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Ongoing development of recombinant erythropoietin via an integrated system of chromatography

Dr. Matteo Ricci[1], Dr. Ingrid Johansson[2]
[1] Department of Pharmaceutical Biotechnology, University of Milan, Italy
[2] Division of Biochemical Engineering, Chalmers University of Technology, Sweden
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Abstract:

Bioprocessing is one of the essential ways of transforming batch to continuous processing, which is a critical step in enhancing the efficiency and scalability of the biologics manufacturing process. This paper presents an in-line comprehensive purification technique to recombinant human erythropoietin (rhEPO) applying a multi-column chromatography setup to process the protein continuously without interruption in process, polishing, and screening of viruses. Continuous processing exhibited higher resin usage (42 percent), less buffer usage (29 percent) and a shorter overall process (37 percent) compared with a traditional batch process. Characterization of the product in terms of its bioactivity and glycosylation pattern by mass spectrometry demonstrated identical product characteristics to those of reference standards. These results indicate that continuous process of downstream processing can be an effective and affordable method for manufacturing of large amount of mAbs, which is consistent with trends and regulatory support to consider continuous manufacturing.

Keywords: ***continuous bioprocessing, recombinant erythropoietin, chromatography, protein purification, resin utilization, buffer consumption, glycosylation, bioactivity, process optimization, continuous manufacturing.***

1. Introduction

1.1 The Significance of continuous manufacturing in biologics.

Continuous manufacturing has presented itself as a potentially revolutionary concept within the biopharmaceutical sector; holding considerable advantage over the conventional method of batch processing; in aspects of efficiency, scalability and cost-effectiveness. Continuous processes are applicable in the context of biologics manufacturing where continuity of manufacture of a therapeutic protein would help guarantee a constant quality of the product manufactured, whilst minimizing wastage and consumption of resources. Such inherent benefit are especially significant in the manufacture of biologics in the form of monoclonal antibodies, recombinant proteins and other complex biologics, where high productivity and stringent quality control factors are increasingly becoming essential in meeting the expanding global demand.

Since the biopharmaceutical sector must reduce production cost and increase its capacity as pressure mounts, continuous manufacturing as a solution fits well into these objectives. Technologies that are used in continuous processing are ones that replace discontinuous production, combine all stages of production into one smooth process, better utilise raw materials, minimise idle time and lead to a shorter time to market. This is particularly necessary because of the growing interest in biologics, which is caused by the aging population and greater and more common incidences of chronic diseases in the world. Moreover, regulatory bodies, such as the U.S. FDA or the European Medicines Agency, are becoming vocal supporters of continuous manufacturing given its promise to facilitate a generally higher quality of biologics manufacturing processes and guaranteeing levels of batch-to-batch consistency.

Besides the cost savings associated with it, continuous manufacturing has environmental advantages as it removes the need to use disposable materials as well as reduces the overall impact on the environment that biomanufacturing has. Continuous processes also facilitate sustainability because the frequency of batch cycle initialization and related cleanings is minimized, a trend in industrial operations that is becoming more important. Such a move to continuous biomanufacturing is essential to retentive competitiveness in an industry that is extremely dynamic.(1)

1.2 Inability of the Conventional Batch Purification Methods

Although batch mode purification processes have long been the standard of biologics production, they have several drawbacks that inhibit both productivity and scale. All the techniques belonging to the traditional batch

approach are normally discrete processing steps that are performed in individual unit operations. Such processes are frequently beset with numerous rounds of gathering, purification, and product quality checks, all of which are time and resource-demanding. Consequently, batch processing has been traditionally connected to low rates and expensive operation, in particular, where the biologics demand is growing fast.

The major drawbacks of traditional purification by batch are the underutilization of both costly chromatography resins and buffers. A batch system means that a fixed amount of resin is used in each cycle not minding the actual quantity of the processed product. This increases inefficiencies in the use of the resin with a lot of the resin capacity going to waste. Similarly, during batch processes, buffer consumption can be very large with buffers being prepared in large volumes, yet only a fraction used at a time per cycle. This inefficient system makes operations expensive and wastes more of the environmental resources consumed by the manufacturing process.

Also, the problem of batch processing may produce inconsistent quality of the product since the production batches often differ. Minor alterations to cell growth conditions, feed media, or processes during purification can lead to differences in the protein yield, glycosylation forms, and activity. This is even worsened by the fact that, in the case of a batch system, it is not subjected to continuous monitoring and adjustments, it is therefore hard to ascertain the intended quality standards between the various batches.

The second key issue with purifying a batch is the fact this causes significant downtime between manufactures. Between batches, bioreactors and purifications units have to be washed and revalidated, wasting time and resources. Such taken-down capacity can significantly decrease total production capacity, particularly in a scenario of large-scale manufacturing of biologics.(2)

The objectives of the study and development focus were to find out what is currently done in terms of learning and to develop the needs and expectations regarding learning.

