Custom-designed solutions expand engineering operations
New engineering facilities have been built to help meet the growing interest in tailor-made solutions for large-scale columns and systems. The new facilities are purpose-designed and include a new product management system which will facilitate the path from specification to final product.

ÄKTAFPLC—the new standard in protein purification
Interest in proteins has never been greater as research swings from describing the genome to studying its expressed products. In timely fashion, FPLC, the system which revolutionised protein purification in the 80’s, has received its biggest ever update. ÄKTAFPLC combines all the advantages of the ÄKTAdesign platform with the established biocompatibility and reliability of FPLC, making it well prepared to meet the challenges of modern research.

STREAMLINE 100 – mid-range expansion
An increase in the range of column dimensions means that there is now a STREAMLINE column for capturing product from bioreactors with volumes up to 100 litres.

Towards large-scale purification of plasmid DNA and a viral vector intended for human gene therapy
Delivering genetic material safely and effectively to its target cell is a major challenge. The use of plasmids and modified viruses are just two potential transport systems under investigation. If these are to be viable delivery vehicles, then they must be able to be purified in large quantities. This article reports on two scalable purification methods.

Methods of inactivation of transmissible spongiform encephalopathies (TSEs) as applied to production chromatography processes
Mad-cow disease increased public awareness of infections derived from TSEs. The causative agent is believed to be the prion protein. Removing this tenacious and resilient protein has proved difficult. This article reviews the most successful methods used to diminish its infectivity.

Meeting reports
22 – Conference on Affinity Chromatography: April 16-17, 1998, San Diego, California, USA.

ÄKTAFPLC faxback
For fast information about the new standard in protein purification, cut out the form and fax to your nearest office.

Fast Trak Course details
Course details from the Fast Trak Centers around the world.
Interest in new and innovative products such as CHROMAFLOW columns, and STREAMLINE systems and columns for expanded bed adsorption, is growing rapidly. For the engineering department in Uppsala, this growth has meant a move into larger and more modern facilities for testing and developing product lines. New facilities were also a solution to meeting the increasing trend for tailor-made solutions.

One of the factors contributing to the growing interest in large-scale columns and systems is the stage many customers have reached with drug development. Many pharmaceutical companies are now proceeding from clinical trials at pilot-scale towards production and thus require manufacturing-scale equipment. This increasingly involves custom-designed solutions.

Close to customer
Developing tailor-made products involves close co-operation with the customer. And what customers ask for can vary tremendously: one customer may simply say that he needs to purify a certain substance and ask for a suitable solution. Another may send a thick loose-leaf binder with complete specifications down to a very detailed level where every nut and bolt is carefully defined. This puts vastly different demands on the team in Uppsala. In the first instance, it is necessary to understand the customer’s applications requirements and translate them into suitable hardware. In the second instance, it requires in-depth knowledge of mechanics and materials sciences in order to interpret the customer’s specifications and, where necessary, to suggest alternatives.

Established procedures
So how does the engineering department meet these differing demands? The department has acquired a great deal of experience over the years, having delivered more than 700 systems and 5000 columns world-wide. Based on this experience, it has established routines for developing a custom-designed system or column. The path to final product closely involves the customer, and includes extensive quality control and functional testing along the way, in addition to that done during installation. From placement of an order to delivery takes approximately 4–7 months per system. This time being very much dependent on the complexity of the customer’s specifications.

Multi-discipline expertise
Initial contact with a customer usually comes via a sales specialist, whereupon a project manager is appointed in Uppsala.
Choice of materials
Materials traditionally used in pharmaceutical production include high quality stainless steel of grade 316L or equivalent. However, there are several alternatives to stainless steel which offer excellent resistance to salts and other agents used in biopharmaceutical production; these include polypropylene, Hastelloy* and PVDF. Plastic materials like polypropylene are robust, chemically resistant and often more cost-effective. Today about 60% of systems are manufactured in polypropylene.

Specialist assembly
Assembly of system and column components usually takes place at one of our qualified subcontractors. Throughout the assembly period workshop inspections are frequently carried out by the project manager to assure quality of workmanship.

