COMPARISON OF SINGLE AND MULTICOLUMN PROCESSES WITH MECHANISTIC SIMULATION

Application to oligonucleotide purification by ion exchange

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INTRODUCTION

- **Multicolumn Chromatography** is a strong possible alternative to single-column chromatography for improving process performances. **Mechanistic simulations** can be used to investigate **several process options** and quantify **key performance indicators.**
- In this poster, we show an illustration of a rational comparison between the single-column process and the Multi-column Counter-current Separation Gradient Purification (MCSGP) process performed with the software Ypso-Ionic® applied to the example of oligonucleotide purification by ion exchange chromatography.

EXPERIMENTAL OBSERVATIONS

This case study was inspired by the experimental data from [1], 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 done 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

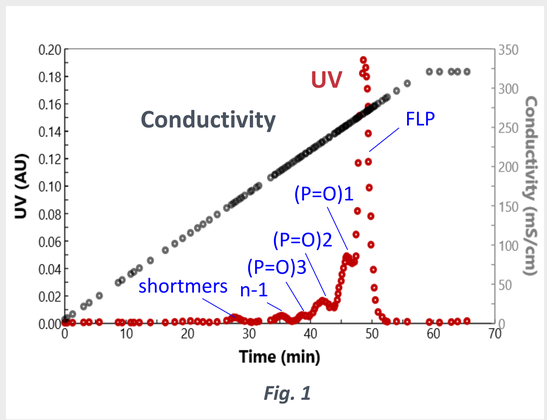


Fig. 1

This separation was done at lab scale under low loading conditions with a long gradient. It is therefore expected that the productivity (resp. the eluent consumption) is lower (resp. higher) than what is commonly observed under industrial preparative conditions. Nevertheless, the methodology described in the following remains valid.

PRESENTATION OF THE MODEL

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

- 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 with only 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).*

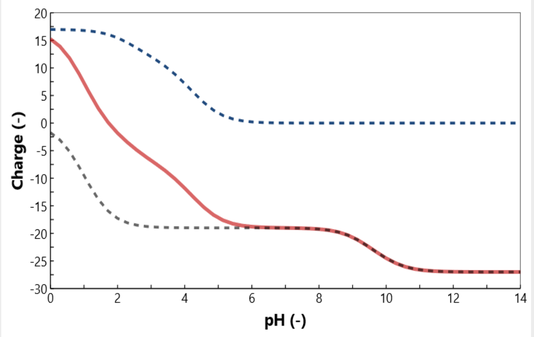


Fig. 2

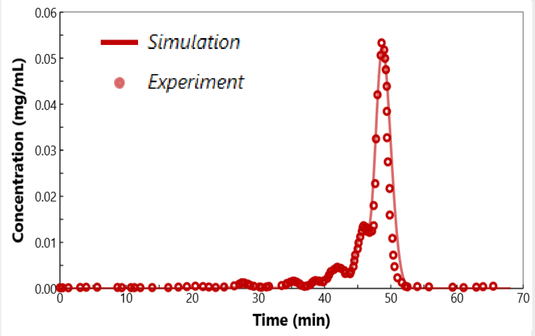
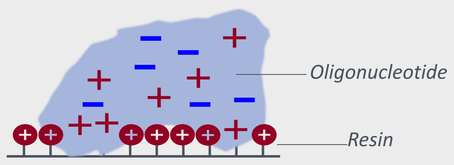


Fig. 3



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).*

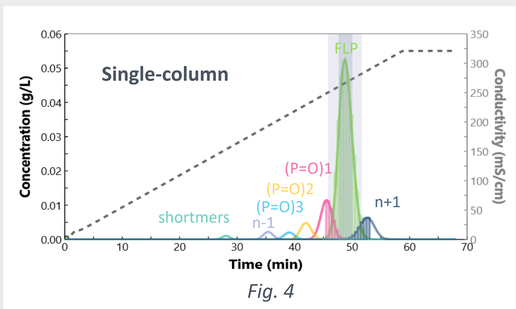


Fig. 4

The MCSGP operating parameters were determined based on the empirical design approach [2] as follows:

- Two identical columns of 1 mL
- Target purity of 96%
- Collection from 47.5 to 50 min (shaded in grey in *Fig. 4*)
- Recycling from 45 to 47.5 min and 50 to 52 min (shaded in light grey *Fig. 4*)
- Maximum flowrate of 2 mL/min
- Recycling of the fractions at a target salt concentration of 0.1 M

