Closed-loop optimization of chromatography column sizing strategies in biopharmaceutical manufacture

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Abstract

BACKGROUND: This paper considers a real-world optimization problem involving the identification of cost-effective equipment sizing strategies for the sequence of chromatography steps employed to purify biopharmaceuticals. Tackling this problem requires solving a combinatorial optimization problem subject to multiple constraints, uncertain parameters, and time-consuming fitness evaluations.

RESULTS: An industrially-relevant case study is used to illustrate that evolutionary algorithms can identify chromatography sizing strategies with significant improvements in performance criteria related to process cost, time and product waste over the base case. The results demonstrate also that evolutionary algorithms perform best when infeasible solutions are repaired intelligently, the population size is set appropriately, and elitism is combined with a low number of Monte Carlo trials (needed to account for uncertainty). Adopting this setup turns out to be more important for scenarios where less time is available for the purification process. Finally, a data-visualization tool is employed to illustrate how user preferences can be accounted for when it comes to selecting a sizing strategy to be implemented in a real industrial setting.

CONCLUSION: This work demonstrates that closed-loop evolutionary optimization, when tuned properly and combined with a detailed manufacturing cost model, acts as a powerful decisional tool for the identification of cost-effective purification strategies.

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Keywords: biopharmaceutical manufacture; antibody purification technologies; downstream processing design; evolutionary computation; closed-loop optimization; process economics

NOTATION

$\bar{x}$	Candidate solution
f	Objective function to be optimized
k	Total fixed number of chromatography steps in a manufacturing process
h_i	Column bed height at chromatography step i (cm)
d_i	Column diameter at chromatography step i (cm)
$n_{CYC,i}$	Number of cycles each column at chromatography step i is used for
$n_{COL,i}$	Number of columns operating in parallel at chromatography step i
V_i	Total volume of resin available at chromatography step i (L)
T_i	Processing time of chromatography step i (h)
M_i	Mass of product entering chromatography step i (g)
DBC_i	Dynamic binding capacity of the resin used at chromatography step i (g/L)
P	Annual product output (kg)
D	Annual demand (kg)
m	Number of Monte Carlo trials

μ	Parent population size used by the search algorithms
λ	Offspring population size used by the search algorithms
I	Number of decision variables to be optimized by the search algorithms

INTRODUCTION

Monoclonal antibodies (mAbs) represent the fastest growing category of therapeutic biopharmaceutical drugs due to their unique binding specificity to targets. Yet, their manufacture is

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Figure 1. Typical flowsheet for an antibody manufacturing process.

costly and time-consuming. The manufacturing process for mAbs can be divided into two phases (Fig. 1): *upstream processing* (USP) and *downstream processing* (DSP). In USP, mammalian cells expressing the mAb of interest are cultured in bioreactors. Following cell culture, the broth moves to DSP, where the mAb is recovered, purified and cleared from potential viruses using a variety of operations including a number of chromatography steps, such as affinity or ion-exchange chromatography. Chromatography operations are identified as critical steps in a mAb purification process and can represent a significant proportion of the purification material costs (associated, for example, with the use of expensive affinity resins and large amounts of buffer reagents). While alternatives to traditional column chromatography platforms are emerging, industry practitioners are still reluctant to perform major process changes.^{1,2} At the same time, it is important to determine how best to use existing production facilities for mAbs.^{3,4} This is particularly challenging given the significant improvements in USP productivities that have been accomplished over the past decade with higher mAb concentrations (*titters*) being achieved in cell culture. These improvements have not been matched in purification capacities, leading to concerns over purification bottlenecks and the desire to continuously optimize the design and operation of existing chromatography steps. Hence, to efficiently exploit these cell culture improvements, and account for the growing market for therapeutic mAbs, it has become critical to identify cost-effective purification processes.^{1,2,5}

An approach to tackle these issues, which is also adopted here, is to develop simulation models of mAb manufacturing

processes and locate promising chromatography setups using computational methods. For example, Joseph *et al.*⁶ present a simulation model to identify windows of operation for the column diameter, bed height and loading flowrate of a chromatography step using productivity and cost of goods (COG) as performance criteria. A model to find combinations of protein load and loading flowrate that meet yield and throughput constraints has been developed by Chhatre *et al.*⁷ The discrete-event simulation framework proposed by Stonier *et al.*⁸ allows the selection of optimal chromatography column diameters over a range of titers. The methodology used by these authors^{6–8} consists of selecting and evaluating specific values within the full range of variation of the critical parameters. However, such an approach may not be feasible for larger decision spaces as considered here, where the number of possible permutations of options means that they can no longer be examined individually. Recently a set of mathematical programming approaches to address chromatography column sizing and sequencing problems were presented by Liu *et al.*^{9,10} These methods validated the outcomes of the evolutionary algorithms created in this paper for small problem instances but were found to reach limitations when attempting to solve large problem instances under uncertainty with commercial solvers. This drives the need for more efficient combinatorial optimization methods in this domain.