When the mechanical and electrical assembly of a system has been completed, the system is brought to Uppsala for software configuration, comprehensive functional testing, final adjustments and calibration. Customers are also invited to come to Uppsala to perform their own testing or inspection before delivery of the system. The system is again thoroughly tested at the customer’s site to assure it is performing as specified. The documentation binder, which contains every piece of documentation accrued to the system, is handed over to the customer after completed installation and testing.

Column packing studies
In our testing facilities we also carry out extensive packing studies on columns and media. These studies are designed to ensure optimal performance of media and column together as a separating unit, to give smooth and immediate start-up. Procedures include evaluation of column packing by measuring height equivalent to a theoretical plate (HETP) and asymmetry values. The knowledge and expertise gained from extensive testing form the basis of our column packing recommendations and also lay the foundations for new product development, as was the case with FineLINE columns and SOURCE media. Packing studies are performed on all products lines. This is an on-going process in our programme to continuously improve product performance. For details of our packing studies, see Downstream #26.

* Trademark of Haynes International Inc.
Downstream 27

**ISPPP ‘98**

18th International Symposium of Separation and Analysis of Proteins, Peptides and Polynucleotides

1–4 November 1998, Hotel Hilton, Vienna, Austria

The symposium will address issues of separation and analysis of proteins, peptides, polynucleotides and related biomolecules with modern techniques. It will also discuss the new challenges to protein purification introduced by genomics and proteomics. Lectures, posters, workshops and exhibits will cover the fundamentals of both chromatography and electrophoresis of biomolecules, development of biological and chemical libraries for affinity ligands, industrial chromatography of biomolecules and validation, PCR and related techniques for analytical and preparative purposes, the impact of genomics on protein separation, and much more.

Full programme details are available from the Symposium Secretariat:
Come In Congress-Meeting-Incentive Organisation
Gabriele Prohazka
Alserstasse 32/3, 1090 Vienna, Austria
Tel: +43 1 402 471412
Fax: +43 1 402 471414
E-mail: come-in@magnet.at
Web site: http://www.boku.ac.at/iam/isppp98/

**BIOEUROPE 98**

Bioseparation and Bioprocessing of Biological Molecules

7–9 September 1998, Queens’ College, University of Cambridge, England

This is a three-day programme of lectures by international biotechnologists and scientists. Lectures will cover a range of topics associated with the separation and processing of therapeutics, including current developments in upstream and downstream processing, gene therapy, transgenics, regulatory affairs, process economics, and technology implementation. Further details and a full programme can be obtained from the organiser:
Dr G. Subramanian
Tel/Fax: +44 1227 720819
E-mail: gsub@globalnet.co.uk

**Fast Trak assistance**

Support for columns, media and equipment is also available separately through Fast Trak Services. They can provide courses, seminars and workshops covering all aspects of industrial chromatography, help you speed up validation by having Installation Qualification (IQ) and Operational Qualification (OQ) documentation available for our systems and columns, and offer advice about how to meet regulatory requirements. Fast Trak provides Validation Support Files for UNICORN control software and columns, as well as Regulatory Support Files for media. These files contain documentation such as material properties, certificates of analysis, toxicological studies, Material Safety Data Sheets and much more; all designed to aid validation and speed up time to market.

**Complete solutions**

Custom-designed solutions are an enjoyable challenge. Our new and modern engineering facilities have increased our capacity, helping us to meet the wide ranging and growing number of customer requests. And although you may be contacting one of our representatives close to you, the engineering department in Uppsala, Fast Trak Services worldwide, or experienced personnel in our regional centers, all are available to ensure that all your needs are fulfilled. Our specialists cross discipline boundaries to provide complete solutions and comprehensive support.
ÄKTA FPLC – the new standard in protein purification

ÄKTA FPLC is the newest addition to the ÄKTA design platform of chromatography systems. This platform comprises a number of systems all sharing a common way of working, the common denominator being UNICORN control and data evaluation software. ÄKTA FPLC is a development of the original and innovative FPLC system on the ÄKTA platform, and is intended for lab scale protein purification in the microgram to milligram range. It uses the latest version of UNICORN, version 3.0 for Windows® NT, is fully automated, biocompatible and can be used with all aqueous chromatographic techniques. Measuring 380x480x470 mm, ÄKTA FPLC is very compact and occupies little space on the lab bench. In fact, it’s small enough to be placed inside a cold box or refrigerator.