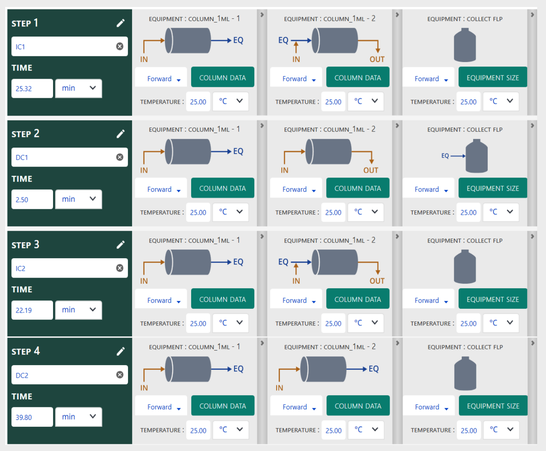


Fig. 5

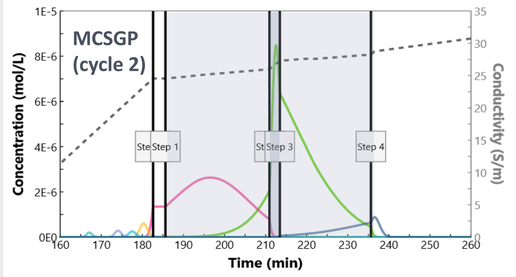


Fig. 6

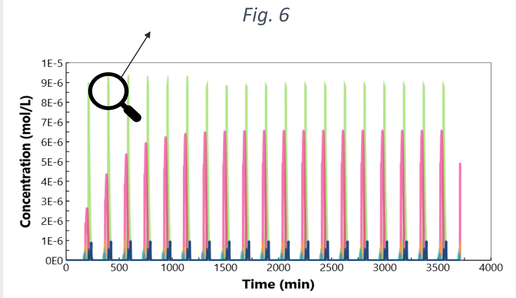


Fig. 7

The MCSGP was entered in Ionic *(Fig. 5)* and simulated over 20 cycles *(Fig. 6 and Fig. 7).*

Note that the **empirical design approach** of the MCSGP by no means provides an optimized process, but rather a **starting point** that can then be further fine tuned [3].

Comparison of the MCSGP with the single-column

- For the single-column process, performances were directly derived from the existing chromatogram without further optimization. Fractions of 0.5 min were collected (Fig. 4) and several pooling strategies were tested *(Fig. 8).*
- The single-column and MCSGP processes were compared at a target purity of approximately 96% *(Table 1).* The MCSGP was found to improve product yield to the detriment of eluent consumption and productivity.
- The **higher yield** of the MCSGP can be explained by the recycling of impure fractions. For comparison purposes, several single-column simulations were performed with different loadings, gradient slopes and flowrates (at constant column length and particle size) and it was found that it is *not possible* to achieve a yield of 98.6% with a single-column.
- The **eluent consumption** is tightly linked with the inline dilution necessary to decrease the salt concentration in recycled fractions. Smaller recycling windows and a flatter gradient profile are expected to decrease the eluent consumption of the MCSGP.
- The **productivity** is strongly linked with the system size. With the empirical design approach, 2 columns identical to the one used in the single-process are used. A more refined design of the MCSGP could be performed with shorter columns with a view to increasing productivity.

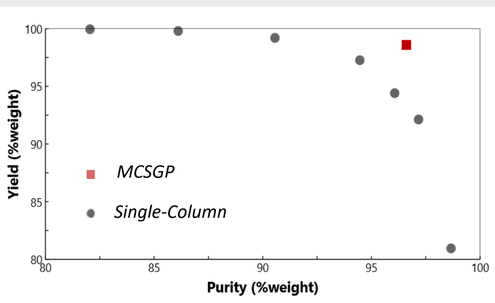


Fig. 8

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

Table 1

CONCLUSION

- Mechanistic simulation is a powerful tool capable of **assessing several process options** based on **rational comparisons** of technical performances.
- In the considered example, and by direct application of the empirical design approach, moving from single-column to the MCSGP process improved the **product yield**, but increased **eluent consumption** and decreased **productivity**. Mechanistic simulation can be used to further improve process performances.
- The final process choice depends on the objectives and constraints.

References

[1] Deshmukh et al. OPRD (2002) 4, 205-213
[2] Steinebach et al. J. Chrom. A (2017) 1492, 19-26
[3] Kim et al. J. Chrom. A (2022) 1681, 463487

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