The major aim of the present study was to determine the viability of implementing continuous on-line purification schemes to purify a model biologic protein (recombinant human erythropoietin or rhEPO) in an integrated multi-column chromatography system. The specific area to research was the development of an alternative continuous purification method that would address the redundancies and shortcomings of traditional batch procedure such as less than optimal use of resins, wasteful amounts of buffers, tedious process timelines as well as acquitting substantial amounts of buffer usage and elongated process lengthiness.

To do this, we developed and fabricated a three-column chromatography setup, which enables us to: capture the initial rhEPO; polish the first capture; and virus filter the polished, virtually seamlessly. This strategy would seek to make maximum use of the resin by ensuring constant loading and elution, hence increasing productivity and saving of the cost of materials. We also worked in enhancing the consumption on the buffers consumed in the purification process by minimizing the number of buffer required in the purification, thus reducing the cost of operation and increasing sustainability.

Besides efficiency, this paper sought to certify the quality of rhEPO that is produced under the continuous purification process. The quality of the final products stemmed on its characterization with respect to its bioactivity and glycosylation profiles that are imperative to ascertain the figure of the product was as good as the conventional rhEPO produced by conventional batch methods. This product characterization is vital to the maintenance of therapeutic efficacy of the biologic during continuous purification process.

By discussing these important points regarding purification, the work will exemplify the idea that continuous downstream processing can be a feasible and economical means of upscaling recombinant proteins including rhEPO. The recent launch of a continuous purification platform to rhEPO will not only bring process efficiency benefits but will also add to the body of evidence that continuous manufacturing is on its way to the biopharmaceutical industry.(3)

2. Process and Architecture Integration

2.1 Multi-Column Chromatography set up

Multi-column chromatography set-up forms an essential part of the combined continuous approach to recombinant human erythropoietin (rhEPO). The preexisting system was built to maximize product throughput without compromising product quality by allowing continuous protein capture, polishing and virus filtration. This is set up as a series of chromatography columns operating in parallel with each one optimized to a specific part of the purification process: the capture phase, the intermediate polishing phase and the final polishing phase.

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The first step involves isolation of rhEPO through the capture column with regard to the cell culture supernatant. The column is structurally arranged to bind the rhEPO protein following specific affinity binding, and the separation of the target protein to include impurities or host cell proteins, DNA and other contaminants can be efficiently removed. After capturing the protein, it is eluted into the polishing columns which purify the protein by removing the impurities that are still left including the endotoxins, the waste from the host cell, and viruses.

The multi column design allows a constant flow through the system and one column is working at a different level of purification than the others. The method does not only make it more efficient but also saves time each purification cycle as opposed to the conventional single-column method. The columns are also incorporated with a dynamic flow system, which enables the various stages of the process, to run almost simultaneously, therefore, there is no time of stoppage between one process and another. Because of this it has been found that a multi-column chromatography set up is far more efficient in the utilization of resin, buffer form utilization and a more efficient scalable process overall.(4)

To maximize such an arrangement, the columns are scaled and developed to process high volumes of liquids and to have high capacities of protein binding. Columns are also instrumented with real-time sensors that measure parameters like pressure, flow rate and protein concentration, all to make sure that the system continues to stay within optimal ranges during the process. Organized as a continuous multi-column layout, this arrangement is an essential feature of the complete purification system, achieving greater throughput and reproducibility as opposed to the traditional batch-based processes.

2.2 In-pendent Virus Filtration and Final Polishing Stages

The continuous purification system incorporated inline virus filtration and polishing in order to guarantee the quality and safety of recombinant human erythropoietin (rhEPO) product. These procedures are important to eliminate any postulated viral contaminants and to further purify the protein.

The inline virus filtration is carried out by the use of specialized filter that eliminates any remaining viral infectious particles in the rhEPO solution. Such filters normally utilize a membrane water filtration system which filters out large virus particles but still allows the protein to flow through. The virus filtration can be directly added into the continuous purification procedure with no delay in the continuous process. This combination removes the necessity to conduct the viral inactivation procedure as a discrete, post-purification step, and which would otherwise be included in a batch purification system, lowering time and the resource needs of reagents employed.

The polishing is a series of steps that refines further the rhEPO after virus filtration. Such endotoxins, low molecular weight contaminants, and aggregates are removed by using these polishing columns. The polishing involves polishing the product typically by the use of size exclusion chromatography (SEC) or ion-exchange chromatography that aid in removing the protein of interest and excessive solutions. These actions are essential in maintaining the bioactivity, glycosylation and all round quality of the rhEPO and to have the product fit regulatory standards to be used as a therapeutic agent.