Reliable
The heart of the system is based on reliable pump technology. Pump P-920 is a development of the P-500 pump used in FPLC System. It produces accurate, reproducible, pulse-free flow and precise gradient formation. It is compatible with high salt solutions and it can deliver flow rates of up to 20 ml per minute.

Easy to learn and use
Chromatography is a tool used by a variety of scientists in a variety of applications. As a tool it must be easy to learn and use. UNICORN, the brains of the system, is pre-programmed with method templates, knowledge about the columns and system set-ups. All the user needs to do is to select the appropriate techniques, template, column and system set-up, then press run. There is even a built-in Adviser ready to help when needed. ÄKTA FPLC is extremely user friendly and exceptionally knowledgeable. As much expertise as possible has been packed into the system so that you don’t need to be an expert chromatographer to use it, but you will get expert help.

In 1982, protein purification underwent a revolution with the introduction of FPLC System, which resulted in drastically decreased purification times. Over the years FPLC has been continuously developed to include a complete range of separation chemistries, automation, UNICORN control software, improved monitoring, compatibility with modern chromatography matrices, etc. Its performance and reliability have made it an “industry standard” with over 50,000 citations in the literature. Now FPLC takes a leap on to the ÄKTA platform.
Convenient and flexible
The system is small and compact, yet it can accommodate a number of extra valves, sample pumps, air sensors and columns for different system configurations to meet the needs of different applications and different laboratories. These accessories are simply clipped on to the gate rack. The gate rack can be opened if more space is required, or extended if longer columns (1 m) are used.

New UNICORN for Windows NT
This new version of UNICORN, v. 3.0 for Windows NT is designed specifically to comply with GLP/GMP requirements and to meet customer demands for networking and multi-tasking. The improvement in networking allows you to check the progress of a separation from any other UNICORN workstation in the network. Remote surveillance possibility means that you can avoid having to sit in the cold room with your equipment and that you can follow progress in several separations in parallel.

UNICORN is common to all ÄKTAdesign systems and provides a consistent way of working. The user interface is the same whether the application is a routine MAb preparation, purification of a pioneering new receptor, or a large-scale downstream process. This consistency reduces training time, the risk of introducing errors and facilitates scale-up/transfer of methods from the lab bench to the production hall.

Application areas for ÄKTAFPLC
In our own laboratories and in collaborations with experts from research groups, ÄKTAFPLC has been tested in several applications involving characterisation studies, structure and function studies, and optimisation of methods. The following are brief summaries intended to give you an idea of the wide-ranging potential of ÄKTAFPLC. Full details are given in the respective Application Notes.

Characterisation studies

In one collaboration, ÄKTAFPLC was used to isolate a integral human cell membrane protein expressed in E. coli. When purity demands are very high, purity should be assessed by characterisation of the size and charge of the protein to avoid erroneous conclusions from further structure/function studies, for example with SDS PAGE. The target protein was a recombinant histidine-tagged cytochrome bo3 ubiquinol oxidase expressed in E. coli. Capture was performed on a HiTrap Chelating column at cold room temperatures in the presence of non-ionic detergents. The collected fractions were analysed by SDS-PAGE for purity (Fig. 1). Under non-denaturing conditions, anion exchange chromatography on Mono Q HR 5/5 (Fig. 2a, b) confirmed the high purity and charge homogeneity observed with SDS-PAGE. Gel filtration on Superdex 200 HR 10/30 size gave essentially one peak, indicating an homogeneous monomeric protein.

Fig. 1. SDS electrophoresis on PhastSystem using PhastGel 8–25%, silver staining.

Fig. 2. Chromatographic characterisation on Mono Q HR 5/5. Fraction 1 (a) eluted in one main peak, fraction 2 (b) gave a single sharp peak.

