A Review on Process Intensification of Biopharmaceuticals for Downstream Purification

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Abstract –The process development for biopharma product purification had been a challenge and developed in a few years to attain a good yield of having better control over the process. Also, techniques were diverted to have Process analytical technology (PAT) and quality by design (QBD) approach to meet the demand of the recent technology. The biopharma process mainly relies on chromatographic and precipitation operations. Development of continuous precipitation in the tubular reactor and continuous chromatographic separation utilizing simulated moving bed chromatography or periodic counter-current chromatography operations becomes the new challenges in front of the biopharma industry. Few studies have focused on the development and monitoring of continuous precipitation platform for downstream process, and has proved significant productivity and yield as well as purity in comparison with batch. The adoption of this technology can be proven a better alternative for the batch processes; also it will prove better product quality.

Key Words:Tubular Reactor, Productivity, Downstream Process

INTRODUCTION

The current state of biopharma operates on batch mode; there is huge potential to convert this process into continuous processing in especially downstream processing. For biopharmaceuticals it very necessary to have this production at low cost and high productivity.[1]The complete process for the manufacturing of a biopharma or biotech product is divided into two categories named as upstream processing and downstream process. The upstream defines the cultivation of the cell and subsequently getting the product out of it. Whereas downstream deals with getting the final product purified, which involves the number of unit operation such as cell disruption, cell separation employing filtration or centrifugation, capture by affinity or precipitation or selective extraction in case of active pharma ingredients, also removal of process scale impurities and product-related impurities and formulation of finally purified product. The operation involved in downstream processing is more than the upstream and hence it also leads to an increment of cost of production. The challenge in the pharma industry to gain productivity, also process control, for example in continuous chromatography, getting continuous purification done is very complex and not much flexible.[2]The studies were also diverted to real-time monitoring of bioprocess; this involves the incorporation of flow cells into the process steps. The model describes the residence time distribution and has control of pH, conductivity and turbidity. This is applied to the continuous purification of biopharmaceuticals.[3]

Continuous Downstream Purification

Process intensification operation results in less time for process completion due to the omission of the hold steps and high productivity and low shutdown cost, and by reduces labor requirement. The continuous processing of biopharmaceuticals involves the conduction of unit operation at a steady state. The advantage of the use of continuous unit operation is, one can start production at a smaller scale and thus reduces capital costs and reduces footprints and higher automation. The initial efforts in continuous processing were focused on upstream processing specifically the use of continuous (perfusion) reactor for high cell density cultures. Now it has been shifted towards downstream processing and

especially towards chromatography. The main objectives of downstream processing are cell separation, clarification of process stream. The removal of process-related impurities and product-related impurity. The main operations involve centrifugation, homogenization, filtration, refolding, chromatography, membrane separation, extraction and precipitation. Cell lysis is when the product is intracellular. High-pressure homogenization and centrifugation can be used optimized in a continuous mode. Over the past decade, the research has focused on the continuous biotech unit operation and such kind of studies can be useful for the industry to produces efficient biopharmaceuticals. [4]

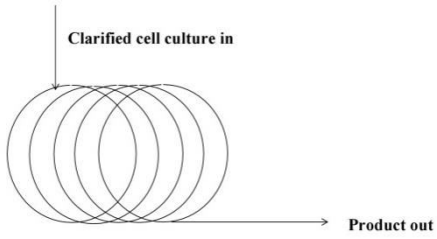

Figure 1 Diagrammatic Explanation of Process intensification of Biopharmaceuticals

A study on continuous refolding of protein like proteases was demonstrated in a continuous tubular reactor. The steps for refolding include dilution of protein, then pulse refolding and use of various temperature strategies to refold the protein. Previously people used to refold the protein in batch mode (as in batch reactor) but now the tubular reactor could be a solution for continuously refolding protein. Here there is the use of tubing and pump occurs so one can easily scale up the process up to a large scale with flexibility. It has a high surface to volume ratio that has an advantage over the heat transfer and achieving better temperature control. [5]