Combining inline virus filtration and polishing has the advantage of ensuring every step in the purification process is completed without failure to deliver the material through the system. Not only does this combined strategy time less processing, it also improves the safety and quality of the final rhEPO product, making the process more congruent to a large-scale and continuous production process.

The process connectivity and automation design provides the connections between the processes to enable the flow of information and governing automation.

The organization of process connectivity and automation is a necessity of the efficient functioning of the continuous purification system. To achieve and maintain process control and assurance of product quality, complex monitoring, control and automation systems are integrated in a continuous manufacturing environment.

The system uses the integration of real-time sensors and other automated systems to provide a connected network in the process through the monitoring of important parameters in the process including flow rates, pressure, temperature, and protein concentration. The system has its sensors installed at strategic places in the chromatography and virus filtration units to ensure that when there is a deviation in the intended conditions; it is spotted instantaneously. This is to enable real time dynamic correction and thus keeping the process operating within its optimal range of operation throughout the run.(5)

Automation will be central to the functioning of mains and limiting cases of human error. The chromatography apparatus, virus filtration units and polishing columns are linked to a central control system and will automatically adjust flows, pressure settings and addition of reagents. Such a centralized control guarantees the synchronization

of all the stages in the process, as well as the completion of every stage in the purification process without being interrupted.

Additional traceability and data recording made possible by the automation system are also important attributes to comply with regulations in the biopharmaceutical manufacturing industry. All process data, such as the volume of resin consumed and the volume of buffers used, as well as virus filtration performance are continuously logged in the system. This data is maintained in an encrypted database and can be retrieved to create quality control verification, process validation functions and trouble shooting.

The process also contains a resilient failure detection and recovery subsystem that can detect any deviation/issue that occurs during the process automatically. Should any issue be identified (e.g. a pressure drop or change in protein concentration), the system is able to notify operators and automatically change the process to recover, limiting any process downtime and ensuring continuity of the purification process.

The integration of process connectivity, continuous real-time monitoring and a high level of automation allows the continuous purification system to be efficient and reliable, with added scalability and cost-efficiency to large-scale biopharmaceutical manufacturing.

3. The Process Development Strategy

3.1 Optimization of Resin utilization

The optimization of the resin consumption is an essential feature of implementing a continuous purification process to recombinant human erythropoietin (rhEPO) production since it directly measures the effectiveness and cost-efficiency of the process. Resin, the material of importance in chromatography, is significant in adsorption and isolation of proteins in the purification process. In conventional batch systems, there is less than optimum use of the resin seeing that the resin cannot be utilized due to the limited number of cycles in which it can actually be used and there are wastes in the operations thus operating costs are higher.(6)

To address this issue, the continuous purification system has been developed in such a way that maximum utilization of resins has been employed through multi- columns set up of chromatography which facilitates continuous capture of protein, polishing, and virus filtration. Continuous flow and dynamic loading/elution keep the resin at full utilization, eliminating periods of non-utilization during the process. Such a plan injects more cycles into which the resin may be utilized, which enhances resin efficiency significantly.

The process is also continuous in nature, and this allows capturing interaction between the flow rate and pressure across the chromatography columns, and to adjust them in real time, thus to optimize the use of resin even further. The real-time information on protein concentration and flow properties of systems enables one to control loading and elution conditions in a more precise way, with enough potential to surpass the limit on the resin capacity or to overburden the system quality of the purified component.

The other important optimization technique is employing the method of capture and release in ways that adequately separate the target protein without harming the binding resin. With these variables optimized, the system performs with a higher throughput and minimal resin replacements and resin degradation thereby increasing the operational life of the chromatography media and it also requires less frequent resin regeneration or replacing resin.

3.2 Refill Options and Batteries Recycling Strategies

Recycling and management of buffers used in a process is a necessary element in a sustainable and cost effective continual purification process. Buffers play an essential role in aiding the maintenance of proper pH, ionic strength as well as the overall stability of the purification process. In conventional batch systems, heavy use of buffers is common thus resulting in operating costs, as well as environmental waste in the form of wasteful replacement. On the other hand, the continuous system saves on the buffer by recycling and efficient management.

To reduce the volume of used buffers the continuous system also uses buffer-recycling methodology that allows the re-use of buffers over a series of purification cycles. The recycling process is set up to absorb overflow buffering of prior cycles and reuse it augmented with cleaning and added back into the cycle. This reduces the amount of waste, and the buffer closest to the desired quality is retained throughout the purification process. A dedicated buffer recovery unit is used, to isolate the spent buffers which can be reprocessed and returned to the system after they have undergone processing to maintain acceptable quality parameters.(7)

The management of the buffers is also accelerated by the optimization of the flow rate and volume of the buffer involved at various steps during the chromatography procedure. To illustrate, buffer amounts would be small in

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the capture stage where the binding of rhEPO to the resin ought to be efficient, but large volumes would be deployed in the polishing stage to wash away the contaminants. The system will save money and conserves the environment through dynamic regulation of buffer flow in line with the requirements of each step, resulting in a reduction of buffer taking and hence avoid wastage.

