

Smart synthesis, membrane filtration and chromatography: For time-efficient and cost-effective production, at the required purity

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Abstract

Molecules reaching the market in the recent years present an ever-growing complexity. In the meantime, purity criteria are more important than ever to ensure patients safety. The combination of these two facts represents a big challenge for the manufacturing industry where the right combination of state-of-the-art synthesis and advanced purification is necessary to meet the demand in a cost-effective and time-efficient manner. This paper presents membrane filtration and chromatography as efficient technologies to reach the required purity level. The use of these technologies at industrial scale is illustrated through a case study including a complex biocatalysis reaction and the complete downstream processing yielding the final, pure product. The scale-up from gram to hundred kilogram scale, process efficiency and timelines are also considered.



Synthesis



Membrane filtration



Preparative chromatography

Introduction

The complexity of the APIs reaching the market has been increasing over the years, the new synthetic molecules developed in the pharmaceutical industry have become larger and more challenging to synthesize. This trend is driven by two major factors: (1) the development of more and more targeted therapies, leading to specifically designed, highly functionalized molecules and (2) the intellectual property mine field, commanding innovators to look for unpatented molecular structures to protect their future markets. In addition, specific and inflexible purity targets have to be met to ensure patients safety and comply with ever tougher regulation while minimizing the costs of production. The combination of complexity and purity represents a real challenge for the chemical and biochemical manufacturing industry which is to master smart synthesis and advanced purification technologies to deliver high quality products. In a first part, this article will present selected purification technologies. Their use to reach the required purity while optimizing the recovery yield and production costs will be illustrated in a second part with a case study, demonstrating that the combination of advanced purification technologies and smart synthesis guarantees meeting tough timelines and purity specifications.

Membrane filtration

Membrane filtration (**Figure 1**) allows for a mild downstream processing to separate the components of a complex mixture depending on their size, to carry out a buffer exchange (diafiltration) or to concentrate of a solution. Depending on the size of the membrane pores (also called the membrane cut-off), different particle sizes are excluded and this determines the type of filtration: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) or reverse osmosis (RO) as depicted on **Figure 2**.



Figure 1: Filtration unit: 4000 L reactor (foreground) and UF membrane filtration units (background, on the wall behind the operator).

This technology is well adapted and routinely used in the food industry to isolate a food ingredient from complex natural mixtures or in bio-pharma industry to purify compounds obtained by fermentation or cell culture. However, this technology can also be implemented

to synthetic processes when similar conditions are met and more conventional processes cannot apply. This is the case for biocatalytic processes where the reaction is carried out in aqueous buffers, the molecules involved are often fragile and temperature sensitive, the resulting mixture can be complex and presents molecules of very different sizes to separate (enzyme, cofactor, product, by-products, etc.). As a result a series of filtration steps with carefully selected membrane cut-offs can be fully integrated within the production process of small molecules, and enable the recycling of expensive biocatalysts (enzymes).

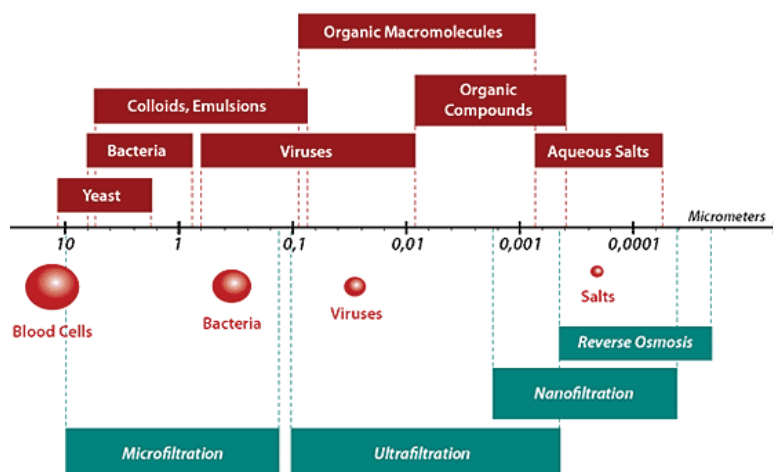


Figure 2: Filtration technologies and their size-based molecular selectivity.