To address this issue, Simaria *et al.*^{11,12} conducted a study on the application of evolutionary algorithms (EAs) to optimize mAb purification processes. EAs represent an example of meta-heuristic combinatorial optimization techniques that function by iteratively evolving a population of candidate solutions using

Figure 2. Schematic of closed-loop optimization. The genotype of a candidate solution $\bar{x}$ is generated on the computer but its phenotype is experimentally prototyped or alternatively realized by running an expensive computation simulation. The quality or fitness $f(\bar{x})$ of a solution may be obtained experimentally too and thus may be subject to measurement errors (noise).

search algorithms based on the principles of natural selection.¹³ Simaria *et al.*^{11,12} found that EAs are efficient optimizers in this domain but also recognized that their performance could be improved by further tuning of the EAs. This study conducts a more thorough investigation on this topic. Beginning with a more formal definition of the chromatography equipment sizing problem, this work proposes guidelines on how to tune EAs for this problem and demonstrates the usefulness of certain EA properties when it comes to incorporating user preferences and final decision making.

This study investigates the application of EAs for the identification of *chromatography column sizing strategies* – defined here by the diameter and bed height of a column, the number of columns used in parallel, and the number of cycles a column is run for – that are cost-effective in terms of COG per gram (COG/g) of product manufactured. This task can be formulated as a combinatorial (single-objective) optimization problem subject to multiple constraints and interacting decision variables, uncertain parameters and expensive fitness evaluations (represented by time-consuming computer simulations).

The type of problem considered here – where solutions are evaluated by simulation rather than computing some function

available in algebraic form – is commonly referred to as a *closed-loop optimization problem*.^{14–16} The term *closed-loop* suggests that the setup in such problems establishes an interactive loop between an optimizer and the experimental platform, represented here by the simulator. Figure 2 illustrates the closed-loop setup commonly used in experimental optimization including in this work. Over the years, evolutionary algorithms (EAs) have proven to be efficient, flexible and robust optimizers for a number of closed-loop problems in areas such as shape design optimization, quantum control, drug discovery, analytical biochemistry, fermentation media optimization, marine biosurfactant production, batch distillation design, and biopharmaceutical portfolio management combined with capacity planning.^{17–27} For a detailed introduction into the field of closed-loop optimization including challenges, applications, and algorithms employed, please refer to Allmendinger.¹⁴

An industrially-relevant case study is used to investigate how to tune some of the EA configuration parameters: population size, degree of elitism, number of Monte Carlo trials (needed to cope with uncertain parameters), and constraint-handling method. The fitness landscape of different scenarios of the case study is analyzed also to observe which landscape features pose a particular

Figure 3. Schematic of the closed-loop platform employed comprising an optimization algorithm (EA) linked to a detailed process economics model; the interaction between the two components is supported by a database. The process economics model (its structure is illustrated using a UML diagram) performs mass balance and cost calculations and the EA determines the best chromatography equipment sizing strategies. A chromatography equipment sizing strategy is defined by the column bed height h_i , column diameter d_i , number of cycles $n_{CYC,i}$ and number of columns $n_{COL,i}$ for each chromatography step $i = 1, \dots, k$ employed. Different sizing strategies may yield the same overall column volume but nevertheless vary in the objective COG/g.

Figure 4. A candidate solution (sizing strategy) with $k = 3$ chromatography steps. Each step $i = 1, \dots, k$ is defined by the parameters h_i , d_i , $n_{CYC,i}$ and $n_{COL,i}$.

challenge when optimizing equipment sizing strategies. A comparison between the equipment sizing strategies used by manufacturers (regarded in this paper as the base case) and solutions provided by the EA is presented and a practical procedure based on visual tools for selecting the most preferred sizing strategy is illustrated.

METHODOLOGY

Problem domain: chromatography equipment sizing

The chromatography equipment sizing problem can be represented as a combinatorial optimization problem with the task of finding the most cost-effective chromatography sizing setup for a sequence of chromatography steps used in the purification process of mAbs. A typical mAb manufacturing process, as shown in Fig. 1, consists of a number of packed-bed chromatography steps, with the chromatography sizing strategy selected having a direct impact on metrics such as manufacturing costs and time, and annual product output. To locate effective sizing strategies, the closed-loop setup illustrated in Fig. 3, which links an optimization algorithm with a process economics model, is employed.