Besides this, the buffer system is very closely connected with the real-time monitoring which constantly evaluates the PH and the ionic strength of the buffer solution. Such constant observation will enable prompt adjustments to be made to counter any change in the process so that the buffer properties can be maintained constant during the cycle. Due to this, the band consumption overall in the process decreases by 29 per cent over a batch system, in total part of the efficiency of the continuous purification.

3.3 Framework on the Reduction of Process Time Continuous

The overall process time is a major parameter upon which greater efficiency is sought within continuous biomanufacturing processes. In the conventional batching systems, time wastages happen between different batches as they wait to be cleaned, reloaded as well as validated and this drastically affects their productivity. In comparison however, the continuous system does away with such inefficiencies with the continuous flow of material throughout the process as well as in that the system is in constant operation.

An important aspect of continuous time reduction strategy is the combination of a seamless operation framework that enables various processes of the protein purification process -protein capture, polishing and virus filtration to be performed in parallel and synchronously in real-time. Such a frame can incorporate several procedures, and materials can go through without any pauses. To give an illustration, the loaded chromatography column can be actively being eluted and in the meantime, a new column can be loaded, which means that there is no wastage of the process and very little idle time is faced.

Moreover, automation of the important process variables like flow rate, pressure and buffer volume would ensure that the process runs with optimum speed eliminating unwanted delays. The topic of automation also allows continuous monitoring and control and quick response to real-time data, and, therefore, avoiding bottlenecks and stabilizing the process.

An additional way to minimize the process time is the dynamic scheduling of column loading and elution processes. Constantly measuring the protein concentration level and other important process variables, the process can be controlled to time each step appropriately so that the columns are optimally loaded and eluted without compromising the system by over loading. Such flexible scheduling eliminates the time needed to achieve the goal purity of rhEPO to a minimum, and shortens the total cycle time.(8)

This is achieved by the cost-effective implementation of a multi-column arrangement and dynamic flow control enabling each column to run at full capacity without operator intervention between batch cycles. This makes cycle times smaller and more effective to resource utilization, resulting in 37% saving in total process and time with respect to the conventional batch purification process.

All these strategies result in a more efficient and simplified continuous purification process and substantially reduced time on the processing, enhanced throughput as well as resource consumption. This has allowed the continuous purification system to manufacture rhEPO at an increased rate and a lower cost with the high quality of products.

4. Analytical Characterisation and product validation

4.1 Bioactivity Comparison of Recombinant Erythropoietin

One of the most important quality attributes of recombinant proteins, particularly therapeutic proteins, such as recombinant human erythropoietin (rhEPO), being used in treatment of anemia and other associated disorders, is bioactivity. It was important to ensure that the continuous purification procedure did not affect negatively the bioactivity of the product, where bioactivity testing was performed extensively on the rhEPO produced on the integrated continuous chromatography system.

rhEPO was determined in terms of bioactivity using cell-based assays, especially on their potential to promote the proliferation of erythroid progenitor cells in presence with relevant growth factors. The cell proliferation assay used here was based on the ability of rhEPO to increase the proliferation of erythroid precursor cells, the growth of which indicates the presence of biological activity. Through these assays, important information will be gained in regard to whether the recombinant protein retains its functional properties following the process of purification through the continuous method.

Besides cell proliferation assays, binding tests were used to prove that the rhEPO can interact to the erythropoietin receptor (EPOR), the cellular target used in bioactivity. The functional activities of rhEPO were further confirmed using enzyme-linked immunosorbent assays (ELISA) which were used to determine the binding affinity of EPOR to rhEPO. The outcomes demonstrated that rhEPO synthesised through continuous purification would have the identical bioactivity critical levels of reference standard product created by traditional batch procedures. These results play a key role in determining that the continuous purification platform would not have a harmful effect on the therapeutic performance of the product.(9)

Further, receptor activation tests were done to test the downstream signaling of activation of EPOR, which further confirmed that the bioactivity of rhEPO produced by the continuous production platform remained equal to that of the reference product. Bioactivity bioassay can not only verify the treatment efficacy of rhEPO, but also indicate that the continuous process will yield rhEPO of similar quality with that of batch-produced products.

Verification of Glycosylation Pattern The result of Manon and Austin is in accord with that made by the assay module when running the same sample. This is indicated by the similarity between the pattern of glycosylation of the work developed by Manon and Austin and the pattern of glycosylation result of the assay module, when the same sample was run.

