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Manufacture of oligonucleotides (oligo's) for commercial purposes will require effective large-scale purification methods. Pharmacia Biotech, in collaboration with LifeScience Molecules, carried out feasibility studies on the large-scale purification of an oligonucleotide for therapeutic use.

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The merger of two strong companies.

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Front cover

... shows a close-up of the distinctive nozzle of the CHROMAFLOW column range, (and the new Amersham Pharmacia Biotech logo).

Downstream

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Tested!

Large-scale purification of oligo's on SOURCE

The development of oligonucleotide-based therapeutics is potentially one of the most exciting advances in medicine. Drugs based on these compounds act at the molecular level by interfering with the diseased cell's ability to express its genetic code. One of the biggest challenges facing the commercial production of these DNA medicines is the development of cost-effective manufacturing methods. In order to achieve the required level of purity and productivity, efficient chemical synthesis must be combined with appropriate downstream purification at plant scale. In a collaboration with Zeneca LifeScience Molecules, Pharmacia Biotech carried out feasibility studies on the large scale purification of an oligonucleotide for DNA therapeutic use.

Recombinant DNA technology has had a tremendous impact on the pharmaceutical industry, resulting in new products to treat and prevent diseases, in a switch from mammalian tissue extracts to bacterial, yeast, or cell culture sources, and in the use of monoclonal antibodies in numerous diagnostic tests and several *in vivo* applications. Surprisingly, there are no DNA/RNA medicines commercially available yet. The pipeline, however, does show promise. There are oligonucleotides (oligo's) in Phase II and Phase III trials giving very good results: higher potency, higher specificity and lower toxicity.

Real-life application

Large-scale, commercial manufacture of such complex molecules will require cost-effective production methods for chemical synthesis and chromatographic purification. At Pharmacia Biotech and Zeneca LifeScience Molecules there was a mutual interest to do feasibility studies. Zeneca is pioneering an approach to chemical synthesis based on solution-phase chemistry. They had independently found that SOURCE 15Q was a suitable chromatography medium for lab-scale oligonucleotide separations, and sought a partner to study large-scale purification. We were interested in a real-life test on the scalability of SOURCE media and FineLINE columns.

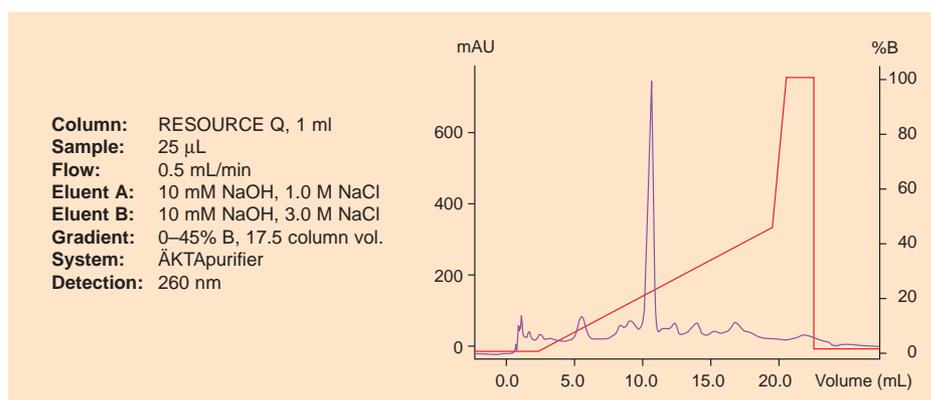


Fig. 1. An analytical chromatogram obtained for the crude starting material, analysed by ion exchange chromatography.

Purification challenge

The target compound used in the study was an eight residue, all phosphorothioate oligodeoxynucleotide. Despite its relatively short length, this compound represents a significant purification challenge due to its propensity to form intra- and inter-chain hydrogen bonds, potentially complicating the separation of full length from truncated species.

Prior to the purification schedule, all the protecting groups on the phosphates and bases were removed chemically, and the product was recovered in water followed by washing with organic solvents. Following deprotection and removal of the DMTr group, low molecular weight contaminants were separated from the product by process-scale gel filtration with Sephadex G25, at the same time the product is transferred to an aqueous solution suitable for further processing. The purity of the crude product at this stage was approximately 39% (Fig. 1).



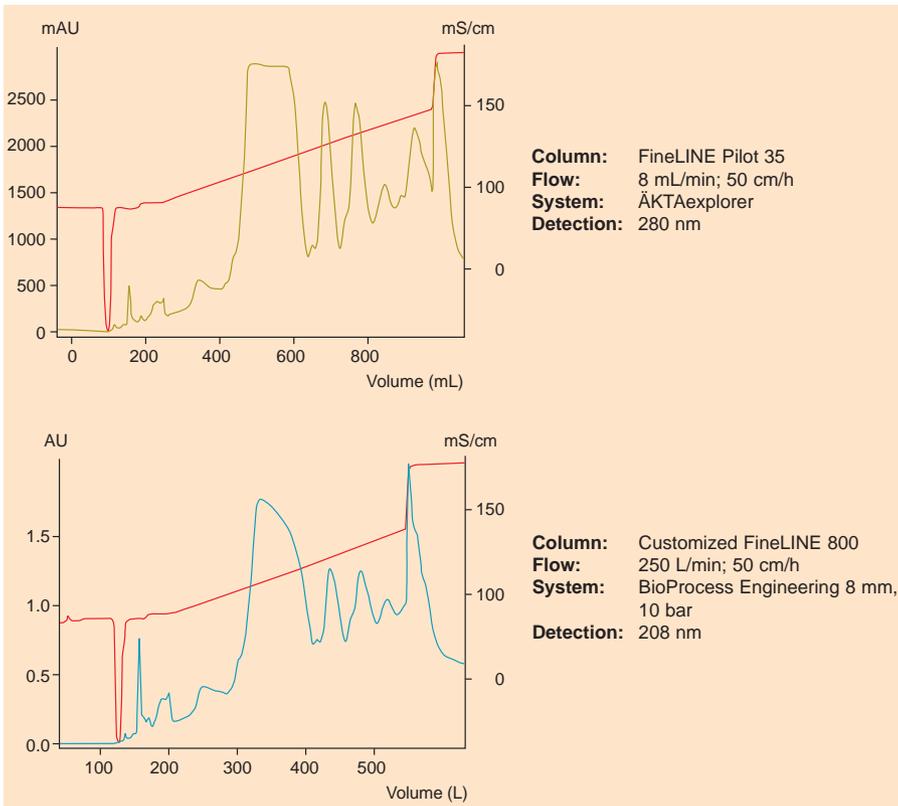
Collaboration between Pharmacia Biotech and Zeneca LifeScience Molecules has verified the use of SOURCE at large-scale.

