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Continuous integrated manufacturing of therapeutic proteins

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With the growth of our understanding of biopharmaceutical processes, a transition from classical batch to continuous integrated manufacturing of therapeutic proteins is taking place across laboratory, clinical and commercial scales. Encouraged by regulatory authorities, this transition is favoured by new emerging technologies as well as by the development of better simulation models. The current status of continuous cell culture and downstream processes and requirements for their successful integration are discussed in this article, with specific reference to product quality attributes.

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Introduction

The transition from batch to continuous manufacturing is a common step in the development of an industrial sector, as it occurred in the last century for chemical, petrochemical and mechanical industries. In general, the main drivers are production costs and product quality. Lower in-process volumes coupled with higher levels of automation lead to significant reductions in both investment and operation costs [1]. On the other hand, the intrinsic reproducibility of steady state operation provides high and constant product quality. Indeed, continuous operation requires a good understanding of the process fundamentals and the capability of properly controlling its operation. This explains the tendency of manufacturing technology to be developed first in the batch form and only after reaching a certain maturity to evolve into continuous operation.

It appears that the pharmaceutical sector has now reached this maturity level and is about to undertake this important step: not only in the area of small molecules [2], but also in the more recent one of large biomolecules. More efficient manufacturing technologies are now requested also in this sector for various reasons. The large product quantities required by the often chronic character of protein based therapies, coupled with the large costs of GMP production spaces make the footprint reduction particularly attractive. On the other hand, the new competitive scenario brought up by the biosimilar markets, makes production costs of primary interest. These aspects are currently debated at different levels, where batch and continuous production technologies, based on stainless steel or single use, are compared considering different types of production volumes ranging from clinical trials to full commercial scale [1,3••,4]. Of course there is not a single winner, and the best manufacture strategy changes for different scenarios. Nevertheless it is to be recognized that continuous operation has clearly conquered its own space, which is now universally recognized in spite of the strong skepticism that surrounded it until only a few years ago.

Biopharmaceutical products need to fulfill high quality requirements in a strongly regulated environment. In general, these therapeutic proteins are heterogeneous in structure due to intra- and extracellular modifications of physical, chemical and biological nature which take place all along their manufacturing process [5,6]. These include posttranslational modifications (PTMs) such as N- or O-linked glycosylation, oxidation, deamidation and C- or N-terminal amino acid sequence variations as well as higher order modifications, such as aggregation and fragmentation [7,8]. In addition, we have process related impurities such as DNA and HCP, which have to be controlled below critical levels (e.g., in the order of 100 pg DNA per dose or 100 ppm of HCP). Together, these product characteristics make up a specific profile of critical quality attributes (cQAs) that determines the biologic activity, safety and therapeutic efficacy of a given therapeutic protein [9]. This characteristic profile has to be matched across the entire production process so as to meet the validated and closely controlled (FDA, EMA) product specifications.

In this review we focus more on the quality of the final product than on the issues related to the economy of the production process. This refers to the removal of both process and product related impurities, but also to the

reduction of the product heterogeneity in terms of isoforms distributions, like in particular glycoforms and charge variants.

Figure 1 shows two examples of end-to-end continuous integrated manufacture of monoclonal antibodies [10•,11•,12,13]. The sequence of operations is the same as in the classical batch production scheme based on mammalian recombinant cell technology [1,4], but here each operation is realized in a unit with continuous (or cyclic) inlet and outlet streams. In both cases the bioreactor is operated in the perfusion mode, followed by the classical protein A capture step with virus inactivation and finally polishing and filtration. The middle plots (1a and 1e) represent, as a function of time, the product concentration in the stream leaving the entire unit, together with the concentration of some typical product related (aggregates and fragments; 1a) and process related (host cell proteins (HCP) and residual protein A leached in the capture step; 1e) impurities. It is seen that the steady state achieved by the unit is rather stable at least for the few weeks considered in the two cases. On the other hand, the two lower panels in Figure 1c and f represent the decrease in the main impurities concentrations along the downstream section of the production unit, thus illustrating the role plaid by each individual purification step.

Before achieving steady state conditions, these units undergo a transient behavior, which is largely dominated by the bioreactor, which exhibits the largest residence time, in the order of days, compared to the purification units, which are more like in the order of hours. Considering that the perfusion bioreactor is a stirred vessel, its transient to steady state is expected to be in the order of 2 to 4 reactor volume changes. This refers however to the hydraulic behavior of the reactor and can be affected by the dynamics of the cell metabolism when subjected to an environmental change. This interaction was investigated using isotope labeling of complex nucleotide and nucleotide sugars by $^{13}C_6$ glucose. The delay of the metabolite turnover in a steady state perfusion culture was analysed as a function of the exchange rate in isotope fraction in the feed [14]. It was found that intracelluar CHO cell metabolite fingerprints achieve steady state in about four days when operated at common perfusion rates around 1 reactor volume per day. These fingerprints remained unchanged at constant bioreactor operation for several weeks, but changed when switching to different operating points [15,16]. For this, the consistency of the underlying cellular transcriptome and proteome was investigated by next generation sequencing and hyper reaction monitoring (HRM) proteomics. In both cases fold-change distributions converged to steady state between days three and seven [17]. The equilibration of intracellular processes and bioreactor hydrodynamics (extracellular environment) translate in constant posttranslational modifications of the protein product. For example, N-glycan distributions, achieving steady state in the order of seven days have been observed [18].

Upstream processes

The bioreactor is the primary source of product variability in PTMs and of its chemical and physical alterations [19]. Since in a fed-batch bioreactor there is no outlet stream, all waste, by-products and the target protein accumulate inside the unit. The cell density increases in a continuously changing environment, where depletion of key nutrients is controlled through proper additions, while the accumulation of undesired by-products (eg, ammonia and lactate) eventually compromise cell viability. In a perfusion reactor instead, two outlet streams, the harvest (equipped with a cell retention device) and the bleed, are used to control the concentration of product and cells in the reactor, respectively. This allows eliminating toxic metabolites thus achieving very high viable cell density values [20,21] but on the other hand limits the final titer to levels significantly lower than in the corresponding fed-batch reactor while increasing media throughput.

When considering product quality, the perfusion reactor exhibits two clear advantages as shown in Figure 2. In Figure 2a the behavior of cell density and viability as a function of time are compared. It is seen that the continuous time change observed in the fed batch is contrasted by the constant behavior of the perfusion reactor at steady state. A similar behavior is exhibited by all other species in the reactor, which causes PTMs to continuously change in the fed-batch reactor thus leading to a much higher heterogeneity of the final product [22,23•,24]. This is illustrated in Figure 2b, where the glycan fractions in the two reactors are compared as a function of time. A second fundamental aspect refers to the residence time distribution, which is compared in Figure 2c for the two reactors. It appears that the perfusion reactor exhibits a reasonably short and narrow residence time distribution, typical of stirred vessels, with an average value equal to the ratio between the reactor volume and the perfusion volumetric flowrate, which is typically of the order of days. On the other hand, the residence time distribution in the fed-batch reactor is very wide with proteins remaining in the reactor anywhere between one day and the entire duration of the culture. This generates much more product and process related impurities deriving from the chemical and physical modifications of the target protein mentioned above, such as aggregates, fragments or various types of charge variants [18,21]. In Figure 2d the charge variant distributions of the same antibody, but produced once in fed-batch and once in perfusion, are compared after the capture step. It is worth noting in this context that for this reason perfusion bioreactors are routinely employed since many years to produce particularly unstable recombinant protein formats (i.e. Infliximab — Janssen Biotech, Basiliximab — Novartis,

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