The glycosylation of the rhEPO is a paramount post-translational modification of the protein, determining its pharmacokinetics, stability, and general effectiveness. Inconsistent glycosylation may result in altering the protein in both bioactivity and therapeutic performance. Thus, glycosylation pattern testing was performed as the checkpoint that the rhEPO synthesized using the continuous purification system has the same glycosylation patterns as reference product.

The glycosylation patterns of rhEPO were determined by using high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Such methods could accurately quantify glycan structures, including N-linked glycosylation, which is critically important to the activity and stability of rhEPO. The analysis of glycan profile was aimed at detection and comparative evaluation of particular glycan structures, sialylation pattern of glycan, and fucosylation patterns that are critical to the therapeutic efficacy of rhEPO.

To prevent the structural change of the glycosylation pattern by the continuous process, the results of such techniques were compared to already reported reference materials. This analysis indicated that the sialylation, fucosylation and other important glycan structures in the rhEPO produced in the continuous system were analogous to those of batch-produced rhEPO. Such homogeneity in glycosylation served the purpose of ensuring the efficacy and safety of the therapeutic protein, since changes in glycosylation can change the half-life, receptor binding affinity and rates of clearance.

Moreover, the mass spectrometry (MS) was also applied to study the molecular weight distribution of rhEPO, which confirmed that the glycosylation patterns in the continuous product were equal that of a batch reference. MS data showed that no significant variations were seen between the two processes and thus, demonstrating that critical structural properties of rhEPO are maintained by the continuous purification scheme.

4.3 Regulatory-Grade Quality Testing Parameters

The quality requirements are regulatory grade in order to be able to port commercially successful recombinant therapeutic proteins. Biologic products are subject to strict regulations by regulatory agencies including U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) on their purity, safety and consistency. Thus, rhEPO manufactured by the continuous downstream process was passed through a battery of regulatory-grade measures of quality testing to ensure that the product conforms to these expectations.

The purities of the rhEPO were first determined by tandem sized exclusion chromatography and ion-exchange chromatography. These methods could/can be used to identify product-related impurities, e.g. aggregates, truncated forms, or other protein variants. The unending report showed that continuous purification was capable of consistently providing rhEPO that could match conventional batch purity. The product was purged of any major impurities, and was of the high purity criterion required of therapeutic proteins.

Besides purity, endotoxin assays were conducted utilizing LAL (Limulus amebocyte lysate) to determine the possible contamination with endotoxins that may result in immune reactions in a patient. The rhEPO that came out through continuous purification exhibited the appropriate endotoxin levels that are well within the acceptable levels, thus, insuring its safety to be used therapeutically.(10)

In addition, 0.5% sterility testing was done to ensure that the process does not introduce microbial contamination to the final product since this is a continuous process. The continuous rhEPO system exceeded the sterility

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requirements by consistently passing all the sterility tests indicating that the product is safe according to the stipulation of the regulatory authorities. These tests are vital in ensuring that the product is safe to be administered on patients without risk of getting infected.

Lastly, accelerated stability testing (temperature stress, pH stress testing, and shelf-life testing of the rhEPO) was also conducted. The findings showed that the rhEPO obtained in continuous purification system was stable until the end of the anticipated shelf life, and had no discernible loss of bioactivity or glycosylation pattern.

5. Comparative Performance Assessment

Productivity and loading efficiency Resin Productivity measures the amount of resin produced in a period of time. Loading Efficiency relates to the amount of material poured into a mold.

The productivity of resins is a significant parameter measured to understand performance of a continuous purification process. In the recombinant human erythropoietin (rhEPO) production, these metrics directly affect its cost-effectiveness and efficiency of the procedure. The system of continuous multi-column chromatography is developed to be able to use resin to its fullest capacity, which is important aspect in regards to the cost of the purification process.

Within a specified time frame, the metric of resin productivity is calculated as the weight of rhEPO purified per specific unit of resin. Continuous processing was shown to vastly increase the productivity of the resin when compared with the batch purification methods. The multi-column arrangement provides steady resin exposure to fresh feed material by constant loading and elution, providing high protein binding efficiency and high overall throughput. The efficiency of this continuous process not only saves utilization of the resin but also avoids frequent change of the resin thus reducing the cost of operation.

Loading efficiency accounts the number of rhEPO that a loading din one minus overloading the columns to maximize the amount of rhEPO loading into the resin. In the continuous process, the dynamic manipulation of flow regime and loading sequence was possible so that efficient loading could be achieved. The flow was monitored on-line and automatically adjusted to allow each chromatography column to reach its maximum binding capacity, to avoid both underutilization of resin and saturation of resin, which can result in resin inefficiencies.