BioProcess Engineering System was the system used in this study to run the customized FineLINE 800 mm diameter column packed with SOURCE 15Q.



From ÄKTAexplorer to customized FineLINE

A number of pilot-scale purifications were carried out using ÄKTAexplorer to run a FineLINE Pilot 35 column packed with SOURCE 15Q (35 mm inner diameter, 140 mm bed height, 135 mL bed volume). During these studies, loading and gradient were optimized. The separation was scaled up to a custom designed FineLINE column (800 mm inner diameter, 140 mm bed height, 70 L bed volume) keeping the buffer composition, loading per volume medium, bed height, gradient volume per column volume and flow velocity constant. In this way, large amounts of crude material (after gel filtration) could be applied in each cycle with the customized FineLINE column. Cycle time, including re-generation, was about 2.5 hours.



Encouraging results

Results from the pilot-scale and large-scale experiments were very similar (Fig. 2) and quashed earlier concerns that resolution and product recovery would suffer in the scale up. Pooling those fractions which surpassed the purity acceptance level (95%) gave a final purity of about 97.8% with a recovery of over 65%. An analytical purity check of the final product is shown in Fig. 3. Reprocessing samples alongside the main peak increased the overall recovery to over 80%.

Fig. 2. A comparison between the two scales of purification. The upper chromatogram is from a run on ÄKTAexplorer with SOURCE 15Q packed in FineLINE Pilot 35 column. The lower chromatogram is from a run on BioProcess Engineering System with SOURCE 15Q packed in a customized FineLINE column. The scale-up factor is 520.

A feasible route

Regardless of the synthetic approach, it will be necessary to purify oligonucleotides before their use as pharmaceuticals. The collaboration with Zeneca described here shows a realistic application of SOURCE 15Q for the purification of an oligonucleotide. Success in the lab could be reproduced at large-scale. A quote from John Parker of Zeneca is a fitting conclusion: “This has been a highly successful collaboration and the results obtained during the course of this project have demonstrated the feasibility of both synthesizing an oligonucleotide by a scalable solution phase route, and the subsequent purification of the target molecule at large scale.”

Reference

Synthesis and purification strategies to address plant scale manufacture for DNA therapeutics. Poster presented at Europa Bio 97 – First European Bioindustry Congress, Amsterdam, The Netherlands, 25–27 June, 1997. Garner, G.A., McClean, M.J., Parker, J.R., Forsberg, B., Johansson, H.J., Sparrman, M.

Zeneca LifeScience Molecules was founded in October 1995. By a realignment of existing businesses, various complementary technologies could be meshed together with the aim of enhancing the focus on the Healthcare and Agrochemical industries. Zeneca LifeScience Molecules works through partnership collaborations to provide product and manufacturing solutions to meet its customers' needs.

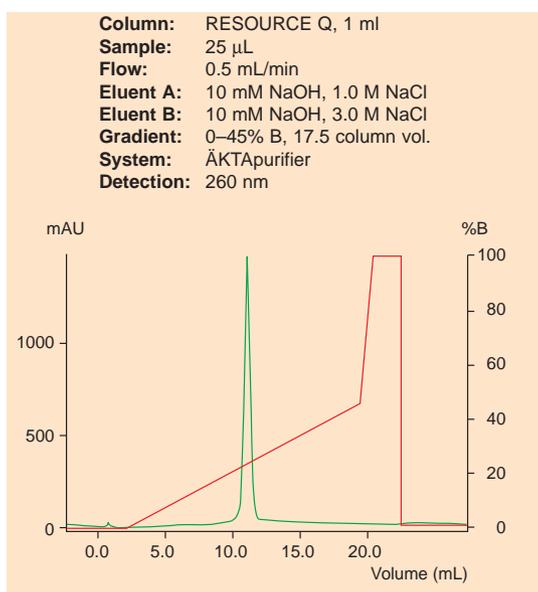
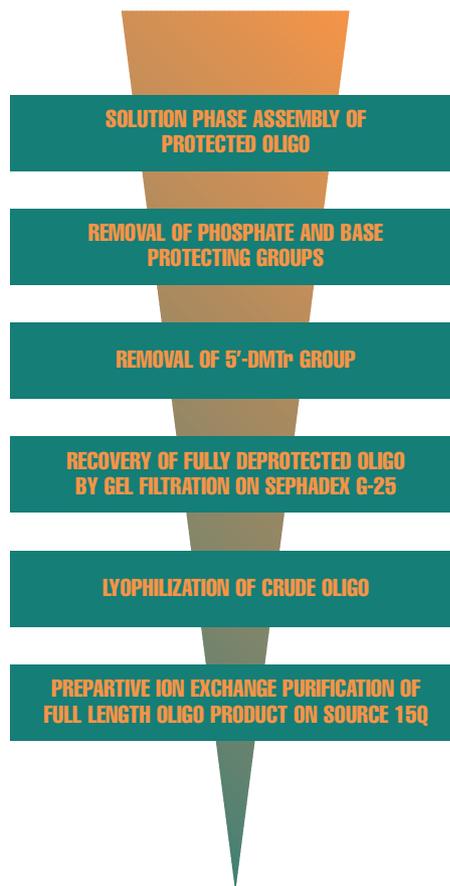