* Trademark of Rohm & Haas Inc.
Structure and function studies

Isolation of highly labile proteins in an active form is a well known obstacle in protein studies, particularly in structural biology where only an active, homogenous preparation is likely to give crystals adequate for X-ray diffraction studies. A purification scheme was developed for deacetoxycephalosporin C synthase (DAOCS), an oxygen-sensitive enzyme involved in cephalosporin/cephamycin biosynthesis. The gene was over-expressed in soluble form in the cytoplasm of *Escherichia coli*. The method was developed by automated screening of different chromatography media, and gradient optimisation, which was facilitated by pre-programmed method templates. The final method involved capture on the anion exchanger Q Sepharose XL (Fig. 3a), hydrophobic interaction chromatography with SOURCE 15ISO (Fig. 3b) and polishing with gel filtration on Superdex 75 prep grade (Fig. 3c). The procedure yielded 10 mg of homogenous, biologically active DAOCS which was used for crystallisation and subsequent 3D structure determination. The total purification time from cell harvest to collection of DAOCS from fractions from gel filtration was 5.7 hours, of which the time on ÄKTAFPLC constituted less than 50%.

For more information about ÄKTAFPLC, ÄKTAdesign chromatography systems or any of the applications work reported here, ask for:

Data Files:
- AKTAFPLC Code no 18-1128-41
- AKTAexplorer System series Code no 18-1124-09
- AKTApurifier Code no 18-1119-48
- Monitor UPC-900 Code no 18-1128-40
- UNICORN control system Code no 18-1111-20

Application notes:
- Rapid optimisation and development of an automated two-step purification procedure for monoclonal IgG antibodies Code no. 18-1128-93
- Purification and chromatographic characterisation of an integral membrane protein Code no. 18-1128-92
- Purification of a labile, oxygen sensitive enzyme for crystallisation and 3D structure determination Code no 18-1128-91

See also faxback, page 25
Fast optimisation

In a third study, the flexible design and functions of the system were found to be extremely helpful for rapid optimisation of parameters important for the capture step in a purification procedure and for creating a routine automated process for two-step purification of mouse monoclonal IgG from culture supernatant. As usual, the aim of the capture step was to concentrate the target molecule and remove the bulk of the contaminants. Affinity chromatography on HiTrap rProtein A was chosen because of its high selectivity for IgG. A pre-programmed method template in UNICORN was used for automatic scouting of binding conditions, allowing automatic control of the salt concentration of the binding buffer. The scouting runs revealed that 2.5 M NaCl was optimal for binding (Fig. 4), and was used during subsequent method optimisation.

The optimal pH for elution was determined by eluting the antibodies with a decreasing pH gradient ranging from pH 7.4 to pH 3 (Fig. 5). The results demonstrated that the peak containing the antibodies was eluted at pH 4.5, which was selected as elution pH in the final method.

Polishing, the final step, was performed by gel filtration on HiLoad 16/60 Superdex prep grade column. The polishing template is already available in UNICORN. The two-step procedure was then automated by expanding the system with additional valves and modifying the template according to the new configuration. The function Method Queue in UNICORN enabled the two steps to be run automatically one after the other. This optimised procedure gave 1.2 mg MAb from approx. 50 ml cell culture supernatant. Recovery from both steps was above 95%.

ÄKTAFPLC and proteomics

With a sound history and a strong lineage, ÄKTAFPLC - designed to handle biomolecules from native and recombinant sources – has timed its entrance well. With the focus today on proteomics and the vast number of proteins emerging from the human genome project, ÄKTAFPLC is sure to quickly establish itself as the new standard in protein purification.
STREAMLINE expanded bed adsorption brings upstream and downstream processing closer by enabling direct capture of biomolecules from the fermentor. Growing interest in this new unit operation has called for a broader range of column dimensions. The introduction of STREAMLINE 100 is a response to this request. The STREAMLINE family now boasts a range of columns with standard diameters of 25, 50, 100 and 200 mm for method development to pilot scale and small-scale production. Larger diameters for full-scale manufacturing operations are available as custom-built columns. The addition of this new dimension is another step in the scalability of STREAMLINE.

Ideal for pilot-scale
STREAMLINE 100 column is ideal for pilot-scale applications, typically handling fermentor volumes from 5 to 100 litres at flow rates between 16 and 31 litres per hour. The column is a 950 cm long glass cylinder with an inner diameter of 100 cm. The column is easy to operate as the adaptor is moved hydraulically; this allows for elution in packed bed mode if required. Recommended settled bed heights lie between 10 and 30 cm, corresponding to adsorbent volumes of 800 ml to 2 litres. STREAMLINE 100 is suitable for use with all STREAMLINE adsorbents.