Chromatography

Several options are available to purify complex mixtures at large scale, among which three preparative chromatography technologies are highly competitive for synthetic molecules: High Performance Liquid Chromatography (HPLC, **Figure 3**), Supercritical Fluid Chromatography (SFC) and Multi-column Continuous Chromatography (often referred to as SMB or simulated moving bed techniques).



Figure 3: Prochrom[®] DAC LC300 HPLC column (in front of the operator) with Hipersep[®] L system (behind the operator) for production under cGMP conditions.

The critical choice of a chromatography technology for a given purification often depends on the scale at which the separation is to be carried out (**Figure 4**) and on the nature of the mixture to purify. More fragile biomolecules are often separated on resin-based stationary phases, which are less pressure stable than typical silica-based stationary phases used in HPLC and require Medium or Low Pressure Liquid Chromatography (MPLC or LPLC).

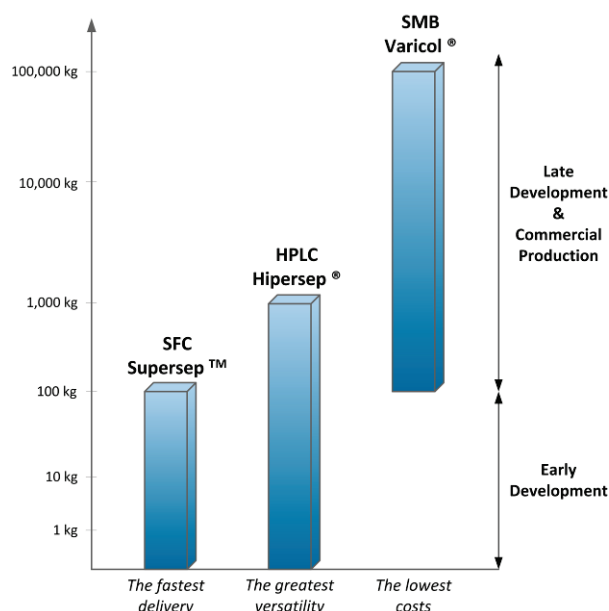


Figure 4: Chromatography technologies and their application span as a function of scale and development stage.

Preparative HPLC is well suited for small to medium scales (up to metric tons). For SFC, the major part of the mobile phase is constituted by supercritical CO₂; the technology is mostly suited to small scales (up to a hundred kilogram) and allows for an improved separation and process throughput. Finally, continuous chromatography presents several columns in a loop and is well suited for medium to large scale production (up to 100's tons per year) due to low product dilution and operating costs. It is routinely used at pilot and commercial scale for the separation of binary mixtures (such as racemic mixtures), and also as a two-step process for purifying complex mixtures in large scale applications. Preparative HPLC is based on the same principle as analytical HPLC, only on a considerably larger scale, with increased injection volumes and stacked injections to maximize productivity. A common misleading belief states that chromatography cannot be evaluated precisely unless pilot runs on larger scale equipment are implemented. It must be affirmed that the main advantage of chromatography is the very predictive assessment of the results on large-scale based on automated screening results obtained from just a few of experiments and sub-gram amounts of material, due to a direct scalability (a 1-million-fold linear direct scale-up has been demonstrated).¹ As a consequence, an accurate estimation of productivity and costs at large scale is obtained very early in the process development phase. In addition, advanced simulation tools allow for a rapid, reliable development and straightforward validation of the process. Nowadays, chromatography has proven to be robust and reliable for the production of commercial APIs and constitutes the purification method of choice, as illustrated in the case study developed below.

Combining state-of-the-art synthesis and advanced purification technologies

The coupling of chemistry, high-performance chromatography, and membrane filtration enables the efficient synthesis and purification of highly charged or polar molecules that are somewhat thermally labile. Because of their charge or polarity, these molecules tend to be water-soluble and can or do exist as salts. Their purification necessitates the use of ion exchange and reverse osmosis or nanofiltration. The organic structure is purified by classical chromatography and membrane filtration allows for the concentration of the purified product under extremely mild conditions and prevents any issue linked to thermal instability. Final drying is achievable with spray drying for instance. This type of combination can, for example, be used for saccharide derivatives, nucleotide derivatives, complexes with metals or other species. Applications can include various pharmaceuticals like contrast agents; cosmetics; certain functional ingredients and other high-end chemicals, such as electronic chemicals.