Fig. 3. An analytical chromatogram obtained for the final product.

Scalability

As a general comment, scalability can never be assumed to be automatic. It requires considerable development on our part to ensure that the performance of a separation medium can be retained when it moves from the lab to the manufacturing facility. In particular, the design of a column can have an important impact on the ability to pack the medium to high efficiency, to obtain symmetrical peaks, to retain capacity and to clean effectively. Thus, in practice, scalability depends on the design of the distribution system, adaptors, seals, etc. as well as work which we do to test the reliability of packing procedures with selected media and the efficiency of cleaning procedures with sodium hydroxide as the most commonly used agent.

Validation issues for antisense drugs

A recent Chemical and Engineering News article entitled “Optimistic about Antisense” describes new clinical results and the promising outlook for synthetic oligonucleotide drugs (1). Clinical results have been positive in the treatment of Crohn’s disease, RSV (respiratory syncytial virus), and HIV (Human Immunodeficiency virus), to name a few. Furthermore, the earlier problems associated with synthesis costs and the ability to make sufficient quantities have been solved. As more products enter clinical trials, firms are now spending more time focusing on validation requirements.

In a new field such as antisense drugs there are few, if any, specific regulations, guidances, or points to consider. However, since the antisense oligonucleotides are similar to other drugs, such as synthetic peptides, sufficient information exists for manufacturers to understand what is necessary. Furthermore, regulatory authorities appear quite willing to discuss the approach a firm will take towards validating both synthesis and purification of oligonucleotides. As stated by Kambhampati, *et al* from the US FDA (Food and Drug Administration), “The regulatory challenge presented with oligonucleotide therapeutics is to provide the identity of the oligonucleotide, and demonstrate its quality, purity, and strength/potency using both those characteristics that are the same as all other drugs, as well as those that are unique” (2).

To be addressed

The FDA’s “Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances” (3) states that the following issues should be addressed: description and characterization, synthesis/method of manufacture, process controls, reference standard, and specifications/analytical methods. For phosphorothioate oligonucleotides, S.T. Crooke has described the characterization of oligonucleotides to include proof of structure, base composition analysis, base sequence, ratio of phosphorothioate to phosphate linkages, molecular weight,



BPG columns are used by Hybridon Inc. in the manufacture of oligonucleotides. BPG columns are glass columns designed for biopharmaceutical production. They are available in a range of dimensions and volumes. Photo courtesy of J. Kannair.

and trace metal content (4). At this time, there is no requirement for controlling stereoisomers (see Reference 2).

According to the document on synthetic peptides, the following issues should be addressed in the “synthesis/method of manufacture” section: starting materials, synthesis flow chart, detailed description of synthesis, and purification. For oligonucleotides, starting materials include amidites and primer supports. Certificates of analysis are required. Once vendors are qualified, the end-user may accept a certificate of analysis and perform only an identity test. A Parenteral Drug Association (PDA) task force has described how to certify suppliers (5).

For both synthesis and purification, a development report, which refers back to properly documented laboratory notebooks, should describe the rationale for selecting a given process. For purification, it is necessary to provide a flow chart and narrative, and all raw materials used for purification should have specified acceptance criteria. In the purification of oligonucleotides, this may include solvents, buffers, and chromatography media. The principles of validating purification processes are described fully in “Handbook of Process Chromatography” (6).

Qualification

Equipment qualification must also be performed for both synthesis and purification. Installation and operational qualifications (IQ/OQ) of both the equipment and control systems are necessary. The professionally prepared IQ/OQ packages and Fast Trak Purification Services available for UNICORN controlled systems enable the end-user to reduce considerably the time and resources required to qualify OligoProcess and BioProcess Systems which are used for synthesizing and purifying antisense drugs.



“Antisense oligonucleotide therapeutics are more similar to most other drugs than they are different.”

(from Ref.2)

Fast Trak Purification Services offer prepared IQ/OQ packages for validation purposes.

Avoid delays

With the intense pressures to get into the clinic and first to market, validation requirements are often pushed aside. This approach, however, can lead to regulatory delays. Validation requirements for manufacturing antisense drugs are rather straightforward, since these products are very similar to traditional synthetic drugs. As with all validation, a scientific approach and common sense are recommended.

References

1. Optimistic about antisense. *Chemical and Engineering News* 75 (1997) 35–39. Rawls, R. L.
2. Regulatory concerns for the chemistry, manufacturing, and controls of oligonucleotide therapeutics for use in clinical studies. *Antisense Research and Development* 3 (1993) 405–410. Kambhampati, R. V. *et al.*
3. U.S. FDA CDER/CBER, Nov. 1994, Rockville, MD USA
4. Commentary:regulatory issues affecting oligonucleotides. *Antisense Research and Development* 3 (1993) 301–306. Crook, S.T.
5. PDA Supplier Certification Task Force, PDA Journal 43 (1989) 151–157.
6. Handbook of Process Chromatography: A Guide to Optimization, Scale-up, and Validation. Sofer, G., Hagel, L. Academic Press, London, 1997. Available from Amersham Pharmacia Biotech, code number 18-1121-56.