Arrests target molecule
In expanded bed adsorption with STREAMLINE, biomolecules such as proteins are captured directly from crude feedstocks in a single pass operation, without the need for any prior dilution or clarification. The principle of operation is common to all STREAMLINE dimensions. In the bottom of the column is a unique liquid distributor. When buffer is pumped through column, the settled adsorbent beads, which have a uniform density distribution, are lifted in such a way that they form a stable expanded bed. Flow of the target molecule through the column is arrested by the expanded adsorbent, while particles and other debris flow freely through the column. The target molecule is then eluted from the column in packed bed mode, partially purified.

Hygienic design
Like all STREAMLINE columns, this new dimension is designed for use in environments which demand high standards of hygiene. Materials of manufacture include borosilicate glass, electropolished stainless steel, polypropylene and EPDM rubber, all of which are compatible with the solvents commonly used in biopharmaceutical production. Sanitisation studies performed on STREAMLINE 200 show that the column design is sanitisable with 0.5 M sodium hydroxide.

This new column can be operated with a manual or automatic STREAMLINE system, or as a stand-alone unit. It conforms with all EU Machine Directives and is CE marked. For further information about this or other STREAMLINE columns please ask for the Data Files or contact your local representative.
Process development with STREAMLINE columns

Method scouting with a conventional packed bed
Selection of STREAMLINE adsorbent and determination of optimal conditions for binding and elution are best achieved through screening of clarified feed in a small packed bed column.

Suggested materials:
Unclarified feed
STREAMLINE 25 column
STREAMLINE adsorbent, 50–150 ml
Fermentor volume, 0.25–5 litres

Scale up to pilot scale
Verification of the optimised methods for the final process can be carried out by scaling up to STREAMLINE 50, 100 or 200.

Suggested materials:
Unclarified feed
STREAMLINE 50 column
STREAMLINE adsorbent, 200–600 ml
Fermentor volume, 1–20 litres

STREAMLINE 100 column
STREAMLINE adsorbent, 0.8–2 litres
Fermentor volume, 5–100 litres

STREAMLINE 200 column
STREAMLINE adsorbent, 3–9 litres
Fermentor volume, 20–300 litres

Optimisation in expanded bed mode
The search for optimal throughput, sample load, dilution factors, flow rates during sample application and elution, etc is carried out with the smallest STREAMLINE column diameter, using crude sample and bed expansion.

Suggested materials:
Clarified feed
Standard column XK 16/20
STREAMLINE adsorbent, 20–30 ml
Optimisation at laboratory scale

STREAMLINE columns
STREAMLINE 50, 100 and 200
Code no. 18-1127-35
STREAMLINE 25
Code no. 18-1112-02
STREAMLINE adsorbents
STREAMLINE SP and DEAE
Code no 18-1111-73
STREAMLINE rProtein A
Code no. 18 1115-67
STREAMLINE Q XL and SP XL
Code no. 18-1123-81
STREAMLINE chelating
(ask for details)

Application Notes
STREAMLINE Scale up
Code no. 18-1118-68
STREAMLINE SP and DEAE
Cleaning-in-Place
Code no 18-1115-27

Recovery of Biological Products IX
May 23-28 1999, Whistler, British Columbia, Canada

This conference has established itself over the years as the forum for discussion and development of new areas in the exploration and commercialisation of biological products, especially within bioseparations and bioprocessing. The established format of morning and evening sessions will be followed, with emphasis on both oral and poster presentations by academic and industrial experts on separation science and related fields. Key themes will be “Speed in Development” and “Decision Making During Process Development”.

For more information contact:
Joan Saluzzi
Rhema Association Management
PO Box 411106 Tel: 415 487 9857
Fax: 415 487 9803 E-mail: society@hooked.net
Web site: http://www.novo.dk/R9/
Cancer, atherosclerosis, osteoporosis, arthritis and Alzheimer’s disease are all examples of disorders characterised by specific changes in the activities of genes. Even infectious disease usually provokes the activation of identifiable genes in a patient’s immune system. Gene therapy aims to treat such illnesses at the molecular level by delivering DNA to affected cells so that the cells will produce a therapeutic protein or peptide. The genes are usually first put into vectors such as viruses or plasmids complexed with lipids able to deposit foreign genes into cells. The production of plasmids and viruses for gene therapy is among the challenges currently emerging for large-scale purification technology. This article will describe purification methods for plasmid DNA and an adenovirus vector for use in gene therapy.

