Supplemental Information

Case study data

|     | c     | u     | D   | $F_{\text{max}}$ | $F_L$ | $F_W$ | $F_E$ | $F_R$ | $\phi_L$ | $\phi_W$ | $\phi_E$ | $\phi_R$ | [mg/ml] | [CV/min] | [CV] |
|-----|-------|-------|-----|------------------|-------|-------|-------|-------|----------|----------|----------|----------|---------|---------|------|
| c0  | 15    | 0.95  | 1.5 | 0.03            | 0.02  | 0.02  | 0.02  | 0.02  | 15       | 8        | 3        | 8        |         |         |      |
| c1  | 20    | 0.9   | 1.5 | 0.03            | 0.0296| 0.0296| 0.0296| 0.0296| -        | 10       | 3        | 10       |         |         |      |
| c2  | 20    | 0.95  | 1.5 | 0.03            | 0.0294| 0.0294| 0.0294| 0.0296| -        | 11       | 2        | 11       |         |         |      |

Table 1: Column data for case I. The entire elute phases are pooled directly to the next column and parts outside the pool are considered to belong to wash or regeneration.

|     | Type  | c     | u     | $F_{\text{max}}$ | $F_L$ | $F_W$ | $F_E$ | $F_R$ | $\phi_L$ | $\phi_W$ | $\phi_E$ | $\phi_R$ | [mg/ml] | [CV/min] | [CV] |
|-----|-------|-------|-------|------------------|-------|-------|-------|-------|----------|----------|----------|----------|---------|---------|------|
| cAB | BAE   | 0.15  | 0.80  | 0.33            | 0.083 | 0.083 | 0.083 | 0.33  | 15       | 2        | 2        | 13       |         |         |      |
| c1  | BE    | 0.30  | 0.95  | 0.15            | 0.15  | 0.15  | 0.15  | 0.15  | -        | 6.5      | 0.5      | 3        |         |         |      |
| c2  | BAE   | 2.0   | 0.80  | 2.0             | 0.33  | 2     | 0.33  | 0.167 | -        | 16       | 1        | 3        |         |         |      |
| c3  | BAE   | 0.25  | 0.80  | 2.0             | 0.33  | 2     | 0.33  | 0.167 | -        | 7        | 1        | 3        |         |         |      |
| c4  | BE    | 0.10  | 0.95  | 0.17            | 0.017 | 0.017 | 0.017 | 0.017 | -        | 1        | 1.2      | 3.5      |         |         |      |

Table 2: Column data for case II. The entire elute phases are pooled directly to the next column and parts outside the pool are considered to belong to wash or regeneration. BAE stands for bind-and-elute and BE is buffer exchange.

Equations

Constraints

The constraints which must be fulfilled for the variables $x$ and $y$ are outlined in this section.

Equation (1) describes the relationship that occur when the pool from $i - 1$ is directly loaded into column $i$, with $V$ in the unit [ml/column], $F$ in the unit Column Volumes/time
[CV/min]. During the eluate/load phase, the volumetric flow rate of $i - 1$ is limited by the maximum flow rate of column $i$. This correlation is necessary when the flow is coupled, as it is during elute and load phases.

$$V_i F_{i,L,max} \geq V_{i-1} F_{i-1,E} \iff \frac{V_i}{V_{i-1}} \geq \frac{F_{i-1,E}}{F_{i,L,max}}$$  \hspace{1cm} (1)$$

Constraint equation (2) is directly translated from equation (1) but with $x$, $y$ and the robustness factor. It is called the current FR-limit, where FR is short for flow rate. The robustness factor is included to lower the flow rate in the connection. A value of 1 means that there is no robustness as no flow rate will be lowered, while $R = 1.1$ reduces the flow rate with 10%. A dilution factor is also introduced here which is a multiplier of the eluting flow rate of the previous column. Since the dilution flow rate is added to the stream of the eluting flow rate, the total loading volume of the next column is increased, affecting constraint (2).