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Fast and reliable packing methods, a quick route to an optimized process, plus maximum performance in operation – these are the benefits to downstream operators from our expertise in column packing technology. Years of experience and know-how in column packing technology have culminated in standard testing procedures, and dedicated resources ensure that media and columns are thoroughly tested together.

Column packing studies

– knowing your column inside and out



A well-functioning column is central to a well-functioning downstream process. That's why we carry out packing and performance tests on every new column design to ensure that it performs optimally with the intended media.

Pharmacia Biotech has been developing media and hardware, and packing columns at all scales for many years. The experience gained and expertise acquired has enabled us to develop a series of packing studies and standardized tests to evaluate column performance. Today we have dedicated column packing facilities and dedicated resources at centers in Europe, the US and Japan to carry out such procedures.

Packing – a testing time

The know-how derived from packing media into columns such as BioProcess Stainless Steel, BPG, FineLINE or INdEX, serves as a starting point for packing studies on new columns and media. Packing methods are media specific and are optimized for the different column families. It should be noted, that not all media can be packed optimally into all columns, but there is a range of columns suitable for each media family. Packing studies result in recommended packing methods which are designed to ensure smooth and immediate start up, in other words saving your valuable time for processing instead of losing it in trial and error packing tests.

Basic parameters

Before launch of a new product we carry out a series of established tests at our facilities. Many of these tests and results are reported in our Column User Manuals so that you can repeat them in order to gauge column performance. Some examples follow.

Creating a pressure/flow curve

If there is no information available on the medium to be used, a good starting point is to generate a pressure/flow curve to determine optimal flow and packing rates (full details are given in Column User Manuals). Briefly, this is done as follows. With the appropriate slurry concentration and volume in the column, start pumping buffer through the column at a low flow rate. Slowly increase the pressure in increments and record the flow rate until no further increase is achieved or the pressure limit of the column is reached. Then stop and plot the results. Semi-rigid media will show a curve which levels off at the maximum, see Fig. 1. The optimum packing flow rate is 70–100% of the maximum flow rate. Generally, once the curve has been obtained, the medium should be unpacked and then re-packed at the optimum packing flow rate. If the packing rate is too high or too low, the quality of the packing, and thus column performance, will suffer.

The column should be operated at a maximum of about 70% of the packing flow rate. The main reason being that the viscosity of samples is often higher than the viscosity of buffers.

HETP and A_s measurements

The efficiency of a column depends on how well it is packed. A poorly packed column gives rise to uneven flow, resulting in zone broadening and reduced resolution. It is therefore important to test the column before it is put into routine operation. Such a method should be simple, quantitative and should not introduce contaminating materials. A test we perform on a packed column, and one which we recommend you to do on a regular basis – after storage, after packing, and between runs – is measurement of HETP (height equivalent to a theoretical plate) and A_s (asymmetry factor). This is a quick way of determining the condition of the packed bed.

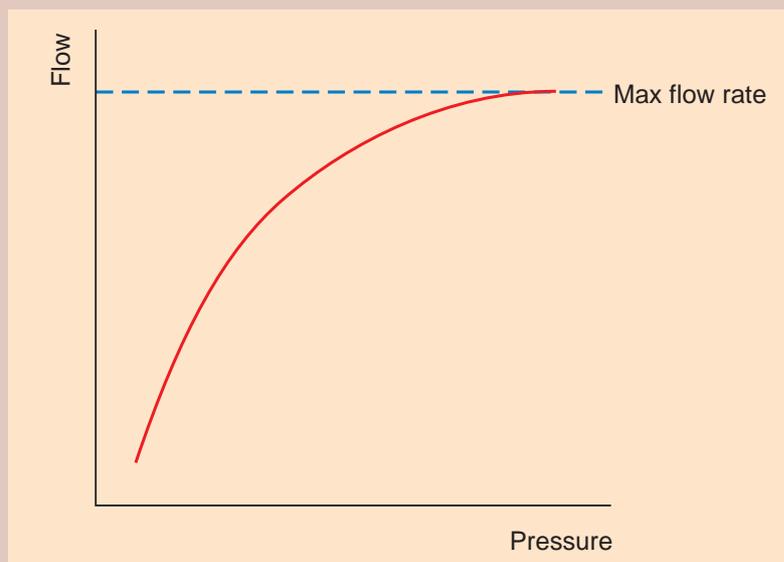
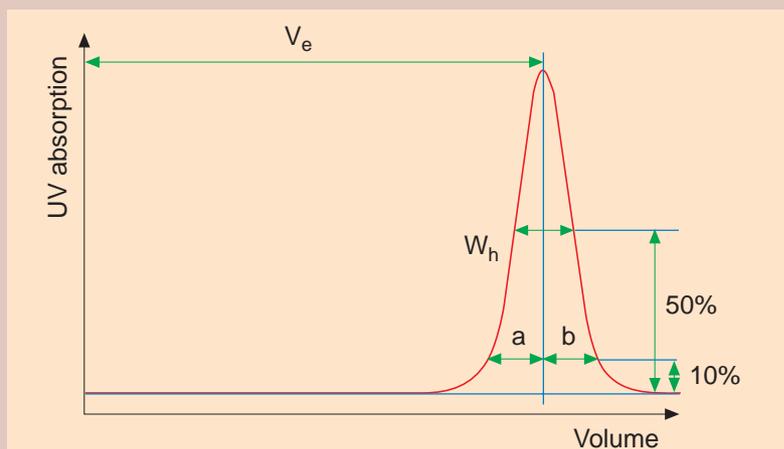


Fig. 1. Pressure/flow curves for semi-rigid media allow the choice of suitable packing and operating flow.



$$HETP = L/N$$

where L = bed height; N = Number of theoretical plates

N is defined by the equation 5.54 $(V_e/W_h)^2$

where V_e = volume eluted from the start of sample application to peak maximum

where W_h = peak width at half peak height

$$A_s = b/a$$

where a = 1st half peak width at 10% peak height

where b = 2nd half peak width at 10% peak height

Fig. 2. UV trace for acetone in a typical test chromatography showing HETP and A_s calculations.

