Advancing process solutions
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Allan Lihme

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Allan Lihme, CTO of Upfront Chromatography A/S, speaks exclusively to *Pharmaceutical Technology Europe* about the biotech arena, Expanded Bed Adsorption, chromatographic technologies and the rewards of establishing partnerships.

Q1: Upfront is a well-known leading developer of customized industrial-scale separation services. How difficult has it been to get where you are now?
Eighteen years ago, when Upfront Chromatography was working with Expanded Bed Adsorption (EBA), Pharmacia (now GE Healthcare) started in parallel with the same technology. EBA promised many advantages in terms of crude feedstock processing, increased yields and operational simplicity, but, because of the regulatory issues and high investments required, we realized that it would be very difficult for a small company such as ourselves to penetrate the biotech area and compete head to head with GE.

Therefore, we changed our priorities and instead focused on applications in the food ingredients and industrial enzymes industries outside of the biotech area. The reason for this shift in focus was two-fold. First, although the regulations in food ingredient business are strict, the industry is more open to new and innovative technology. Second, in the food ingredients industry the volume of raw material is 100–1000 times higher than in the biotech industry. This requirement for large-scale applications meant that our technology was very well suited and commercially viable in the food industry.

Some technical issues needed to be resolved to make the technology work for large-scale applications. So, while Pharmacia's biotech products had some drawbacks that became more obvious as the years went by, we had to deal with those drawbacks immediately. In the biotech area, processes run in a short period and it can take a long time to conclude that the technology is not delivering as expected. In the food related area, drawbacks are visible from the start and technological issues must be solved immediately. We were forced to solve problems related to EBA right from the very beginning and it took us years of hard work to create a really robust process. As a result, customers from the bioprocessing area can now benefit from this experience.

Q2: What were these initial drawbacks of EBA?
The drawbacks of the first generation EBA solutions were associated with the design of the column hardware, the adsorbent beads and the ligand chemistry. First generation columns could efficiently distribute the incoming liquid, but could not accommodate the crude raw materials without clogging up during the process, which made cleaning very difficult. Another issue was air bubbles that could be trapped below the distribution system where they couldn't be easily removed without serious disruptions of the process.

First generation adsorbents were also of a relatively large diameter (100–300 micron) with a relatively low density of approximately 1.2 g/mL. The large size resulted in poor mass transfer kinetics and low dynamic binding capacities. The low adsorbent density resulted in the bed being easily disturbed and extensively expanding, which led to a very high consumption of washing, elution and equilibration buffers and a low resultant product concentration. Finally, first generation ligand chemistry was based on classical ion exchangers that were very popular in packed bed chromatography far downstream of the process. Ion exchangers, however, are not the optimal choice when the adsorption step is moved up towards the crude raw materials because the ionic strength at this point will typically be too high for proteins to bind efficiently. Ion exchangers are also vulnerable to crude raw materials as they contain substances and insolubles that can easily foul the surface. This would eventually lead to poor binding of the product, bead agglomeration and cleaning issues.

Q3: How did these drawbacks affect application of EBA in the biotech industry overall?
Inefficient cleaning of the EBA system precluded the use of the technology for the manufacture of biopharmaceuticals because of the very high regulatory demand for documentation of clean processes. Inefficient binding caused by the large particle size would lead to product yield losses with significant economical impact. The lack of process robustness meant a considerable level of downtime to solve technical issues, and was a practical and economical drawback counteracting the assumed benefits of the technology. The very high buffer consumption, as a consequence of low adsorbent density, was another serious economic drawback resulting in higher costs for buffers, the requirement for holding tanks and a larger floor space, waste issues and increased ultrafiltration capacity needs.

Q4: How did you solve these technical problems?
We addressed the column issues by developing a new column design in cooperation with the Danish Technical University (Denmark). The design includes a rotating inlet device that does not clog up and that, simultaneously, distributes the liquid very efficiently. We also removed all items inside the column that could clog up when applying very crude raw materials. We changed the adsorbent design, so that it solves all the issues mentioned above resulting in a very robust process, efficient binding with high dynamic binding capacity and low buffer consumption. We also developed a new ligand chemistry with salt-tolerant mixed mode ligands that are much less prone to fouling because of the low ligand concentration, but that also provide a very high dynamic binding capacity.

Q5: Where does your success lie?
The reason we are successful today in the biopharma and biotech area is based on the fact that we spent years in the food ingredients industry solving the previously mentioned technical problems and creating robust processes. This experience enables us to provide the biotech industry with mature technology that can deliver what it did not deliver 10 years ago, and we notice significant interest from biopharma companies. They can see that the present Rhobust product line based on our proprietary 2nd Generation EBA is delivering today what Pharmacia failed to deliver 10–15 years ago.

We are now among pioneers of disposable chromatography for downstream processing; for example, within the monoclonal antibodies (MAbs) purification programme we have developed EBA

as a disposable process step and have launched the Rhobust disposable product line.

Q6: Which areas of pharmaceutical market are of interest to Upfront and how do your leading products fare within them?
Protein-based therapeutics are dominating in the biopharmaceutical market. Within this group, MAbs comprise more than half of the market. Isolation of MAbs and delivering solutions for the direct capture of MAbs from the fermentation broth is, therefore, one of our focus areas.

We provide an alternative to classical MAb isolation where you can capture MAbs directly from the fermenter without clarification, filtering or centrifugation. The classical route is to first ferment and then to clarify by centrifugation and/or filtration, taking out cell debris and then applying a packed bed adsorption, typically Protein A in the capture step. However, 2nd Generation Rhobust EBA allows capture of the antibody directly from the fermenter without centrifugation or filtration and allows MAbs to be quickly extracted from the bulk liquid in a concentrated, purified state. This is, of course, not the final therapeutic product, but the process increases antibody yield and enables faster, more flexible processing compared with classical clarification steps.