Case study background

An expert purification partner was required for the production of pre-commercial quantities of a highly polar functional ingredient. The initial project included the full process development for a chromatography step, and the scale up of the complete process from lab scale to commercial scale including a complex biocatalysis step and the downstream process. Chromatography was part of a multi-step downstream process to isolate a chiral compound from a complex enzymatic reaction mixture. Being a CMO, pioneer and leader in the development of preparative and process-scale HPLC, Novasep was selected for this project. Due to a good technology and competence fit, the developer entrusted the CMO and equipment provider with the entire scale-up and manufacturing project, from the enzymatic stereoselective reaction to the isolated final product. After the technology transfer, the process was implemented and scaled up from a few grams to a few hundred kilograms.

Synthesis

The chiral active ingredient targeted resulted from a complex enzymatic process. This bioreaction conversion resulted in a complex mixture of biological material, reagents, intermediates, and desired product. The isolation of pure product required a series of advanced purification steps (downstream processing) including membrane filtration and HPLC chromatography (**Figure 5**).

Purification

The biocatalyst material was removed from the reaction mixture by ultrafiltration (UF) using a 10,000 Dalton molecular weight cut-off membrane, meaning that no selection was obtained on the smaller molecules: their concentration was expected to be the same in the permeate and retentate. However, thanks to a retentate volume 30 times smaller than the

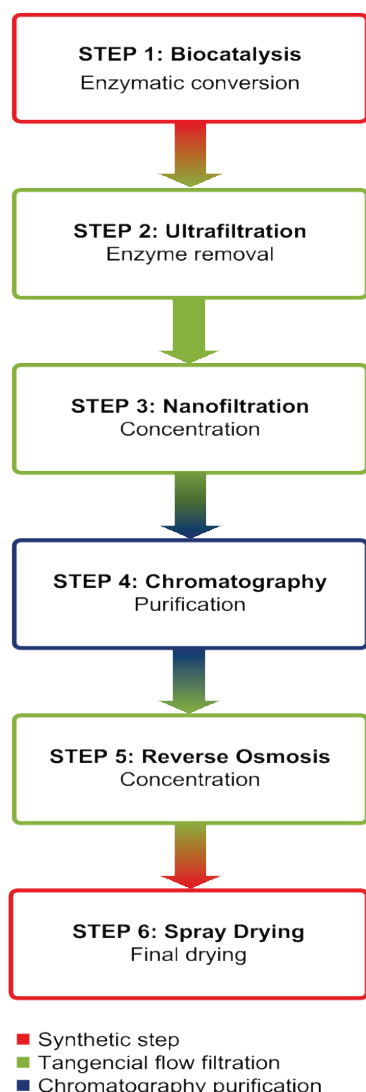


Figure 5: Process overview with the key technologies involved.

higher than initially expected were reached. Finally, the product was isolated by spray drying with a recovery of 87%.

The bio-conversion equilibrium afforded the desired product within a complex crude mixture of biomaterial and smaller molecules. A multi-step downstream processing was implemented to reach the 95% purity target with maximum recovery and a purification yield of 52% was obtained over the five purification steps (three filtrations, one HPLC chromatography and the final spray drying).

permeate volume, the recovery of small molecular weight compound was maximized, and reached 95% for the valuable desired product.²

At this stage, the product remained in dilute solution within a complex aqueous mixture, together with seven undesired compounds. The mixture was concentrated by nanofiltration (NF): impurities were partially removed while the overall volume was reduced in preparation for the HPLC step. This step was very important since it determined the quality of the feed for the chromatography which in turn impacted the quality of the separation. A concentration close to 45 g.L⁻¹ of the desired product was obtained with a 90% recovery yield over the two filtration steps.