In the following the decision variables, objective function, constraints, and uncertain parameters, to which the chromatography equipment sizing problem considered here is subject to, are described.

Decision variables

Figure 4 shows the encoding used to represent a solution $\bar{x}$ to the chromatography sizing problem. For each chromatography step $1 \leq i \leq k$ (k is the total number of steps) four discrete decision variables were defined related to the sizing and operation of chromatography columns: bed height h_i and diameter d_i of columns, number of cycles $n_{CYC,i}$ each column is used, and the number of columns $n_{COL,i}$ operating in parallel. That is, the problem is subject to $k \times 4$ discrete variables in total. This captures the trade-offs of using large columns with a single cycle versus smaller columns with multiple cycles as illustrated in Fig. 3. Small changes in bed height were accommodated to account for typical ranges seen in industrial applications and it was assumed that such changes would not affect product quality or recovery. The use of multiple parallel columns per step was also incorporated so as to determine whether this offered significant advantages that might outweigh current preferences to avoid parallel columns due to validation burdens. For each step i , the variables define the (i) total volume of resin V_i available for the purification of a product at that chromatography step, and the (ii) processing time T_i that the chromatography step takes; both parameters are calculated according to standard mass balance equations as follows:²⁶

$$V_i = \pi \cdot d_i^2 / 4 \cdot h_i \cdot n_{CYC,i} \cdot n_{COL,i} \quad (1)$$

$$T_i = n_{CYC,i} \cdot h_i \cdot (CV_{BUFF,i} + CV_{LOAD,i} / n_{COL,i}) \cdot u_i \quad (2)$$

where $CV_{BUFF,i}$ and $CV_{LOAD,i}$ are the number of column volumes of buffer and product load applied per cycle, and u_i is the linear flowrate of the resin used at step i .

Objective function

The objective f is to find a chromatography sizing setup that yields minimal cost of goods per gram (COG/g) of product manufactured. The COG includes both direct (resource) costs (e.g. resin, buffer and labor costs) and indirect costs (e.g. facility dependent overheads, such as maintenance costs and depreciation), and is divided by the total annual product output P to yield the metric COG/g. The COG/g values are obtained by running a detailed process economics model, which simulates the different purification steps based on mass balance and cost equations as defined by Farid et al.²⁸ A more detailed explanation of the COG/g components is presented in Simaria et al.¹² A key feature of this model is the impact of processing time on COG/g. The annual demand D is an input of the model and it is used to calculate the bioreactor size assuming a given number of batches is produced annually in the facility. However, if a particular equipment sizing strategy leads to long processing times, it may not be possible to meet the total number of batches and hence the annual product output would be below the production target. This penalizes the objective function through the decrease of the denominator in COG/g (i.e. the total amount of grams of product produced).

Constraints

The problem is subject to two types of constraints:

1. Each chromatography step $i = 1, \dots, k$ needs to satisfy a resin requirement constraint to ensure that the resin volume V_i available for purification at step i is sufficient to process the mass of product M_i entering that step, given the resin's dynamic binding capacity DBC_i and the maximum utilization factor κ . Formally, this constraint can be defined as

$$V_i \geq M_i \cdot \kappa / DBC_i \quad (3)$$

Solutions violating this constraint are considered infeasible and handled using one of the constraint-handling strategies introduced later.

2. There is also a demand constraint to ensure that the amount of product manufactured P is sufficient to satisfy the annual demand D , or $P \geq D$. This constraint may be violated for column sizing strategies with long chromatography processing times T_i . Using COG/g as the objective function (recall that the product output P is in the denominator of this metric) was found to be sufficient to cope with this constraint.

Uncertainties

Uncertainty related to the product titer can have a significant impact on the annual product output P . As the equipment sizing is a function of an expected titer value for bioreactors through to chromatography columns, titer fluctuations can cause (i) failure to meet demand (if titer is lower than expected), or (ii) product waste (if titer is higher than expected and equipment capacity is insufficient to process the excess). Other sources of uncertainty (e.g. yield, and processing times) may be present and are realistic but are not considered in this paper.

Search algorithms

To gain insight into the behaviour of evolutionary search algorithms on the chromatography sizing problem, four types of search algorithms were considered: a simple generational genetic algorithm (SGA), a genetic algorithm with generation gap (GA-GG), a genetic algorithm with a $(\mu + \lambda)$ -ES reproduction scheme (GA-ES), and a population of stochastic hill-climbers (PHC). Algorithm 1 shows the pseudocode of the search algorithms and the way the constraint handling strategies (which are introduced below) are managed.