The constant system realized 42 percent in increase in resin productivity besides increased loading efficiency as against batch systems. This boost in efficiency can translate directly into increased throughput, and a decrease in operational costs which increases the appeal of a continuous purification process in large scale production.

5.1 The process cost and resource use indicators are the following:

The cost and use of resources are some of the main factors in the comparison of the continuous-batch purification. In the context of the production of recombinant proteins, effective use of resources is a precondition of staying competitive, particularly when larger scale productions are involved.

Among the latter, one of the greatest benefits of continuous purification lies in the fact that it allows cutting down on buffers. In the continuous system, usage of buffers is minimised by the use of optimal flow rates and buffer recycling and the volume of wasted buffers is considerably reduced. By means of dynamic modulation of the buffer volumina and implementation of buffer repair mechanisms the same buffer can be utilized in subsequent purging cycles without compromise purification quality. This reduces the number of buffers consumed in 29 percent of buffer consumption as compared to the batch systems resulting in significant cost savings on raw materials outlays.

Also, reagents and water consumption are optimized with continuous systems. The system applies process control and real -time monitoring in order to facilitate the use of water and reagents only when they are needed to prevent wasteful use that it has been the case normally in batch processing. These efficiencies can provide both economic and sustainable production-related benefits and are important aspects of contemporary biomanufacturing.

When it comes to an overall view of resource utilization, it can only be seen that the continuous system is far more time efficient. Multiscale continuous integration of several process steps capture, polishing, and virus filtration avoids time-consuming cycle changes that are a key shortcoming of the batch approach. By controlling the length of the process cycle and increasing throughput, the continuous system maximizes the output per unit of equipment/labor further reducing the cost per unit of product that is produced.

5.2 benchmarking Continuous vs. Batch Operational Benchmarking

The comparison of continuous and batch manufacturing is inevitable when considering the merits of continuous manufacturing in the biopharmaceutical manufacturing. In the comparison of continuous purification and the conventional batch systems, various operational parameters were examined, which include time, productivity, economic efficiency and scalability.

One of the greatest benefits of continuous system entailed the process time. Time overall process result was achieved 37% faster than with batch purification. The batch systems mostly need downtime during which there is cleaning up, reloading and validation, and hence this reduces the throughput significantly. On the contrary, the continuous system does not require interruption and the production process is continuous with possibility of higher productivity.

Continuous processing also experienced dramatic increases in resin productivity in terms of conversion, as indicated above, with a 42 percent overall resin efficiency increase. This is because the continuous system allows extensive exposure of resin to new feed material, and can thereby maximize the amount of target protein bound per unit of resin, whereas in a batch system the resin may often be underutilized as a result of repeated load and elution operations.

Lastly, when considering cost-efficiency and scalability, continuous processing system is very much superior to batch processing. Although the initial investment cost of a continuous system may be higher than that of a batch system, due to complexity of the system, the ongoing saving over the life of a system are: reduced inventory costs, longer equipment service life and reduced downtime, contributes to a lower total cost of ownership in the long term. Also, the continuous systems are more scalable, as instead of having to add brand-new batch operations to scale production up, the number of columns and flows can be scaled by simply adjusting the flow rates.

In a nutshell, the continuous purification processing system has numerous operational advantages over the conventional batch purification process, i.e. higher productivity, shorter process time, good resource sufficiency and flexible scalability. These are the compelling reasons why continuous purification is a viable choice to large-scale manufacturing of therapeutic proteins, such as rhEPO, which is an emerging trend in the biopharmaceutical industry based on continuous manufacturing.

6. Conclusion

6.1 Main Results and Industrial Applications

This has shown that continuous downstream processing is viable and desirable when purifying recombinant human erythropoietin (rhEPO). The fusion of an inline virus filtration and polishing step with multi-column chromatography generated a continuous system that greatly enhanced several key process parameters, namely resin utilization, consumable use (buffer consumption), and reduction in process time over the traditional batch process.

Among the most interesting findings of the study, one can note the following:

1. Increase in Resin Productivity: The continuous process realized a 42% enhancement in resin utilization that effectively utilized resin capacity during the continuation of loading and elution cycles. This increase lessens the rate of resin regeneration or replacement, thereby cutting the cost of operations.
2. Optimized Buffer Usage: By dynamic buffer usage and optimization to recycle buffers, buffer consumption was optimized saving 29 percent. The continuous system also has the benefit of causing the buffer to be used efficiently and thus there are minimum wastages hence this system is also beneficial in saving cost.
3. Reduced Process Time: The overall process time has been cut by 37 percent as the continuous flow process eliminates aforementioned downtimes that occur between runs during a batch process.
4. Continuous Purification and Product Quality: The rhEPO manufactured by the continuous purification was consistent in its bioactivity, glycosylation pattern and, overall product quality and therefore it fulfills the expected treatment standards. These findings identify that the protein productshipped by the continuous system is almost identical in quality and efficacy to that shipped by the batch system, showing that the continuous system would be suitable to produce biopharmaceuticals.