$$x_i \geq y_i R_i D_i$$  \hspace{1cm} (2)$$

The flow rate must not exceed the maximum of the previous column’s which is described by constraint equation (3) and is called the previous FR-limit. Same principle as above regarding the robustness factor.

$$y_i \leq \frac{1}{R_i} \frac{F_{i-1,max}}{F_{i,max}}$$  \hspace{1cm} (3)$$

The absolute capacity of each column must be greater than the first column’s and the product sum of the intermediate recovery yields, which is why constraint equation (4) also must be fulfilled for all connections. Note that the product sum of $x$ is equal to the last column volume divided by the first, e.g. $\prod_{j=1}^{3} x_j = x_3 x_2 x_1 = \frac{V_3}{V_2} \frac{V_2}{V_1} \frac{V_1}{V_0} = \frac{V_3}{V_0}$. Equation (5) is only used, and instead of equation (4), when column $i$ is volume dependent, i.e. it is a buffer exchanger or a size exclusion column. Since they are not used simultaneously, both are referenced to as the capacity limit.

$$\prod_{j=1}^{i} x_j \geq \frac{c_{0,\text{weight}}}{c_{i,\text{weight}}} \prod_{j=0}^{i-1} u_j \iff x_i \geq \frac{c_{0,\text{weight}}}{c_{i,\text{weight}}} \prod_{j=0}^{i-1} \frac{u_j}{x_j}$$  \hspace{1cm} (4)$$
\[
x_i \geq \frac{\phi_{E,i-1}}{c_{i,\text{volume}}} \quad (5)
\]

As \( y \) is a ratio between non-negative flow rates, it can’t be zero or below. This is described by equation (6) and is used as a bound in the optimization.

\[
y_i > 0 \quad (6)
\]

**Single or multiple optimal points**

To determine if there are multiple optimal choices or a single optimal corner in the connections, the inequality in equation (7) must be satisfied. If it is not satisfied, the capacity line is to the left hand side of where the flow rate constraint crosses the maximum flow rate line in figure 2, and there is only one corner solution.

\[
\frac{1}{R_i} \frac{F_{i-1,\text{max}}}{F_{i,\text{max}}} \leq \frac{c_{o,\text{weight}}}{c_{i,\text{weight}}} \prod_{j=0}^{i-1} u_j \prod_{j=1}^{i-1} x_j
\quad (7)
\]

**Multi-objective optimization**

The individual objects are the total volume of the columns, equation (8), and the total process time of all connected steps, equation (9). Subindex \( n \) represents the total number of column connections in the sequence which is one less than the number of columns.

\[
V_{\text{tot}} = V_0 + V_0 \sum_{i=1}^{n} \prod_{j=1}^{i} x_j \quad (8)
\]

\[
t_{\text{tot}} = \sum_{i=1}^{n} \left( y_i \frac{\phi_{E,i-1}}{F_{i-1,E}} \right) \quad (9)
\]

The competing objectives are weighed against each other with a parameter, \( w \), ranging from 0 to 1 in equation (10) which produces a Pareto front with volume and total time on the two axes.
\[
\text{minimize } \ w \frac{V_{tot}}{V_{tot,min}} + (1 - w) \frac{t_{tot}}{t_{tot,min}} \\
\text{w.r.t. } x, y \in \mathbb{R}^n \\
s.t. \text{ Equations (2) – (6)}
\]

\(V_{tot,min}\) and \(t_{tot,min}\) were minimized based on equation (8) and (9) in order to normalize the objectives. The robustness factor is 10% in all steps and cases, i.e. set to 1.1.

Case study II has a different objective instead of the total process time. There is instead another parameter that is affected by the optimized column sizes and is also a cost bearer; total buffer consumption. Thus, another Pareto front is created in equation (11) with buffer consumption per mg produced product, \(B\), and total column volume, \(V_{tot}\).

\[
\text{minimize } \ w \frac{V_{tot}}{V_{tot,min}} + (1 - w) \frac{B_{tot}}{B_{tot,min}} \\
\text{w.r.t. } \ V_0 \in \mathbb{R} \\
x, y \in \mathbb{R}^n \\
s.t. \text{ Equations (2) – (6)}
\]