Purification of plasmid DNA

Although a wide range of plasmids are now routinely purified at the lab bench, the methods used are often based on one-use kits. In addition, the production of plasmid DNA for preclinical toxicology human trials, and ultimately for an approved pharmaceutical indication, requires the development of a process that reproducibly meets the quality and regulatory standards for the manufacture of a biopharmaceutical.

For gene therapy, a plasmid should be in the supercoiled form and essentially free from bacterial chromosomal DNA, RNA, proteins and endotoxins. Target levels for supercoiled plasmid and impurities in plasmid preparations are listed below, (2).

<table>
<thead>
<tr>
<th>Content</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>undetectable</td>
</tr>
<tr>
<td>Supercoiled plasmid</td>
<td>&gt;95% nucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>&lt;2% nucleic acid</td>
</tr>
<tr>
<td>Chromosomal DNA</td>
<td>&lt;1% plasmid</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>&lt;100 EU/mg plasmid</td>
</tr>
</tbody>
</table>

The common host for plasmid DNA production, as for recombinant proteins, is *E. coli*. Plasmids extracted from *E. coli* by alkaline lysis are recovered in the clear lysate supernatant obtained after centrifugation. However the plasmid only accounts for a small percentage of the total nucleic acid content of the lysate (the higher the plasmid copy number, the higher the...
Sample preparation

Frozen *E. coli* cells harbouring 4.5 kb plasmids were used and cell lysis was performed essentially as described by Sambrook *et al* (1).

Calcium chloride (0.2 M) was chosen as a precipitant to reduce the amount of RNA, chromosomal DNA and open circular plasmids in the clear lysate. RNA and chromosomal DNA were reduced by about 50% and 70% respectively. About 10% of the plasmid is lost during this step. The precipitation was integrated with the alkaline lysis procedure, and hence no additional handling step was required. The conductivity was adjusted by dilution, where necessary, and the clarified lysate at pH 5.5 was then applied directly to the gel adsorbent without any change of buffer.

Chromatography

Since RNA and DNA have similar charge properties it has been difficult to capture plasmid DNA selectively on anion exchangers. In this application 99.4% pure supercoiled plasmid was obtained in a two-step, rapid and scalable chromatography procedure using Q Sepharose XL for capture and SOURCE 15Q for polishing. Operating conditions were chosen to capture plasmid selectively from the pre-treated lysate.

Vectors are the vehicles being developed for delivering genetic material to the target cell in gene therapy. Vectors based on viruses can be effective vehicles for gene transfer due to their specialised mechanisms for binding to specific types of cells and delivering their payload. Viral vectors containing therapeutic genes, can be administered directly to patients, in vivo, or used to treat cells in vitro before being administered to the patient.

Viruses presently being developed for vectors for both in vivo and in vitro use include retroviruses, adenoviruses, herpes simplex viruses and adeno-associated viruses. Each type of viral system offers both advantages and disadvantages with respect to efficiency and accuracy of delivery, contra potential infection. For example, retroviruses integrate copies of their genes permanently into the chromosomes of the cells they invade, offering long term stability; however they lack specificity for host cells and can deliver their genes into a variety of cell types. Adenoviruses are considered relatively safe since they cause nothing more serious than the common cold; however, the body has a strong immune reponse against them.

There are other vector systems under development that do not use viruses, for example those based on liposomes and DNA conjugates. Liposomes can be designed to carry plasmids – flexible loops of DNA that multiply naturally in bacteria – in which original genes have been replaced with therapeutic ones. Liposomes are non pathogenic, but are less effective at transferring the genetic material into the cells.

Other studies are focused on non vector delivery, such as direct injection of naked DNA into the patient. DNA can be taken up and expressed by some cells, but stable transformation is rare.

In all cases, research is focused on enhancing the benefits and negating the drawbacks of each of type of delivery system so that safe and efficient delivery of the gene to its target cell is achievable.
Capture

Q Sepharose XL was found to show the highest binding strength for plasmids of various sizes when compared to several other ion exchangers (Fig. 1). Method optimisation was performed on a 1 ml column (Figs. 2a and 2b) and the purification then scaled up to a 10 ml column (Fig. 3). Conditions used in the capture step are outlined in fig. 4 and analysis data are shown in Table 1. Note that using a lower flow velocity during plasmid elution increased the yield by 20%.

