Novel resin technology for purification of peptides

Rohm and Haas Advanced Biosciences has expanded its range of resins to support peptide and protein synthesis, with the emphasis now on improving product purity through the use of highly specific resin systems. Dr Marlin Kinzey demonstrates the advantages of one of the company’s new resins for the polishing of peptide products.

Rohm and Haas is a $7 billion global specialty materials company with core competence in functional polymer chemistry. The company has a long history of support for the pharmaceutical industry. It has been active in the field of polymeric ion exchange resins for almost 60 years, and has supplied polymeric media to the pharmaceutical industry for more than 40 years. The company is well-established in polymeric reversed phase resins for bioseparations.

On this basis the company founded its Advanced Biosciences business unit, which uses Rohm and Haas core technology in functional polymers to develop novel products for the biopharmaceutical industry.

New resin for therapeutic peptide and protein manufacture

The company recently introduced Amberchrom™ HPR10 Resin designed for therapeutic peptide or protein manufacture using recombinant DNA technology. There are several chromatography steps designed to isolate the product and establish its purity. This starts first with capture-concentration, and is followed by one or more intermediate purification steps, and is completed with final polishing. The goal of the new product is to improve the state-of-the-art in final polishing, where Rohm and Haas Advanced Biosciences believes there is a demand for new solutions.

Properties of an ideal polishing medium

The ideal polishing medium would embody a number of attributes. First, the ideal medium would be mechanically stable for high pressure use. Mechanically stable media support high solvent flowrates for high throughput. Second, the medium would be chemically stable to ensure it can be cleaned and sanitised in place with rigorous protocols, ideally with 1N NaOH.

Third, the medium would be highly selective with high resolution in order to resolve trace impurities from the target compound. In order to be commercially viable and add value, the innovation must be cost effective. The combination of these three factors - mechanical stability, chemical stability, and high resolution - translates into a cost-effective solution in final polishing.

These were the three design criteria that were used for the new product development. Amberchrom HPR10 is a 10 micron monosized polymeric resin with a pore size that is optimised for the production of therapeutic peptides, proteins, and oligos. The resin is highly chemically stable and has very good pressure stability, making it suitable for industrial operation.

Mechanical stability

Polishing media must be able to withstand pressures between 20 and 60 bar. One severe drawback of first-generation polymeric media was their lack of mechanical stability. Compared to rigid silica gels, early polymers were highly compressible under high pressure and could not be operated easily with industrial equipment. When compressed at 60 bar, first-generation polymers lose between 35 and 40 per cent of their total volume. This means the bed is impermeable and has very low flow capacity. The new technology is a much more rigid polymer structure. The benefit of this is that its mechanical stability enables much higher flowrate capacity.

Looking at scalability, data generated in a 30 cm diameter column from Novasep demonstrated that the flowrate capacity of the product was excellent using a traditional solvent such as acetonitrile. Good flowrate capacity is seen even with a viscous solvent such as propanol. The flow capacity for earlier polymeric media would have been a fraction of this. Also, under constant pressure conditions the height of the bed did not shrink further over time. This addresses a further complaint about earlier polymeric media - not only were they compressible during initial operation, but they kept compressing over further usage.

The medium must also be mechanically stable to solvent polarity changes during a typical chromatographic cycle. Earlier polymeric resins had the tendency to shrink and swell when exposed to solvent cycling, which destroyed column performance. Rohm and Haas Advanced Biosciences's data demonstrate unchanging high performance in simulated use, indicating excellent stability to cycling.

The Rohm and Haas plant at Chauny, France is the largest ion exchange resins plant worldwide, with a capacity of more than 50,000 tpa.
Chemical stability
The ideal medium would be capable of CIP / SIP using 1N NaOH solution. Amberchrom HPR10 is a highly crosslinked polymer, with a chemically inert surface that can be cleaned and sanitised in place according to rigorous protocols with 1N NaOH. This means first the column can be cleaned routinely for prolonged lifetime, which translates into cost improvements. It also means that manufacturing equipment can be fully cleaned and sanitised to prevent product carryover, and endotoxin and viral contamination.