All four algorithms began the search with the same initial population containing μ randomly generated solutions. The algorithms used also the same mutation operator, which selected a decision variable value at random from the set of possible values. SGA used uniform crossover and random flip mutation as the variation operators, and binary tournament selection (with

replacement) for parental selection; for environmental selection, it replaced the entire current population with the offspring population. GA-GG and GA-ES differ from SGA in the environmental selection step only. With GA-GG, the new population was formed by selecting the fittest μ solutions from the combined pool of the offspring population and the two fittest solutions of the current population. With GA-ES, a greater degree of elitism was employed and the fittest μ solutions from the combined pool of the current population and the offspring population were selected. PHC maintained a population of stochastic hill-climbers, which, at each generation g , independently underwent mutation and replaced their parent if it was at least as fit.

Accounting for uncertainty

To account for titer variabilities, m Monte Carlo trials (based on the titre probability distribution $\text{Tr}(2.6, 3.0, 3.4)$) were performed for

Algorithm 1. Pseudocode of the search algorithms with constraint handling strategies

Algorithm 1 Search algorithms with constraint handling strategies

Require: G (maximal number of generations), μ (parent population size), CHS (constraint-handling strategy selected), Algo (search algorithm selected)

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1:  $g = 0$  (generation counter),  $Pop = \emptyset$  (current population),  $OffPop = \emptyset$  (offspring population),
    $AllSolsEval = \emptyset$  (genotypes and their moving fitness average values of all solutions evaluated so far),
    $Stoch$  (boolean variable indicating whether uncertainty is present or not)
2: while  $|Pop| < \mu$  do
3:   generate solution  $\bar{x}$  at random; evaluate( $\bar{x}$ );  $Pop = Pop \cup \{\bar{x}\}$ 
4: while  $g < G$  do
5:    $OffPop = \emptyset$ 
6:   for  $i = 1$  to  $\mu/2$  do
7:     if Algo = PHC then
8:       Mutate solution  $\bar{x}_{2,i-1}$  and  $\bar{x}_{2,i}$  of  $Pop$  to obtain mutants  $\bar{x}'_{2,i-1}$  and  $\bar{x}'_{2,i}$ 
9:       evaluate( $\bar{x}'_{2,i-1}$ ); evaluate( $\bar{x}'_{2,i}$ ); replace  $\bar{x}_{2,i-1}$  with  $\bar{x}'_{2,i-1}$  if  $f(\bar{x}'_{2,i-1}) \geq \bar{x}_{2,i-1}$ , and  $\bar{x}_{2,i}$  with  $\bar{x}'_{2,i}$ 
         if  $f(\bar{x}'_{2,i}) \geq \bar{x}_{2,i}$ 
10:      else
11:        generate two offspring  $\bar{x}^{(1)}$  and  $\bar{x}^{(2)}$  by selecting two parents from  $Pop$ , and then recombining
          and mutating them
12:        evaluate( $\bar{x}^{(1)}$ ); evaluate( $\bar{x}^{(2)}$ );  $OffPop = OffPop \cup \{\bar{x}^{(1)}\} \cup \{\bar{x}^{(2)}\}$ 
13:      if Algo = SGA then
14:         $Pop = OffPop$ 
15:      if Algo = GA-ES then
16:        form new  $Pop$  by selecting the best  $\mu$  solutions from the union population  $Pop \cup OffPop$ 
17:      if Algo = GA-GG then
18:        form new  $Pop$  by selecting the best  $\mu$  solutions from the union population of  $OffPop$  and the two
          fittest solutions of  $Pop$ 
19: evaluate( $\bar{x}$ ) { % this method manages the repairing and evaluation of a solution  $\bar{x}$ 
20: if  $\bar{x}$  violates Equation (3)  $\wedge$  (CHS = RS1  $\vee$  CHS = RS2  $\vee$  CHS = RS3  $\vee$  CHS = RS4) then
21:    $\bar{x} = \text{repair\_CHS}(\bar{x})$  % repairing is realized by calling the selected strategy CHS
22: obtain fitness  $f(\bar{x})$  (i.e. COG/ $g$ ) by running simulation model (perform  $m$  Monte Carlo trials if  $Stoch =$ 
    $true$ )
23: if  $\bar{x}$  violates Equation (3)  $\wedge$  CHS = RS5 then
24:   penalize fitness by  $f(\bar{x}) = f(\bar{x}) - c$ 
25: if  $Stoch = true$   $\bar{x}$  then
26:   if  $\bar{x} \in AllSolsEval$  then
27:     compute moving average fitness of  $\bar{x}$  and also update its fitness in  $AllSolsEval$ 
28:   else
29:     compute moving average fitness of  $\bar{x}$  and add  $(\bar{x}, f(\bar{x}))$  to  $AllSolsEval$ 
30: }
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each candidate solution. The fitness of a solution was then the average of the COG/g values across the m trials, and this average was updated if a solution happened to be evaluated multiple times during an optimization procedure.