HETP

As it is generally not possible to use the real sample for testing performance, alternatives such as 1% v/v solution of acetone, or sodium chloride are used. The test sample should not react chemically with the medium or column. Sample volumes (1) are typically between 0.5% and 2% of the total bed volume, depending on bead size. Distilled water or a simple buffer is often used as the eluent. Use a UV-monitor to detect acetone or a conductivity meter to monitor sodium chloride in the eluate. From the resulting profile, the HETP and A_s values are calculated. Media-specific HETP test conditions are reported in our media instructions.

The calculated plate number will vary with the test conditions, and should only be used as a reference value. If results are to be compared, then equipment and conditions must be kept constant. Any change in dead volumes, solute, solvent, eluent, sample volume, flow rate, temperature, etc, will affect results.

HETP values can be included in the acceptance criteria for column use in the production hall. Often, the concept of “reduced plate height” is used to compare performance of different columns, but remember to account for pre- and post-column effects.

$$\text{Reduced plate height} = \frac{\text{HETP}}{dp}$$

where dp = the diameter of the beads.

As a guideline, a value <3 is normally acceptable, although in large scale chromatography the HETP value can lie between 3 and 5 dp .

A_s factor

The peak should ideally be symmetrical. Asymmetry is most commonly due to interaction of the sample with the matrix, or poor packing. Peaks with extreme leading edges indicate channelling from packing at too high a pressure or flow rate. Tailing edges imply too low a packing pressure. A change in the shape of the peak is usually an early indication of deterioration of the packing.

The calculated peak asymmetry factor should be as close as possible to 1.

A general guideline is: for $L < 30$ cm: A_s 0.8-1.8,
for $L > 30$ cm: A_s 0.8-1.5
For gel filtration, A_s is 0.8-1.2

where L = bed height



Extensive studies

In addition we do extensive tests related to normal operation. These include:

- Determining optimal packing pressure
- Determining optimal packing flow rate
- Comparison of constant pressure with constant flow packing
- Repeated column packing with HETP and asymmetry measurements
- Repeated stability testing (measuring HETP and peak asymmetry after exposing the column to specified pressures for defined times)
- Defining optimal slurry concentrations for media
- Defining optimal packing solutions (e.g. water, ethanol)
- Minimizing packing time and column handling

As with media, column design and development is a continuous process. Columns are modified to accommodate expanding media ranges, or after input from users. The above tests are routinely applied to new and modified column designs, and are also an integral part of media development projects.

Behind the column

Every column design has an accompanying User Manual which lists recommended media and recommended packing methods. Other information includes typical HETP and A_s , pressure/flow curves, and useful packing test results. You'll also find full details of column specifications, materials and chemical compatibility, construction diagrams, maintenance and service procedures, spare parts and accessories lists as well as other tips and hints to help you achieve optimal column performance right from the start. For a deeper insight into column packing methodology, we offer standard and customized courses through our Fast Trak Purification Services.

Complete solution

We aim to know our columns inside and out. All new column designs are subjected to packing tests; likewise, new separation media are packed and tested in relevant columns. Only by developing columns, media and packing techniques together can we provide you with a “complete solution” and offer a high level of practical help.

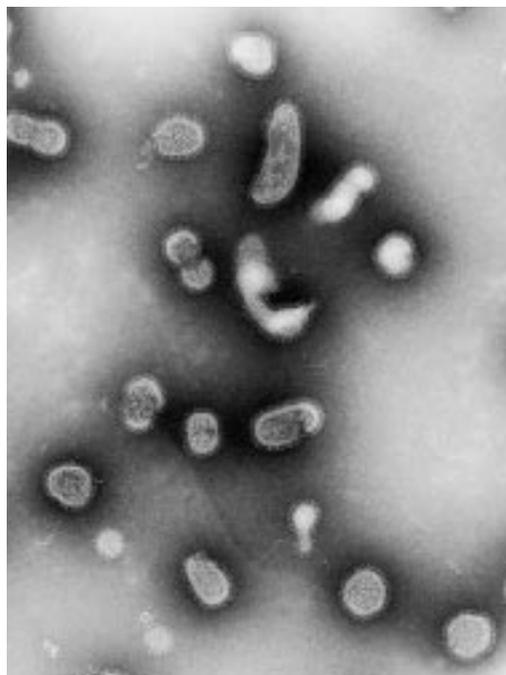
Our aim is to provide chromatographic unit operations with media, columns and systems working optimally together from the lab bench to the production hall.

Reference

1. Effect of sample volume on peak width in high-performance gel filtration chromatography. Hagel, L. J. *Chromatogr.*, 324 (1985) 422-427.