Few studies on monoclonal antibody purification give insights into continuous precipitation of monoclonal antibody precipitation. The precipitant was a combination of zinc chloride and polyethylene glycol. The tubular reactor was made up of silicon tubing having a diameter of 0.48 cm. the reactor has a Y joint and harvested fluid was continuously passed through one side and precipitant passed through the other side. The yield of mAb was greater than 97 % and precipitant concentration was above 15 mM and 7 % PEG. The reduction of host cell proteins was obtained by 60 %. The mAb yield of the fully integrated process was 80 %. Thus it can be a cost-effective method for purification of proteins like monoclonal antibodies, which involves high-cost resins for purification of proteins. [6] The continuous precipitation of the antibody was studied by a few people, which gives a prominent result. The capture step was done by using PEG 6000 precipitation and Zn^{++} was also used for multistep precipitation. The obtained yield was 95 % with a purity of 97 %. The purity obtained which is comparable to that of the protein A chromatography, also process scale impurities like HCP and HCDNA were comparable to chromatographic separation. The process is roboust and needs not to have a surge tank for operation. This can be integrated into other operations such as viral filtration and ultrafiltration. [7] The continuous precipitation of mAb by using PEG was developed sequentially. The initial step was process-related impurity removal and then precipitation of product can be done. The mAb's were precipitated by pH adjustment and PEG supplementation, which gives a yield of about the 86 – 94 % lowering the impurities by 7200 – 15000 ppm. The method was tired in a continuous tubular reactor by converting the time for reaching equilibrium into the residence time. The efforts were made in terms of combining the TFF concerning the precipitation to achieve yield in the process.[8]

Few studies focus on the economic perspective of recombinant antibody production; the chromatographic steps become obsolete and can be performed continuously. The cost of goods analyses was done for the generic chromatography based antibody standard purification. The continuous precipitation based purification, and hybrid coupling to the continuous purification such as upstream batch process. [9]

The continuous precipitation of IgG from CHO cell culture was studied in a tubular reactor. The precipitation involves the use of salts such as $CaCl_2$ and organic solvent. The protein A capture step was replaced by precipitation and yield was obtained towards more than 90 %. Also, there was a significant reduction in host cell protein and host cell DNA observed. The continuous precipitation in the tubular reactor is low cost and high yield giving process, also there is ease of

scalability in this process. The precipitation can be performed in both ways, like batch and continuous mode. The tubular reactor gives an advantage over the cooling and counters current operation with comparison to the batch reactor. [10]

Continuous purification of proteins utilizing crystallization in a tubular reactor was developed. A small lab scale stirred tank with a cooled tubular reactor in bypass mode was developed for continuous crystallization of lysozyme. The protein was crystallized by a combined method of crystallization the one which is salting out and another is cooling crystallization. The crystal growth was enhanced by recrystallization in bypass mode and the yield was achieved 90 % and the antibody yield of in terms of space time was 12 g/l hr. [11] The continuous crystallization of lysozyme in tubular reactor was achieved successfully, the crystals generated having particles size 15 to 40 µm. The reactor having dimensions of 2 mm inner diameter and 13 m overall length. The total flow rate was 1.9 mm/s and the production rate achieved was 1 g/h. The crystallization of protein in the formulation has added advantages in terms of suspendable formulation, good syringe ability and injectability. [12] Another study on continuous refolding of proteins (auto protease) was done in the tubular reactor. The inclusion bodies obtained from expression were dissolved continuously in a tubular reactor and refolded continuously. The reactor having a configuration like a 3.2 mm internal diameter and having a variable length. The steady-state was achieved during the operation and higher utilization of reactor volume and smaller holding times also the better flexibility.[13] The study deals with the process integration of bioprocessing and control over the process. These three approaches were proposed, the first one is a modular approach, the second is an adaptation and the third id the merger approach. The modular approach deals with the consideration of each unit operation and integration of each unit operation. Adaptation deals with the modification of the unit operation to integrate the process. The third one is, merging all unit operations to one single unit operation. The approaches are evolving over the period and there is a need to find out analytical tools to find out real-time monitoring of critical attributes of quality and control over the process. [14] There are few examples which has developed the continuous chromatographic process for recombinant blood coagulating factor VII, this leads to higher productivity and reduced buffer consumption. In continuous process the time required for CIP and washing will get reduced. A study done coiled reactor which is basically plug flow reactor and it was developed for continuous refolding of granulocyte colony stimulating factor (GCSF). The design gives the effective refolding of protein in tubular reactor. The performance was compared with respect batch process and achieved more yield than batch with nearly equal purity. The final outcome of this study improve the product quality by using quality by design and process analytical technology and thus increase the productivity by employing continuous purification. [15]

CONCLUSION

The continuous purification has wide application such as reduced buffer consumption, increased productivity and reduced number of steps for the preparation of vessels and purification process. Use of continuous precipitation has much potential to convert the process from batch to continuous. Also few studies give an idea about the continuous crystallization of proteins which can be a future perspective to develop single step crystallization for purification process. Integration of modern online tools for monitoring and optimizing the performance of production can leads to better control over the process.

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