Handling of infeasible solutions

Five constraint-handling strategies were analyzed to cope with infeasible solutions (violating Equation 3). Four of them (RS1, RS2, RS3 and RS4) repaired infeasible solutions, i.e. modified the genotype of a solution, while strategy RS5 avoided repairing in some way in order to prevent drift-like effects in the search direction (which may occur due to repairing).

The four 'repairing' strategies iteratively increased the values of the decision variables (associated with a particular chromatography step i), one variable at a time, until Equation 3 was satisfied or until the maximum value of a variable was reached, in which case the value of another variable was increased. The sequence in which the variables were modified affected the search. To investigate this effect, different sequences, represented by the strategies RS1 to RS4, were analyzed. The strategy, RS1, applied repairing according to the decision variable sequence $d_i \rightarrow n_{CYC,i} \rightarrow h_i \rightarrow n_{COL,i}$ (where i is the chromatography step violating Equation 3); this sequence represents typical rules applied in equipment sizing scale-up models. The strategy, RS2, employed the inverse sequence of RS1, i.e. $n_{COL,i} \rightarrow h_i \rightarrow n_{CYC,i} \rightarrow d_i$. The strategies, RS3 and RS4, switch between different repairing sequences during an optimization procedure. While RS3 chooses at random between the two sequences employed by RS1 and RS2, the strategy, RS4, chooses at random among all possible repairing sequences (note, there are 4! different sequences to choose from) whenever it needs to be repaired. The approach employed by RS4 would be plausible, for example, if no prior knowledge about promising repairing sequences was available. The strategy, RS5, does not apply repairing but penalizes infeasible solutions by degrading their fitness by a large fixed penalty value c .

The closed-loop platform comprising the search algorithms and process economics model was coded in C# (Microsoft Visual Studio 2010, Microsoft Corporation, WA, USA) which was linked to an input/output database in Microsoft Access (Microsoft Office 2010, Microsoft Corporation, WA, USA).

Case study description

An industrially-relevant case study was considered to demonstrate the ability of the framework to generate cost-efficient and robust chromatography equipment sizing strategies. The case study focuses on a single-product mAb manufacturing facility that employs a process sequence as shown in Fig. 1 (with $k=3$ chromatography steps) to satisfy a total product demand of $D=500$ kg/year with an expected titer of 3 g/L. The main details of the chromatography steps used in the model to perform mass balance and cost calculations are presented in Table 1. Titer variabilities were modelled using the triangular probability distribution, $Tr(2.6,3.0,3.4)$. Three scenarios of this case study with different ratios of USP:DSP trains were investigated: 1:1, 2:1 and 4:1. The USP train refers to the number of bioreactors operating (in a staggered mode), and an increase in the USP:DSP ratio corresponds to a decrease in the DSP window, the time available to perform chromatography. The range of possible decision variable values is $15 \text{ cm} \leq h_i \leq 25 \text{ cm}$ (11 values), $50 \text{ cm} \leq d_i \leq 200 \text{ cm}$ (16 values from a set of commercially available column diameters), $1 \leq n_{CYC,i} \leq 10$ (10 values), $1 \leq n_{COL,i} \leq 4$ (4 values), $i=1,2,3$; i.e. there are $(11 \times 16 \times 10 \times 4)^3 \approx 3.5 \times 10^{11}$ sizing strategies in total. The

Table 1. Key input parameters for the $k=3$ packed-bed chromatography steps in the case study

Input parameters	$i=1$	$i=2$	$i=3$
Dynamic binding capacity (g/L)	35	40	100
Linear velocity (cm/h)	350	200	300
Buffer volume (CV)	35	25	10
Resin price (\$/L)	13000	630	1100

Note: CV = number of column volumes.