Effective virus removal and inactivation with STREAMLINE

The effectiveness of two procedures to remove and inactivate viruses was evaluated during a process to purify anti HIV-1 human monoclonal antibody by expanded bed adsorption with STREAMLINE rProtein A. The viruses introduced into the process were Influenza virus 25A-1 and Poliovirus Type 1. The results show that the procedure using detergents in the chromatographic buffers was successful in reducing the viruses.



Influenza virus, magnification factor 40,000. Photo courtesy of the National Veterinary Institute, Dept. of Virology, Uppsala, Sweden.

A study was performed during the purification process for anti HIV-1 human monoclonal antibody to evaluate virus removal and inactivation. The first part of the study investigated the effects of the washing and elution buffers used in the chromatographic process with STREAMLINE rProtein A, while the second part involved separate incubation of the

virus in the acid elution buffer. The viruses introduced into the purification process were Influenza Virus 25A-1, a lipid-enveloped RNA virus, and Poliovirus Type 1, a non-lipid-enveloped RNA virus.

Process and test procedures Part 1

The system used to capture and purify the anti HIV-1 human monoclonal antibody is illustrated in Fig. 1. STREAMLINE rProtein A adsorbent was expanded and equilibrated by passing buffer 1, see Table 1, upwards through the system at a linear velocity of 300 cm/h. Culture supernatant spiked with the virus was introduced into the expanded adsorbent at the same velocity. Buffer was then reintroduced to wash out unbound material. A switch to buffer 2, pumped through at a much lower flow velocity, approx. 5 cm/h, allows the adsorbent to settle.

Buffer 2 contains Benzonase* and detergent. It is circulated through the column for at least 8 hours at low flow velocity so that the DNA/RNA can be

digested and the virus inactivated. After this step, the system is re-equilibrated with buffer 1 at the higher flow velocity until the UV absorbance returns to the baseline. The adsorbent is allowed to settle again before elution of the antibody with buffer 3 in downward flow. The system is regenerated, then cleaned and sanitized with buffers 4 and 5.

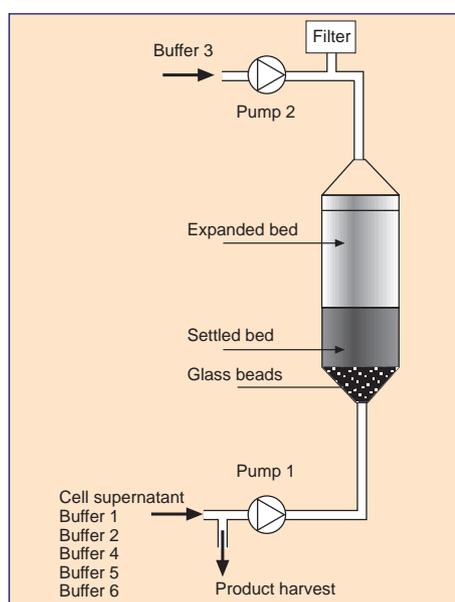


Fig. 1. Schematic representation of the system used to purify anti HIV-1 human monoclonal antibody with STREAMLINE.

Table 1. Experimental materials and conditions.

Cell line:	Rec. CHO derived IAM-anti HIV-1 monoclonal antibody MCB I. Passage no. 70.-80. p
Fermentation conditions:	Pefused fluidized bed reactor
Affinity adsorbent:	STREAMLINE rProtein A
Buffer 1:	100 mM glycine, pH 7.5
Buffer 2:	100 mM glycine, 1 mM MgCl ₂ , 1% Triton X-100, 100 U/mL Benzonase, pH 8.5
Buffer 3:	100 mM glycine, 100 mM NaCl, pH 3.5
Buffer 4:	100 mM glycine, pH 2.5
Buffer 5:	100 mM citric acid, 0.5% Triton X-100
Buffer 6: (storage buffer)	20% ethanol
Virus solutions:	Influenza Virus 25A-1 produced on MDCK cells at 34 °C. Poliovirus Type 1 produced on vero cells at 37 °C

* Benzonase is a recombinant endonuclease active against all RNA and DNA. It is a registered trademark of Nycomed Pharma A/S.

Table 2. Virus removal and inactivation effect of chromatography buffers 1, 2 and 3 on Influenza Virus 25A-1 and Poliovirus Type 1.

Sample No.	Sample	Influenza Virus 25A-1 Total TCID ₅₀	Poliovirus Type 1 Total TCID ₅₀
1	Cell supernatant spiked with virus	9.28 x 10 ⁹	1.20 x 10 ¹²
2	After sample loading	6.88 x 10 ⁹	2.14 x 10 ¹²
3	Entire flow-through	2.12 x 10 ¹⁰	1.18 x 10 ¹²
4	Entire wash with buffer 1	9.10 x 10 ⁶	9.94 x 10 ⁹
5	Start of the flow-through with buffer 2	–	–
6	End of the flow-through with buffer 2	–	–
7	Entire wash with buffer 1	<5.74 x 10 ²	6.90 x 10 ⁶
8	Elution with buffer 3	<8.93 x 10 ¹	3.05 x 10 ⁵

Table 3. Inactivating effect of acid pH buffer 3 on Influenza Virus 25A-1 and Poliovirus Type 1.

Eluate sample	Influenza Virus 25A-1 TCID ₅₀ /mL	Poliovirus Type 1 TCID ₅₀ /mL
Virus solution used for spiking	2.7 x 10 ⁷	1.12 x 10 ¹⁰
Immediately before sample treatment	<4.27	2.40 x 10 ⁸
5 min before sample treatment	<4.27	1.35 x 10 ⁸
10 min before sample treatment	<4.27	1.35 x 10 ⁸
15 min before sample treatment	<4.27	1.35 x 10 ⁸
30 min before sample treatment	<4.27	1.35 x 10 ⁸
120 min before sample treatment	<4.27	2.40 x 10 ⁸

Sampling and testing

Samples were taken from the spiked cell supernatant and column eluent at different stages of the process and analysed to evaluate the effectiveness of the procedure. Virus particles in all samples were quantified by a TCID₅₀ assay, see Table 2. The content and purity of the anti HIV-1 monoclonal antibody were determined using ELISA and analytical gel filtration (Superdex 200 HR 10/30 column), respectively.