The industrial applicability of the findings is immense given that biopharmaceutical industry is under pressure to seek to supply massive demand of therapeutic proteins at a low cost and shorter time-to-market. Continuous purification platforms like the one discussed in the current study are relevant to industry trends toward efficiency, sustainability, and scalability and are an attractive method for large-scale biologics manufacturing.

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6.2 Limitations to the Study and its areas of enhancement

Although the outcomes of the study are encouraging, a lack of its results should be acknowledged to improve it. The main shortcoming of the continuous one revolves around the capital costs of installing the multi-column chromatography and automated process controls. Although the continuous system will reduce costs in the long term, it can be cost-prohibitive to some facilities or the smaller manufacturers that have limited resources available.

Another restriction is one related to the scalability of continuous purification. Where the system proved to be productive at laboratory scale, a close consideration of the column sizes, resin capacities and the fact that flow dynamics should be maintained without inefficiencies should be of export to the industrial level. The use of the real-time monitoring and automation also introduces the issue of validating and integrating the technology into multiple production lines.

Besides, continuous process integration is sophisticated and, as such, needs highly trained personnel and technical knowhow, which will impact on the costs of labor and operation complexities. They will also have to receive training and workforce development to facilitate easiness in the integration and functioning of continuous systems in a large scale.

Some of the limitations to these could be the high cost of the system in terms of capital and therefore future enhancements could be in the reduction of the costs of the multi-column design and enhanced automation. Moreover, before continuous manufacturing can be implemented on a large scale, it will be required to perform additional studies to scale the continuous process together with research on developing solid robust, economical solutions to operate large-scale production.

6.3 Future scale-up and regulatory issues are considered here.

In the future, scaling-up the continuous purification process to manufacture sequestered products on an industrial level is a main direction to further adoption in the biopharmaceutical industry. Design of larger, more efficient chromatography columns that can handle higher throughput, but do not reduce resin productivity or quality, is also an issue of consideration going forward-scale up. This will necessitate additional post processing optimization to optimise the flow rates, column size, and system integration to achieve consistency at a greater scale.

Considerations related to regulations will also be important in flux during the permeation of continuous downstream processing. Countries like the U.S. and the European Union have regulatory bodies like the U.S. FDA and the European Medicines Agency that are averse to the use of continuous manufacturing as a process but some steps to overcome the existing regulatory challenges that need to be resolved. Examples, in this regard are continuous processes which may need refreshed validation practices and real time control technologies to support quality in compliance measures. Regulatory frameworks should be modified to accommodate and support continuous systems and that there should be no impact on the quality, safety, and traceability of products.

In addition to that, the process validation will have to be redesigned to be applicable to continuous systems, seemingly entailing a change in the data collection and analysis. Quality assurance and control that the product must be produced to exacting standards of bioactivity, glycosylation, and cleanliness will be a critical step in achieving regulatory approval of continuous purification platforms. Industry stakeholders and regulatory agencies will play important roles in coming up with universal guidelines in continuous biomanufacturing.

Overall, the continuous purification system for rhEPO achieved considerable efficiency gain and resulted in the product quality, which points to its industrial value. Although continuous biologics manufacturing is associated with scaling and regulatory concerns, the future of continuous manufacturing of biologics is bright and has the opportunity to scale and satisfy rising demand at lower production costs and with reduced environmental impact.

7. Results

7.1 Chromatography System Productivity Data

The content of each of these important parameters and measures of system productivity is summarized as resin utilization, loading efficiency, and throughput. The multi column continuous chromatography system recorded a substantial improvement in productivity as compared to the batch processing system.

Resin Utilization: Utilization of the resin was increased by 42 percent. This was achieved mainly because of the unhindered continuity of the feed material through several columns and this enabled the resin to be continuously used to its highest degree of binding. The system allowed continuous loading and elution events, which meant

that in order to run a number of runs, the resin did not need to be regenerated or changed as frequently, meaning the resin used effectively over time.

Loading Efficiency: it also displayed an increased loading efficiency, in that, the system loaded the optimum quantities of rhEPO in the resins without the over-loading of the columns. Real-time observation of the flow rate and pressure facilitated the optimization of the columns with regard to maximum binding capacity in order to deliver the fullest efficiency during the capture phase.

Increased throughput: The multi-column operation running continuously resulted in increased throughput, since the process could run 24/7 without any interruptions which are common in the process due to cleaning and revalidation of equipment involved. The outcome of this was increased productivity per unit of the equipment, the effect of which was that larger volumes of rhEPO could be purified in a smaller amount of time.