These following demonstrates the chemical stability of Amberchrom HPR10. The product was stored in a solution of 1N NaOH at a temperature of 60 Centigrade for one month, and tested at six intervals. There was no change in either the dynamic or the total capacity for insulin, indicating excellent chemical stability.

Resolving power
In addition to lack of mechanical stability, another drawback of earlier polymers was their lack of resolving power. A second key innovation of the new technology was to create monospheric 10 micron beads with high capacity, selectivity and resolution power for peptides and small proteins. The new product was designed with selective pores for peptides and small proteins. This translates into high binding capacity and throughput.

Second, improvements in pore selectivity translate into higher resolution. This translates into higher yields at target purity when the process is scaled up to the plant.

Practical examples of purification processes
As an example of an industrial polishing purification illustrating the innovations in improved resolution of the new product, a commercial bovine insulin, which has a purity of about 92 per cent, was examined. The major impurity at 6 per cent is so-called A21 desamido insulin, which is a de-amidation product. In addition to this product, there are also a host of minor impurities which total about 2 per cent. The goal of polishing this molecule would be to increase the purity to a level to at least 98 per cent and more probably to 99 per cent or higher, while maintaining both high yield and productivity.

The insulin was loaded and separated under industrial loadings and compared with three other products, including two larger-particle size polymeric media, and also a 10 micron silica gel. For a target of 98 per cent purity all four resins enabled a yield of at least 75 per cent to be reached. If 98 per cent purity were the actual target, a choice could be made between lower-pressure operation with the larger resin and higher-pressure operation with the smaller resins.

However, 99 per cent purity is a more typical target. At a high target purity, a 10 micron resin will often be required to meet performance needs. In addition, resolution translates into high yield and productivity.

A chemically robust chromatography resin can not only improve the cost of existing products, but can also enable the production of new products that might not otherwise be feasible to manufacture economically. A peptide targeted for influenza vaccine development was studied based on its obvious relevance to a current issue of global concern. The peptide was very hydrophobic and its solubility is limited. This translates into a high production cost using traditional means. Initially the experiment reproduced literature conditions at acidic pH. High concentrations of organic solvent were needed to solubilise the peptide, and therefore only very low loading levels on the column were possible. This was true for both silica gel and the polymeric medium. Yields under acidic conditions were poor, only 43 per cent on silica and 55 per cent on the polymeric resin.

Obviously this would not be an economical process to scale up to manufacturing, because of its low productivity and high yield loss.

Basic pH conditions were then investigated. This would not be possible using silica, but is enabled with a polymeric medium. At a pH of 10 it was possible to increase the peptide solubility dramatically by nearly sixfold. Retention was also more favorable, meaning lower solvent consumption, and the peak shape improved considerably. Because solubility was no longer restricted, it was possible to load the column to much higher levels - giving a twelvefold increase. This improved the separation and provided a dramatic yield improvement.

When coupled with a near doubling in yield, the above means the productivity increased by a factor of 24, based on medium utilization. On a cycle time basis, this increased by yet another twofold due to shorter elution time. Finally, solvent utilisation decreased by a factor of 17.

This example cannot be generalised to all situations. However, the point is that the new technology provides a chemically robust platform to enable a higher degree of process development freedom than existing technology. This can in turn enable certain types of compounds to be produced with much better productivity, economy, and environmental impact.

The final example illustrates the commercial added value of the new product in a peptide polishing application. Based on equivalent production size columns and loading conditions that are optimised for each medium, it was possible to demonstrate significant economic advantages of the new product. The advantages accrue from longer column life and increased plant uptime. The majority accrue to improved resin lifetime, which is a result of a chemically stable medium. In this scenario, the application of an optimised Amberchrom HPR10 process delivers a 57 per cent reduction in consumables costs in the polishing of a therapeutic peptide at manufacturing scale. 

FURTHER INFORMATION
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