Part 2

In the second part of the study, external time-related kinetics were used to assess the effect of incubating the virus at acidic pH, i.e. in buffer 3, the elution buffer. In

this instance, the purification process was run without virus spiking. Instead, aliquots of the eluate were spiked with virus solution (1/10th of the sample assay) 120, 30, 15, 10, and 5 minutes prior to the TCID₅₀ assay, as well as immediately before.

Virus reduction

The results show that both viruses were effectively reduced during the chromatographic process on STREAMLINE (Part 1). For the Influenza Virus 25A-1, the calculated reduction potential was 8.0 logs and for the Poliovirus, the calculated reduction potential was 6.6 logs, see Table 2.

The incubation experiment (Part 2), gave a reduction of 5.8 logs for the Influenza Virus 25A-1. In contrast, the Poliovirus was relatively unaffected by this incubation, see Table 3.

Combined effects

This process demonstrates the virus removal potential of expanded bed adsorption with STREAMLINE. The process produced anti HIV-1 human monoclonal antibody with a purity of more than 97% and a yield of 95% or more, while at the same time reducing viruses.

For more details about this study please ask for the Application Note: Evaluating virus removal/inactivation in a process to purify anti HIV-1 human monoclonal antibody by expanded bed adsorption with STREAMLINE rProtein A, code no. 18-1127-30.

Acknowledgement

The work reported here was performed by S. Lenz, G. Koller, D. Katinger, A. Assadian, C. Schmatz and H. Katinger, Polymun Scientific Immunobiologische Forschung GmbH., Austria.

Royal visit for STREAMLINE



Howard Chase (centre), University of Cambridge, has been associated with the development of STREAMLINE expanded bed adsorption from the beginning. Here he is pictured explaining the technique to His Royal Highness, Prince Philip (left), during a visit in the spring of 1997. As Chancellor of the University, Prince Philip is a regular visitor. He has a keen interest in technology and is always anxious to keep himself informed of the the university's research.



6th Annual Meeting on Bioseparation and Bioprocessing of Biological Molecules, Queens College, Cambridge, UK, 10–12 September, 1997

This meeting gave an overview of trends in the manufacture of biotechnological therapeutic products.

Affinity chromatography

One of the most interesting approaches to downstream purification is offered by the ability to construct affinity matrices with mimetic ligands. S. Burton, ACL, stressed potentially greater chemical stability combined with lower cost compared with protein ligands. Today, an evaluation (screening of candidate ligand libraries) takes approximately 2 years after the structure of the target molecule has been determined. To be really competitive, however, development time should be less than 6 months. A mimetic ligand for binding an Insulin precursor has already been developed and tested in a lab-scale purification as described by K. Hejnaes, Novo Nordisk.

Transgenic production

Strategies for designing manufacturing facilities for transgenic production were discussed by J. Noble, Foster Wheeler. Here requirements from agricultural authorities (handling of animals) and the processing of milk, determine the flow of raw materials and product, and the level of cleanliness. Of major concern in this type of production is inactivation and removal of potential viral contamination.

Time to market

Manufacturing issues were addressed by D. Low, Pharmacia Biotech, who made the point that “time to market” is generally more important than cost restrictions. He continued his talk with an emphasis on the importance of a process approach (purity, robustness and throughput) during development and the impact of vendor selection in meeting manufacturing needs.

Outsourcing

Advice from K. Christoffersen, Hyclone Labs, was to “Concentrate on your core issues and outsource what can be made better/cheaper elsewhere”. The trend towards contract manufacturing is now entering process development. M. Thiry, Pharos, presented a few cases where contractors had optimized processes.

A word of caution came from R. Woodward, TBTI, who discussed the pros and cons of outsourcing drug development and critical issues in the selection of a contractor. Science, compliance and service are important; if one is missing, the project will most probably fail. He continued his discussion with some consideration of the amount of work/pricing, confidentiality/security, reporting/handling of results.

A very dynamic presentation by J. C. Baker, Eli Lilly, entitled “Unlocking the process – post registration changes”, described how to define whether a parameter is critical or not and how to simplify future improvements in the process by using active process control with reference samples and thereby avoiding the need to revalidate everything.

Regulatory issues

The recent changes in regulatory documentation (almost 30 new guidelines during last 12 months) and their impact on validation of chromatographic processes were reported by G. Sofer, Amersham Pharmacia Biotech. The trends are towards harmonization, viral safety, CMC (chemistry, manufacturing and controls), sections unique to different product areas, and well characterized products (by specification, not defined by the process).

Process control

Strategies for development, characterization and maintenance of reference standards were presented by J. Dougherty, Lilly Research Labs. Primary standards must be established and stored under conditions that allow long term storage without change (-70°C), and serve as a reference for secondary “working” standards used for process control and product release. D. Bonam, GI, talked about validation of chromatographic robustness using statistical methods and the importance of selecting relevant variables for such evaluation.