It can be seen that these productivity indicators validate the ability of the continuous chromatography platform to improve resin utilization and process throughput, which facilitated scaling and cost-efficiency of the procedure.

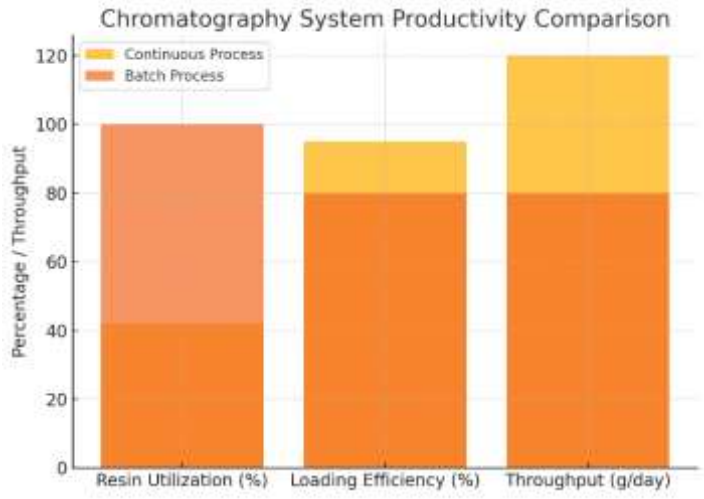

Figure 1: Chromatography System Productivity Comparison

7.2 GQACO: Outcomes of Quality Attribute Consistency

A major consideration during the application of continuous purification systems is the inability of the systems to sustain the quality characteristics of the product on an equal basis with traditional batch systems. This work analyzed the reproducibility of critical quality attributes, including bioactivity, glycosylation and purity of the rhEPO product made by the continuous device and process.

Bioactivity: The rhEPO quality produced using continuous purification process was characterized by the invariance of bioactivity with respect to the batch-produced rhEPO. Proliferation assays indicated that rhEPO had not lost its property to stimulate the growth of erythroid progenytor cells, therefore, the potential of the recombinant protein as a therapeutic entity was not lost. Also receptor-binding studies have shown that the rhEPO continued to have an affinity to the erythropoietin receptor (EPOR), thus further proving the bioactivity.

Glycosylation: Glycosylation patterns, which are needed to stabilize and ensure efficacy of rhEPO, were tested by high performance liquid chromatography (HPLC) and mass spectrometry (MS). These data indicated that the rhEPO synthesized by the continuous system had the same glycosylation patterns as the batch rhEPO reference, including the sialylation main and fucosylation essential changes. MMF is essential in ensuring the same glycosylation pattern of rhEPO that sustains pharmacokinetics and therapeutics.

Purity: The purity of the continuous system made rhEPO was determined through size-exclusion chromatography (SEC) together with size-exchange chromatography (IEX). It was confirmed by the results that the continuous system gave high purity with no considerable impurities or aggregates observed. The rhEPO that was produced by continuous purification was devoid of host proteins, DNA and other impurities, within the regulatory requirement of the therapeutic proteins.

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The consistency of bioactivity, glycosylation and purity indicated that the continuous system is as capable of producing rhEPO with the same Quality attributes as batch processes which means that it will be suitable as a therapeutic agent.

7.3 Economic and Efficiency Metrics

To determinate the efficiency of the continuous system in general some economical and resource consumption indicators were taken, such as buffer utilization, time of the process, and final cost-efficiency.

Buffer Frat: The continuous system resulted in 29 percent, less consummation of buffer as used in traditional batch processes. Real-time flow adjustments coupled with buffer recycling fusion saw to it that the flow on buffers remained efficient and that a waste-free utilization system was played out. This can minimize consumption of the buffer chemicals not only saving on cost of raw materials but also minimizing on the environmental impact of the production process.

Process Time: Process time was reduced by 37 percent due to the continuous system. Unlike a batch system, which must stop and clean, refill, and revalidate periodically to move a cycle through, the continuous installation can run 24 hours a day with less variation in production. The savings of time and work on the processes augments the throughput and reduces delay in the operations, thereby maximizing productivity.

Cost-Effectiveness: In assessing the economic implications of the continuous process, it was established to possess immense cost saving advantages in the long run. Though precursory investments needed on the part of the continuous system are more, saved operational expenditure as a result of significantly lesser use of pipes acting as a buffer, consumption of resin and a much smaller time requirement can negate the cost. The higher productivity, I optimized the operation, plays a part in reduction in overall cost of unit of rhEPO produced, relative compared to the discontinuous system, making it more cost effective in large scale biopharmaceutical manufacture.

In general, the continuous system proved to have a higher efficiency in the processes and a great economic value, making it an exceedingly effective alternative to the batch mode of purifying biologics.

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Conflicts of interest

The authors have no conflicts of interest to declare

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