Table 2. Default parameter settings of search algorithms

Parameter	Setting
Parent population size μ	80
Offspring population size λ	80
Per-variable mutation probability	1 / I
Crossover probability	0.6
Constraint-handling strategy	RS1
Number of generations G	25
Penalty value c	5000
Monte Carlo trials m	25

equipment sizing strategy of the base case is based on empirical rules used by manufacturers: a single column $n_{COL,i}=1$ with a fixed bed height of $h_i=20$ cm is run for a fixed number of cycles $n_{CYC,i}=5$ with the diameter size d_i being calculated such that the resulting total resin volume V_i (calculated according to Equation 1) satisfies the resin requirement constraint (Equation 3). Additionally, an attempt is made to reduce the number of cycles to $n_{CYC,i}=4$ (while keeping the other sizing parameters fixed); this setting is realized if the resulting resin volume still satisfies Equation 3.

The experimental study investigated different settings of the parameters involved in the search algorithms. However, if not otherwise stated, the default settings given in Table 2 were used. Any results shown are average results across 20 independent algorithm runs. To allow for a fair comparison of the search strategies, a different seed was used for the random number generator for each EA run but the same seeds for all strategies. This allows for the application of a repeated-measures statistical test, the Friedman test,²⁹ to investigate performance differences between algorithmic setups.

RESULTS AND DISCUSSION

Before analyzing the behaviour of the search algorithms on the chromatography equipment sizing problem, an indication of the properties of the fitness landscapes spanned by three case study scenarios is given. For this, the adaptive walks method was adopted.^{16,30} Starting from a randomly generated solution, an adaptive walk calculates the fitness of all neighbours of the solution that can be generated with a single mutation step, and selects one of the fitter neighbours at random to move to. If there is no fitter neighbour, then the walk has reached a local optimum and terminates. In this study, 1000 adaptive walks (using a fixed titer of 3 g/L) were performed on the landscape of each scenario, and the length of each walk was recorded. Figure 5(a) shows the distribution of the adaptive walk length in the form of boxplots. From the plot it can be observed that increasing the USP:DSP

Figure 5. Boxplots showing the distribution of the (a) length and (b) final fitness (COG/g) of 1000 adaptive walks for different USP:DSP ratios. The box represents the 25th and 75th percentile with the median indicated by the dark horizontal lines. The whiskers represent the observations with the lowest and highest value still within $Q1-1.5 \times IQR$ and $Q3+1.5 \times IQR$, respectively; solutions outside this range are indicated as dots. $Q1$ and $Q3$ are the 25th and 75th percentile, and the interquartile range is $IQR = Q3 - Q1$.

ratio tends to decrease the average length and variability of an adaptive walk. That is, the landscape becomes more rugged, or, equivalently, the number of local optima increases. This pattern is due to tighter DSP windows, which cause more solutions to violate the demand constraint and thus makes the problem harder to solve. This also causes an increase in the COG/g values as indicated in Fig. 5(b). The next section presents an analysis of how the search algorithms fared for both the deterministic (using a fixed titer of 3 g/L) and stochastic scenario.

Deterministic product titer

Figure 6(a) analyzes the performance of the search algorithms as a function of the population size μ . The aim of this experiment was to understand whether a large population should be evolved for few generations, or a small population for many generations. This understanding is important when optimizing subject to limited resources, such as limited computational power and time constraints. The figure illustrates that: (i) a population size of around $40 \leq \mu \leq 80$ yielded the best performance for the GA-based algorithms (SGA, GA-GG and GA-ES); (ii) GA-ES found the most cost-effective strategies; and (iii) random search outperforms PHC. Small population sizes, or search algorithms employing no elitism, such

Figure 6. (a) Average best COG/g (and its standard error) obtained by different search algorithms as a function of the population size μ ; the number of fitness evaluations available for optimization was fixed to #Evals = 2000, i.e. the number of generations is $G = \lfloor 2000/\mu \rfloor$. (b) Average best COG/g, as a function of the generation counter g , obtained by GA-ES using different repairing strategies. Both experiments were conducted on a chromatography equipment sizing problem featuring a ratio of USP:DSP trains of 4:1. For each setting shown on the abscissa, a Kruskal–Wallis test (significance level of 5%) has been carried out. In (a), GA-ES performs best for $\mu > 40$ while, in (b), RS1 performs best in the range $1 < g < 15$.

as SGA, did not perform well due to the high probability of getting trapped in one of the many local optima of the fitness landscape. Large population sizes converged slowly due to the low number of generations available for optimization. PHC was inferior to random search because the hill-climbers could get trapped in local optima, in which case further improvements were unlikely, while random search kept on generating (at random) new and potentially fitter solutions. (Note, the performance of random search is constant for different values of μ as it depends only on the total number of function evaluations available.)