The HPLC process development and optimization was carried out at Novasep and fully benefitted from its long experience with this technology. The target was to reach a purity over 95% with a maximal recovery and in a cost-effective manner. The HPLC purification was the key step to meet these requirements and numerous parameters were optimized to comply with the specifications: the bed length (amount of stationary phase used), the stationary phase stability (*i.e.* its lifetime), the minimization of the volume of eluent required by batch, the volume of solution injected and the collection time. With optimal parameters, the elution volume was halved compared to the initial process and the recovery reached 90% of the desired product injected (against 50% for the initial process).

The desired compound was obtained at the required purity in a dilute aqueous solution, which was concentrated 30 to 40 times by reverse osmosis to reach the targeted 180 g.L⁻¹ concentration. This step was performed with a 90% recovery yield and performances

Scale up and timelines

The process initially provided to Novasep had been optimized up to a three liter scale which corresponded to the production of a few grams of product per batch. The production was scaled up in three phases to obtain several hundreds of kilograms in the final phase (Figure 6).



Figure 6: Global project timeline, including the production phases and the design and construction of a dedicated workshop.

Phase I consisted of a technology transfer to ensure of the reproducibility of the initial process at the CMO production site. The process was applied and minor improvements on the existing process were carried out. A major part of this step was to fully develop and optimize the HPLC purification step, which was completed within only three month (including stationary phase screening, phase stability testing, and operating condition optimizing). The complete manufacturing of the pure, dry product was fully mastered and 50 g of product were obtained.

The process was scaled-up to pilot scale (batches of a few kilograms each). All unit operations were scaled up without major issues and Phase II afforded 35 kg of product over a two-month campaign.

The final phase aimed at hundreds of kilograms production and required a purpose-built workshop (Phase IIIa). The workshop was designed thanks to Novasep's strong expertise in engineering gained over the years, especially for purification systems in pharma and industrial biotechnology industries. The workshop was fitted with four reactors (1000 to 4000 L) for the biocatalysis itself and the work-up, a Prochrom® HPLC column (800 mm internal diameter) complete with its chromatography system (Hipersep® XL), three filtration units: UF (15 m²), NF (36 m²) and RO (36 m²) and a spray dryer (3 kg.h⁻¹ capacity) to accommodate the full process at pilot and pre-commercial scale. Within this new facility, more than 500 kg of product were manufactured over four months (Phase IIIb), exceeding the customer's expectations.

Conclusion

Overall, a complex process was implemented and scaled up thanks to the uniquely broad range of technologies mastered at Novasep. The expertise in advanced synthesis permitted a straight forward technology transfer for the enzymatic reaction, the strong know-how in various filtration (UF, NF, RO, diafiltration) and chromatography (HPLC) technologies ensured a smooth and reliable downstream processing, including the development and optimization of the HPLC process using proprietary chromatography technology. Finally, a new workshop was designed and built on purpose using the engineering capacity gained by building

turn-key purification units for the pharmaceutical and industrial biotechnology industries. The project was completed in a record time of 15 months, including the scale-up from a gram scale to a hundred-kilogram scale and the design and construction of a dedicated workshop.

The combination of advanced purification technologies enables to obtain purity levels and recovery yields unattainable by classical purification; it may be used to increase the purity of intermediates to improve the yield of difficult or expensive downstream chemistry steps, which sometimes dramatically reduces the manufacturing cost. The combination of biocatalysis, membrane filtration and chromatography is a specific example: the functional ingredient presented here required a moderate purity of 95%, which enabled high recovery at the HPLC unit operation. However, specification as strict as 99.9% purity can be met with chromatography: the product is delivered at the required purity, whatever the threshold. Combined with smart synthesis, the choice of purification technologies best suited to the crude mixture is definitely the key to a time-efficient and cost-effective production.

References:

1. Welch, C.J.; Sajonz, P.; Spencer, G.; et al., *Org. Process Res. Dev.*, **2008**, *12*, 674–677.
2. All the yields and purity data correspond to the average data over the entire commercial-scale production campaign (Phase IIIb: 4 month, 30 batches).

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