PROTEIN THERAPEUTICS

Between Platform Technology and Individual Process

Today more than ever, time-saving and cost-effective techniques are required in the production of protein therapeutics. *By Dr. Alexander Faude, Dr. Dethardt Müller, Dr. Stefan Schmidt, Dr. Birgit Schwab, Rentschler Biotechnologie GmbH.*

Innovative protein therapeutics are currently in the focus of many biotechnology and pharmaceutical companies. In this context, monoclonal antibodies and Fc fusion proteins play an important role. These substance classes are already successful on the market in a wide range and dominate the biopharmaceutical development pipelines. They are usually used as simple antagonists, mostly in tumor therapy. In order to realize a broader spectrum of mechanisms of action, additional protein formats are increasingly being developed. For example, these include multi-specific and multi-functional antibody fragments or novel fusion proteins. They can simultaneously inhibit several cell surface receptors or block various ligands at the same time, but also link different receptors or recruit T cells in the immediate vicinity of tumor cells [1]. Underlying this is a broad range of different molecule types that lack a similar element such as the Fc portion.

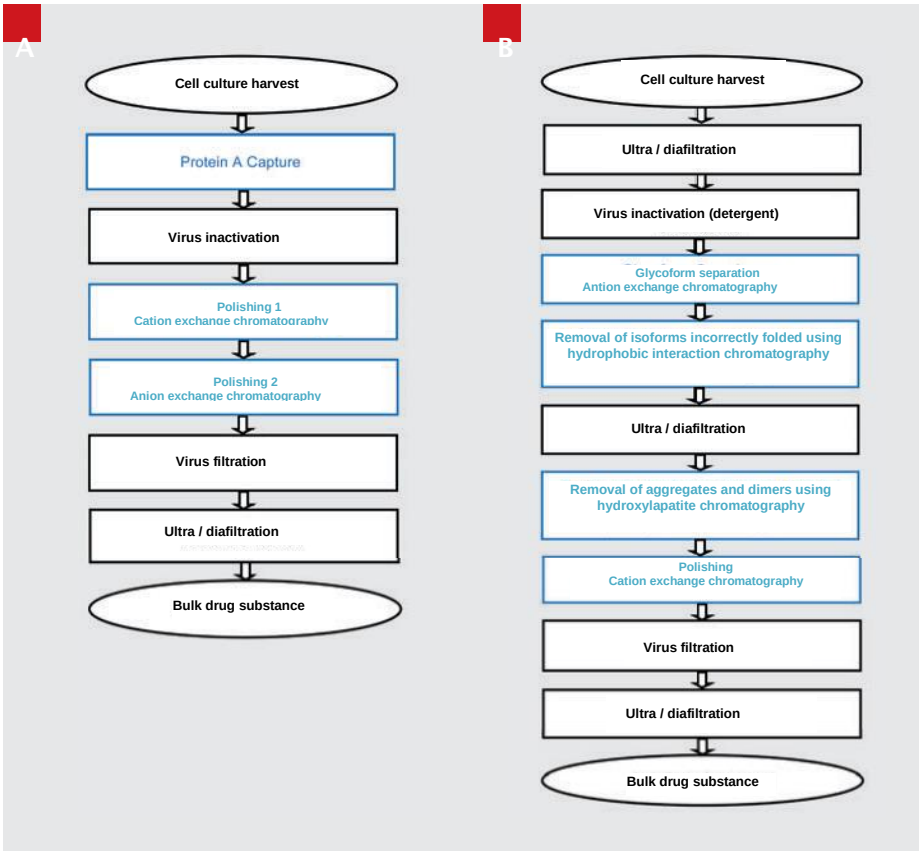

Fig. 1: Comparison of downstream processing variants. A: Platform process for monoclonal antibodies and Fc fusion proteins including three chromatography steps. B: Individual purification process for a glycosylated non-Fc fusion protein including four chromatography steps. The chromatography steps are shown in blue.

Today more than ever, time-saving and cost-effective techniques are required for the manufacturing of these therapeutic agents, which to a large extent are produced using cell cultures. This is especially true for the purification of such complex products into which a large part of the production costs still flows. Costs increase rapidly in the event of a process failure since the pure active substance is closer to being obtained. Therefore, besides efficiency, a reliable robustness is especially required in all process steps.

Purification of monoclonal antibodies and Fc fusion proteins

The question of the existence of an Fc portion is crucial in the purification of proteins from cell culture supernatant. Due to this structural similarity, platform processes can be established for monoclonal antibodies and Fc fusion proteins using standardized and characterized separation media, filter, process solutions and plants as far as possible. As a result, the process development time is reduced. Preparation and implementation of production under Good Manufacturing Practice (GMP) conditions can be realized quickly and cost-effectively. The process platform established by Rentschler includes various chromatography and filtration steps (Fig. 1A). Affinity chromatography with protein A is used to concentrate the active substance and to reduce process-related impurities such as host cell proteins, DNA, production media contents and metabolites. This capture step contributes a major part of the required purification work.