Q7: Who will mostly benefit from this technological improvement?
Our main beneficiaries for disposable chromatography are CMOs that typically work with trials in the early phases of development of a new protein drug, as fast turnover of the products is the key issue for them. The cost of disposable products is returned with greater flexibility and faster turnover of projects: no cleaning or cleaning validations are required and more projects can be completed, resulting in higher revenues — that is the driving force for CMOs to look for fully disposable downstream processing lines and this is where we are contributing.

The biopharma industry is becoming increasingly sophisticated, and the need for shortcuts and increased efficiency in downstream processing is constantly growing. The advantage of using EBA becomes more pronounced the higher the cell density and product concentration because clarification steps become more demanding and yield losses become more severe. In these instances, the enabling nature of our technology becomes more visible, and biotech companies benefit from the advantages we have been offering to the food industry for a number of years.

Q8: Upfront's most recent work led to the development of a fully disposable chromatography system optimized for high yield cell line production. What makes this product unique and what advantages does it present with respect to existing technology?
We are helping several MAb producers overcome the challenge of high density cell cultures in order to obtain high product yields. Clarification, filtration and centrifugation steps of a liquid with high cell density are very demanding and the product losses tend to be very significant if traditional packed bed is applied. Only EBA chromatographic process can handle such cell density without the clarification process.

Q9: How did EBA manage to solve the high cell density issues?
Upfront provided EBA technology that uniquely combined the disposable EBA one-step procedure with direct crude feedstock processing. There is no longer the need to centrifuge cells to release trapped MAbs from the biomass as they can be extracted directly from the fermenter using our 2nd Generation EBA ultrahigh density adsorbent beads.

With EBA, there are also no clogging issues related to scale up. Unlike classical packed bed columns, any bed height can be used without the problem of back pressure. As there is no need for pressurized vessels, our technology is economical and the columns can be disposed of with low capital costs and a process can be run with any peristaltic pump because only very low pressure is needed.

Q10: Can you give other examples of companies working with high cell densities who can benefit from EBA?
An example would be companies working with expressions of microbial systems, such as *E. coli* or yeast. In many instances, these companies are working with cell homogenates where products are not extracted until the whole fermentation broth has been homogenized and the cells have been broken. This creates even greater problems compared with the high cell density culture as it becomes difficult to separate the biomass from the liquid. Companies working with these cell homogenates would benefit by using EBA.

Q11: Cangene Corp. (Canada) is another of your partners, in this case in the development of a process for the isolation of immunoglobulin G directly from blood plasma. Why do you think Cangene approached Upfront to establish this partnership?
Cangene is involved with the isolation of proteins from human plasma, and Upfront's technology provided them with the only alternative to the standard Cohn process commonly used in the isolation of proteins from plasma. EBA can be combined with the Cohn process to provide significantly improved yields, which is particularly important because of the scarcity of human plasma.

Although the Cohn process has been widely used to deliver important therapeutics, it does have some drawbacks and, until now, there has not been an alternative. Our technology represents an alternative to this process and it can also be successfully combined with the Cohn fractionation process to improve it.

The main drawbacks of the Cohn process are the poor yield and the requirement to use ethanol that can destroy the biological activity of some proteins. Human plasma is expensive and the demand for IgG and albumin exceeds supply. Therefore, it is very important for companies to seriously consider the benefits of EBA. The increased yields are a major factor in getting the technique accepted and regularly used as a standard practice in the competitive market of plasma fractionation.

Q12: What about other chromatographic technologies; how well can they work with human plasma?
Other chromatographic techniques, such as packed bed chromatography, are very sensitive to precipitation and fouling of columns. This is a particular problem with human plasma as it is a very complex raw material and when run through a packed bed chromatographic column, extensive clarifying, filtering and centrifuging of the precipitated material must then be undertaken. The industrial production of therapeutic products from plasma requires a reliable process. Our EBA is such a robust process that solves problems associated with the use of packed bed chromatography. Cangene is one of the first companies to establish this process.

Q13: All these collaborations are a testimony of Upfront's business flexibility and adaptability. What have been the biggest hurdles in the establishment of these partnerships and what have been the greatest rewards?
The biggest hurdle is that it takes a long time to build such a partnership. First, we need to build trust in our technology, in our company and our abilities. We have very long sales cycles and it can take a while before large-scale production becomes established. The biggest reward is when we get there — the business is significant and we have a long-term relationship with the customer. From a business perspective this is very rewarding.

Q14: In an industry facing increasingly tougher regulations and stricter standards governing the manufacturing process, how difficult it is to be fully GMP compliant?

This is not really a big issue for us. Our manufacturing facilities are ISO9001-compliant, but as we are not producing pharmaceutical intermediates or APIs, we are a step further away from the actual production. Therefore, our own production of adsorbents is not regulated in the same way as for companies producing APIs. However, the quality of our products is of the highest standards and we are working towards GMP regulations. We are open to audits from our customers so they can assure themselves that our quality systems and production facilities satisfy the necessary standards.

Q15: How does Upfront see the future? Do you have any exciting developments in the pipeline or plans for expansion?
Certainly there are a lot of exciting developments going on! We are undergoing a patenting process, and are focusing on future developments in processes and technologies for therapeutic proteins and MAb purifications. We are also looking at purification of MAb fragments produced from expression systems other than mammalian cell cultures. So you will see new products and processes from Upfront in this field in the next year or two.

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