Animal sources

M. Wisher, MA BioServices, gave a comprehensive presentation on Validation of contaminant removal: removal/inactivation of viruses, removal of host cell DNA/proteins and removal/inactivation of TSEs (transmissible spongiform encephalopathies). Partition methods, like chromatography, are often not considered to be efficient enough, but are easy to model and monitor. In a removal step, he continued, it is not sufficient to show that a virus cannot be detected in the product, you must show where the “removed” virus is located e.g. bound to the column. Changes in the risk assessment of DNA have lead WHO to increase the limit of DNA to 1 ng/dose. Today DNA is considered more of a “general” impurity. A tip for simplifying or even avoiding validation of TSE removal steps is to not include bovine material, or obtain it from a country with a surveillance program such as Australia.

Overall impressions

Although this was a small meeting, there were many opportunities for informal and informative discussions (approximately 70 participants attended). The next similar meeting will take place in Cambridge, April '98.



8th European Congress on Biotechnology, ECB 8, 70th Event of the European Federation of Biotechnology, Budapest, Hungary, 17–21 August 1997

High throughput screening was a topic of the plenary lectures and also of a presentation by Erik M. Eastman, Gene Logic Inc., who stressed how this technique can reduce the discovery phase of a new drug by up to 50%. Natural drug discovery was also covered by K. A. Thompson, M&K Enterprises Inc., who discussed the role of combinatorial techniques in a host to create new metabolites with potential pharmacological activities. Combinatorial biology was the subject of S. Forenza's lecture, Bristol-Myers Squibb Pharmaceutical, who suggested that it should be used to make "unnatural" natural products which surpass both the potency and the quality of "true" natural products.

Environmental issues

A number of lectures concerned environmental aspects of biotechnology. Van Loosdrecht, TU, Delft, suggested that in the future we must consider integrating energy production with production of new feedstocks. Since biomass can be used for fuel, this fact should be taken into consideration when designing the feedstock.

Food industry

Biotechnology is becoming increasingly important for the food industry. M. Linko, VTT Biotech and Food Research Lab, Espoo, described how immobilised yeast has been used for the main beer fermentation step at pilot scale. A. Morgan, Finnfeeds International Ltd., talked about the growing importance of biotechnology for both the micro and the macro feed industry and claimed that it is one of the largest markets for the biotech industry, >5 billion USD.

Recovery processes

Rational affinity ligand design is the way forward, claims Chris Lowe, University of Cambridge. In another presentation, Henrik Valore, Novo-Nordisk, showed data obtained from their process which uses a purpose-designed affinity ligand for the recovery of monomeric insulin produced in yeast.

Rolf Hjorth, Amersham Pharmacia Biotech, described the use of expanded bed adsorption at industrial scale. The scalability of the process was presented including the application of expanded bed adsorption using feed volumes exceeding 10 000 L.

Chromatography

A lively discussion was held by the Chromatography Task Force on the subject "Continuous Chromatography". Continuous chromatography is supposed to be used for purification of a feed that is produced continuously. Lars Hagel, Amersham Pharmacia Biotech, opened by presenting a

"provocative view", questioning the need for continuous chromatography and pointing out that each chromatographic step needs to be optimized in concert with the other steps in the purification scheme. Shushi Yamamoto, from Yamaguchi University, continued the discussion by addressing the productivity of different modes of chromatography. He was "quite pessimistic" and asked "Who needs continuous chromatography?". Alois Jungbauer, University of Agriculture, Vienna, who had suggested the topic, did support the use of continuous chromatography and stated that annular chromatography has the same performance as bed chromatography but is continuous. Guido Zacchi, Lund University, made an analogy with the paper and pulp industry who had switched from bulk processes to continuous processes and now are back into bulk processes again. The conclusion made was that the market need for continuous chromatography is still in question.

Wide ranging topics

Over 1500 scientists attended this congress which covered a wide range of topics: bioreactors, microbial physiology, downstream processing, animal and plant cell culture technology, applied molecular genetics, measurement and control, environmental and safety in biotechnology, to name a few. ECB 9 is planned for July 1999, in Bruxelles.

Second International Conference on Expanded Bed Adsorption

**Napa Valley,
California, USA
21-23 June 1998**



Announcement

The first EBA conference held in Cambridge, UK, in December 1996 was very well received (Downstream #24) and gave participants the opportunity to meet and exchange their experiences in this exciting new technology. As EBA establishes itself as a viable capture step in downstream processing, this second international meeting will give participants another opportunity to discuss further developments and guidelines for adopting this technology in bioproduct recovery. Details are available on the web site: <http://www.bo-conf.com/eba98> or from your local Amersham Pharmacia Biotech representative.

AMERSHAM PHARMACIA BIOTECH

The merger of Amersham Life Science with Pharmacia Biotech was completed on August 6th 1997. The merger brings together two strong companies with complementary business interests to create:

“the world’s leading supplier of innovative systems to help life scientists, biotechnology and pharmaceutical companies discover, develop and produce health-enhancing products with great speed, convenience and safety.”

Amersham Life Science, a leading supplier of products and automated systems for drug discovery, genomics, and cell biology research, was looking for a partner which would provide expertise in engineering as well as software and systems integration. The combination will help meet challenges arising from the growth in sequencing technologies and the need for faster and more cost-efficient drug development.

Pharmacia Biotech, a leading supplier of chemicals, software and instrumentation to the biotech industry

was looking for an injection of new research expertise which will lead into new markets and help achieve its goal of being a global, total solution supplier for every area of biotechnology, from scientific discovery to biopharmaceutical production.

The benefits this merger bring to our customers include:

- Increased speed to market for new technologies
- High levels of R&D spending
- Integration of product portfolios to give complete solutions
- Improved level of customer support

The new company has a new logo too:



amersham pharmacia biotech