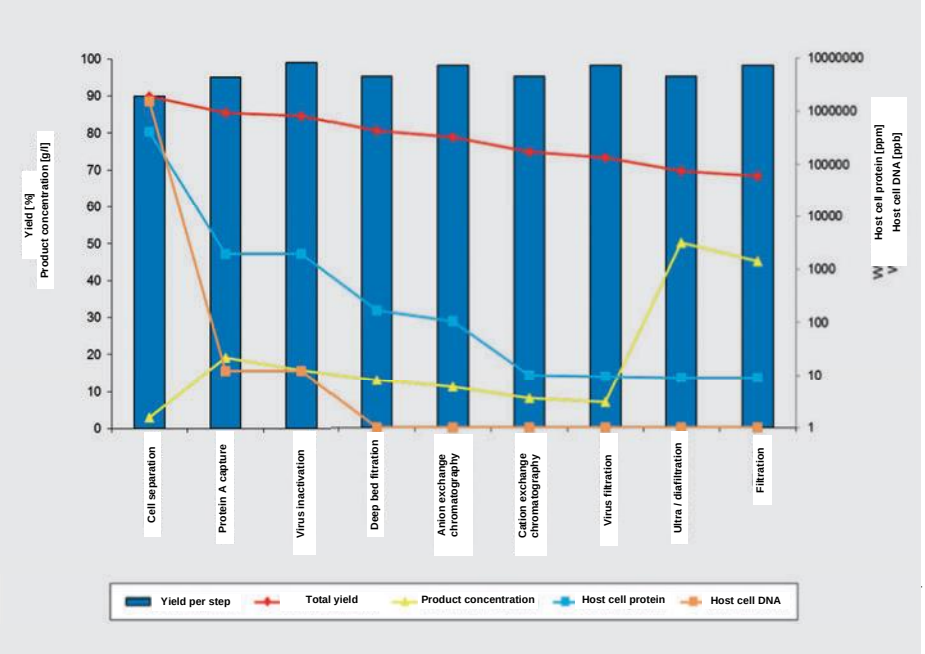

Fig. 2: Production overview of the platform process

Following virus inactivation via pH-lowering and subsequent deep bed filtration to remove precipitated substances and undesired particles, further sequential purification was carried out via anion and cation exchange chromatography. Reduction of product-related (aggregates, fragments) and process-related impurities (host cell proteins, DNA) is the main focus here. The following nanofiltration as well as virus inactivation and anion exchange chromatography contribute to the viral safety of the active substance. The product is concentrated by the final ultra / diafiltration and transferred to a suitable matrix via buffer change. Following a germ reduction filtration, it can be stored in a stable condition. With this process, a yield of more than 70% can be achieved in most cases (Fig. 2).

The plants existing at Rentschler allow purification of up to 3600 liters cell culture harvest. Systems based on single-use or stainless steel as well as hybrid solutions are used thereby. A special advantage of the platform technology is – besides standardization – the high degree of adaption flexibility which provides sufficient freedom for product-specific requirements. Minor changes (in the order of the process steps for example) already allow generating new stable process states achieving the desired robustness as well as product yield and quality.

Purification of recombinant proteins without Fc portion

If the protein does not contain an Fc portion, special requirements for the purification process will be a result of this molecule structure. In many cases, no previous experience can be used, so an individual process must be developed. There is no special capture step based on a specific affinity for most of the proteins without Fc portion. This problem can be avoided by classical ion exchange chromatography. This method offers the required capacity but shows a considerably lower purification factor than the traditional protein A chromatography for antibodies. In addition, the typical virus inactivation at low pH-value is frequently not practicable due to the low stability in acidic conditions. Organic solvents or detergents can be used to solve this problem. For detergents, analytical evidence for their absence must be presented in the further course. In addition, a specific quantification method must be established and validated for each protein, since otherwise the usual Protein A HPLC method cannot be used.

The artificial linking between proteins not related to each other with different characteristics can cause increased aggregate formation in fusion proteins. Approved methods such as Hydroxylapatite chromatography can be used to solve this problem. In addition, incorrectly folded variants frequently arise from proteins which consist of multiple copies of similar subunits (multivalent single chain antibody fragments (scFV) for example). These must be separated from the molecules with the correct conformation. Hydrophobic interaction chromatography can for instance be used here to selectively bind incorrectly folded molecules exposing hydrophobic parts.

Another challenge of non-Fc proteins is compliance with a specific glycosylation pattern. This is essential for example for erythropoietin or enzymes for treating lysosomal diseases, due to the fact that some isoforms show high pharmacological activity. Ion exchange chromatography for instance is for these cases a method of choice.

In general, non-Fc proteins require on average at least one more purification step than the platform process (Fig. 1B).Nevertheless, one can profit from the experience of the platform processes, since modern methods for process development such as design of experiment (DoE) remain the same. Only analytical test must be adapted or newly established.

– Rentschler has extensive know-how in protein purification, whether it is platform technology or an individually developed purification process. This has so far been successfully demonstrated with a multitude of processes for GMP-compliant production of monoclonal antibodies and Fc fusion proteins. In addition, Rentschler has for many years been a specialist in projects which represent a challenge by the search for a suitable affinity step, aggregation problems or demanding glycan profiles. Based on the pioneering work on interferon beta, the expertise in the field of non-Fc proteins has been continually developed with the production of well over twenty completely different molecules (for example fusion proteins, enzymes, blood or growth factors) in recent years.

Literature

[1] The New Generation of Antibody Therapeutics: Current Status and Future Prospects, K.J.Morrow, Insight Pharma Reports 2012

Contact

www.rentschler.de