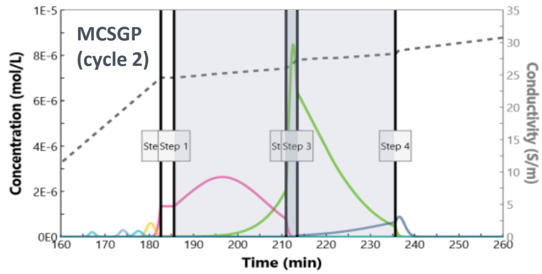


Fig. 5

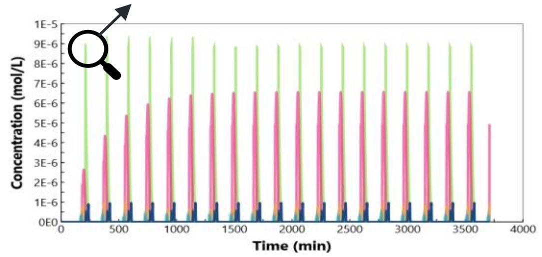


Fig. 6

❑ Comparison of the MCSGP with the single-column

- For the single-column process, fractions of 0.5 min were collected (Fig. 4) and several pooling strategies were tested *(Fig. 7)*.
- The single-column and MCSGP processes were compared at a target purity of approximately 96% *(Table 1)*.
- The MCSGP was found to improve product recovery to the detriment of eluent consumption and productivity.

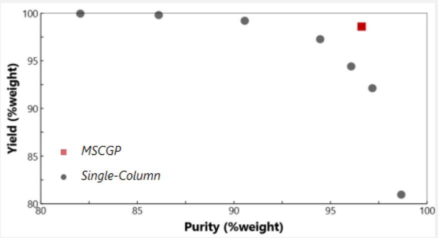


Fig. 7

	Single-column	MCSGP
Purity (%)	96.1	96.5
Recovery (%)	94.4	98.6
Eluent consumption (L/g)	484	805
Productivity (mg/L/min)	2.1	1.2

Table 1

Conclusion

Take home messages

1. Mechanistic simulation is a powerful tool capable of assessing several process options. Ypso-Ionic can provide **unbiased and rational comparisons** of processes based on technical performances.
2. In the example, moving from single-column to the MCSGP process improved the **product yield** by 4%, but increased **eluent consumption** by a factor of 2 and decreased **productivity** by a factor of 2. .
3. In order to implement a fully optimized process, **mechanistic simulation** can be used to fine-tune operating parameters.

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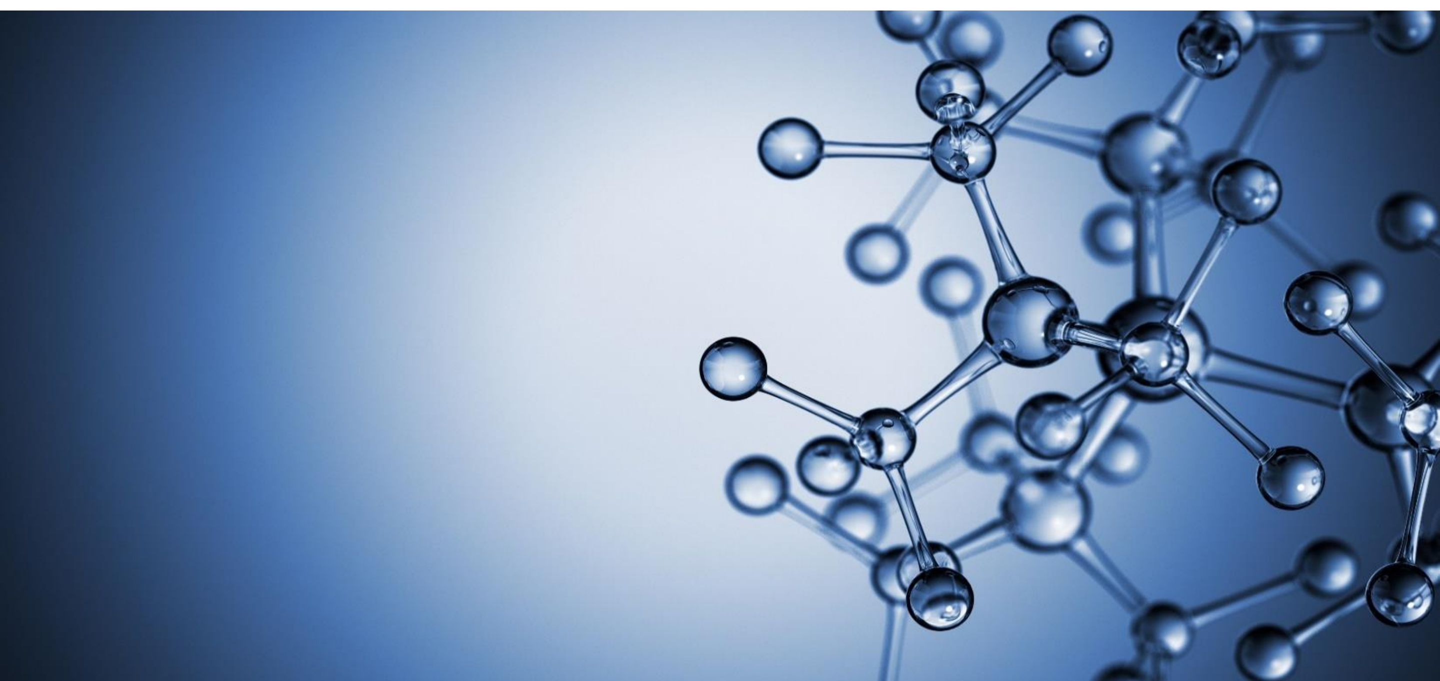

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