Figure 6(b) investigates the performance impact of the five constraint-handling strategies, RS1 to RS5, when augmented on GA-ES (a similar performance impact was present for the other search algorithms). From the plot it is apparent that the constraint-handling strategy employed had an effect on the convergence speed and the final solution quality. Furthermore, it indicated that a repairing strategy (RS1, RS2, RS3 and RS4) performed better than a non-repairing strategy (RS5). The superior performance of RS1 is due to the fact that the variable d_i is modified (increased) first when repairing a solution. Unlike to the other variables, an increase in d_i is often sufficient to just satisfy the resin requirement constraint without increasing the processing time. From the performance

Figure 7. Average best COG/g (and its standard error) obtained by (a) GA-ES and (b) SGA in a deterministic and stochastic environment (using different values for the number of Monte Carlo trials m) as a function of the generation counter g . For each setting shown on the abscissa, a Kruskal–Wallis test (significance level of 5%) has been carried out. In (a), GA-ES with $m = 10$ performs best for $g > 15$, while, in (b), SGA, deterministic, performs best in the range $1 < g < 6$.

obtained with RS2, RS3 and RS4 it can be concluded that if d_i cannot be changed, then either the variable $n_{CYC,i}$ or h_i should be modified to meet the resin requirement constraint.

Stochastic product titer

The performance of the algorithms was then investigated in the presence of uncertain product titers for different values of Monte Carlo trials m . Figure 7 indicates that uncertainty impacts negatively the convergence speed and under certain circumstances also the final solution quality. This impact tends to be less severe as the degree of elitism employed by an algorithm increases (i.e. the performance of GA-ES is less affected than that of SGA). Elitism can help circumventing this issue as it causes a population to converge (quickly) to a (local) optimal region and then exploit this region. However, on the other hand, too much elitism (Fig. 7(a)) may disturb and prevent the generation of innovative solutions; here, optimization in a stochastic environment using relatively small values of m can yield better performance than optimization in a deterministic environment due to the greater randomness in the search. When the optimizer does not employ elitism (Fig. 7(b)), however, any additional randomness in the search may be a burden (because it can cause a population to oscillate between different regions of the search space, preventing or slowing down convergence towards promising regions).

In the following, the discussion will be limited to results obtained with GA-ES, as it was the best performing optimizer. The Monte Carlo trial setting $m = 25$ will be used to account for the trade-off between converging reliably to high quality solutions and being able to escape from suboptimal search regions. Figure 8 shows the sizing strategies for the most expensive chromatography step ($i = 1$) found by GA-ES for the USP:DSP ratios 1:1 (Fig. 8(a)), 2:1 (Fig. 8(b)) and 4:1 (Fig. 8(c)) at the end of the search across 20 independent algorithmic runs. The filled bubble in each plot indicates the best solution found by the EA, and the filled diamond the base case setup, which will be discussed in more detail in the next section. From the plots it is apparent that, when moving from 1USP:1DSP to 4USP:1DSP, the number of cycles tends to decrease as the time available for purification shortens. The EA finds more similar solutions for the scenario 4USP:1DSP than for 1USP:1DSP because the problem is harder to solve, as already indicated in the landscape analysis conducted previously.

Comparison with the base case

Figure 9 compares key performance metrics between the best equipment strategy found by GA-EA and the base case setup for chromatography step $i = 1$ (as this was the most expensive step) for the different ratios of USP:DSP trains in a stochastic optimization environment. The bar chart demonstrates that the EA discovered chromatography column sizing strategies that improved the value of the objective function COG/g relative to the empirical approach to column sizing often adopted by industry (i.e. base case, as described earlier) with savings in COG/g of up to 20% being achieved. The characteristics of the optimal solutions found by the EA depend on the type of scenario being addressed. For 1USP:1DSP, where the DSP window is unconstrained at 14 days, the optimized column sizing strategy employs a smaller column running for more cycles than in the base case. This reduces the amount of resin purchased and thus also the COG/g. In the scenario 4USP:1DSP, where the DSP window is constrained to 4 days, the column sizing strategies are optimized to run faster (i.e. fewer cycles and larger diameters), increasing the output kg produced and thus minimizing COG/g.

The use of stochastic EAs permitted the identification of more robust solutions, better equipped to handle titer fluctuations, as shown by the lower standard deviation values of COG/g and reduced product waste (Fig. 9). In the base case approach, the columns are sized according to the expected average titer without specifically accounting for titer fluctuations. Hence, in situations where titers are higher than expected, the columns may not have sufficient excess capacity to cope with higher product loads, leading to product waste. The sizing strategies found by the EA exhibit higher resin volumes and thus overcome this issue to a certain extent.

Incorporation of user preferences for selection of strategies

For all scenarios, the solutions in the final population found by GA-ES have COG/g values that do not differ by more than 3% of each other, and thus can be considered as valid alternatives available to the decision maker. This allows for the consideration of further criteria and preferences when selecting the strategy to be implemented in the real world, as illustrated in Fig. 10. For example, if space limitations and ease of operation considerations restrict the diameter of columns that can be used to $d_i \leq 1.6$ m, then solutions that do not meet the requirements can be excluded (black bubbles in Fig. 10). Also, due to validation issues there

Figure 8. Column sizing strategies for the most expensive chromatography step ($i = 1$) found by GA-ES at the end of the search across 20 independent algorithm runs (within an uncertain optimization environment) (bubbles) for the scenarios (a) 1USP:1DSP, (b) 2USP:1DSP and (c) 4USP:1DSP. The size of a bubble is proportional to the variable d_1 ; all solutions feature the setup $n_{COL,1} = 1$. The COG/g values of all solutions found by the EA for a particular scenario are within 3% of each other. For each scenario, the filled bubble represents the optimal setup found by the EA. The base case setup is indicated with a filled diamond and was not part of the solution set found by the EA for the scenarios 1USP:1DSP and 4USP:1DSP.

Figure 9. Percentage change in key performance criteria of the best solution found by the stochastic EA relative to the base case for different ratios of USP:DSP trains (COG = average COG/g, sd = standard deviation of COG/g, time = DSP time, kg = average product output, waste = average amount of product wasted due to titer fluctuations). The stochastic values for the base case sizing strategies were obtained by running the same number of Monte Carlo simulations on batch titer as performed for the best solution found by GA-ES.

might be the need to narrow the range of variation of the column bed height. The grey bubbles in Fig. 10 represent strategies that would not be feasible if the bed height was required to be in the range $18 \leq h_1 \leq 22$ cm. Therefore the white bubbles are the strategies that meet all criteria. The plot illustrates that even with these user preferences, the user still has a set of alternative solutions with similar COG/g values to choose from,

Figure 10. Column sizing strategies for the most expensive chromatography step ($i = 1$) found by GA-ES at the end of the search across 20 independent algorithm runs for the scenario 1USP:1DSP. The size of a bubble is proportional to the column diameter d_1 ; all solutions feature the setup of 1 column per step, $n_{COL,1} = 1$. Black bubbles: $d_1 > 1.6$ m. Grey bubbles: bed height $h_1 > 22$ or $h_1 < 18$ cm, white bubbles: meet both constraints.

thus providing greater flexibility to meet pressures for continuous process improvement.

CONCLUSIONS

This paper has considered a real-world problem concerned with the identification of cost-effective equipment sizing strategies for purification processes (with focus on chromatography steps) which

are core to all bioprocesses. The industrial case study was applied to monoclonal antibodies which represent the fastest growing segment of the pharmaceutical industry where a significant focus is on the need for more cost-effective and robust purification processes for different facility configurations. The application was formulated as a combinatorial closed-loop optimization problem subject to (i) expensive fitness evaluations (relying on time-consuming computer simulations), (ii) multiple dependent decision variables (related to properties of a chromatography column), (iii) constraints (related to resin requirements and product demand targets), and (iv) uncertain parameters (related to variations in product titers, which were accounted for using Monte Carlo simulations).

The investigation highlighted that EAs can identify a diverse set of equipment sizing strategies that are more cost-effective than the strategies used in industry. In particular, the analysis demonstrated that an EA performs best when elitism is employed in combination with a small number of Monte Carlo trials, infeasible solutions are handled using a non-trivial repairing strategy, and (when resources are limited) a medium-sized population (a size between $30 \leq \mu \leq 80$) is evolved for a relatively large number of generations. Furthermore, the study illustrated that having available a diverse set of cost-effective sizing strategies (rather than a single one) is beneficial when it comes to account for user preferences and selecting a strategy to be employed in the real world.

Future research will look at extending the equipment sizing problem considered here with relevant decision variables related, for example, to the sequence of a purification process employed and each step's operating conditions. The ultimate goal is to formulate an optimization problem that covers as many relevant aspects arising along the lifecycle of a biopharmaceutical product as possible. The benefit of solving this problem is that decisions regarding the development of a product can be made at an early stage, resulting in potentially significant financial savings.

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