![Fig. 1. Elution of plasmid on different anion exchange media.](Image)

Polishing

Final purity goals were achieved by chromatography on a 6 ml prepacked column of SOURCE 15Q (Fig. 5), using a shallow salt gradient. The content of RNA, chromosomal DNA, and endotoxins was further reduced in this step. The resultant supercoiled plasmid was essentially free from bacterial chromosomal DNA, RNA, proteins and endotoxins (Table 1) and satisfied the target purity for gene therapy applications (Table 2).

![Fig. 2a. Purification of plasmid on Q Sepharose XL, 1 ml column.](Image)

Table 1. Analysis of plasmid fractions from capture and polishing (Figs. 3 and 5).

<table>
<thead>
<tr>
<th>Chromatography process</th>
<th>Q Sepharose XL (10 ml)</th>
<th>SOURCE 15Q (6 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of plasmid applied (mg)</td>
<td>6.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Analysis of plasmid peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nucleic acid (mg)</td>
<td>5.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Plasmid amount (mg)</td>
<td>4.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Plasmid (% nucleic acid)</td>
<td>90</td>
<td>99.4</td>
</tr>
<tr>
<td>RNA (% nucleic acid)</td>
<td>4</td>
<td>not detected</td>
</tr>
<tr>
<td>Chromosomal DNA (% nucleic acid)</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Supercoiled plasmid (% plasmid)</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Yield per step (% of plasmid applied)</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td>Overall yield (% of total plasmid)</td>
<td>78</td>
<td>66</td>
</tr>
</tbody>
</table>

![Fig. 4. The capture step. Chromatographic procedure and conditions.](Image)

CV = column volume, X = volume of the clear lysate, Flow velocity is 60 cm/h during the elution of plasmid and 300 cm/h in all the other steps.
Cleaning in place

After each run both columns were cleaned in place with sodium hydroxide and sodium chloride. Tightly bound chromosomal DNA was removed during this step. The column was then rapidly equilibrated.

Rapid and scalable method

This method is rapid- the two chromatography steps take about three hours and the whole process can be completed within a day. The method is scalable and includes a CIP procedure using 0.5 M NaOH and 2 M NaCl.

Comments

This process was optimised using plasmid with a low copy number. The purification yield is likely to be much higher using plasmid with a high copy number.

Acknowledgements

The work described here is the result of a collaboration between scientists at Rhône-Poulenc Rorer Gencell and Amersham Pharmacia Biotech. A patent has been applied for the process.

Table 2. Target levels for supercoiled plasmid and impurities in plasmid preparations (2).

<table>
<thead>
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<td>&lt;100% EU/mg plasmid</td>
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</tbody>
</table>
Purification of a viral vector

Francis Blanche
Rhône-Poulenc Rorer Gencell

At present, the most efficient and commonly used vectors for gene therapy are based on attenuated or modified versions of viruses. The modified viruses cannot replicate in the patient, but do retain the ability to deliver genetic material. Current clinical programmes at Rhône-Poulenc Rorer Gencell utilise adenovirus vectors. A single step purification of Type 5 adenovirus has been developed using SOURCE 15Q media, giving a product which fully complies with the quality specifications required for clinical grade material (see below).

Vector purification process

```
culture supernatant
293 cells
---
filtration, ultrafiltration
Benzonase* treatment (optional)
---
SOURCE 15Q
---
diafiltration to final buffer
---
Therapeutic virus
```

Vector quality goals (3)

- HPLC purity (see Fig. 7) > 98%
- SDS-PAGE Coomassie purity: Only protein bands corresponding to constitutive viral proteins detected
- BSA: < 50 ng per dose
- Host nucleic acids: < 10 ng per dose
- Host cell protein: None detectable (Western blot)
- Infectivity: Same as CsCl purified

Scale-up

The process was scaled up to 2.5 litres of medium in FineLINE 200 column operated by BioProcess System. Scale up was done keeping load per volume of SOURCE 15Q, flow velocity and bed height constant, Fig. 6. Purity of the virus peak was greater than 98% as determined by HPLC, Fig. 7.

Sample preparation

A “supernatant harvest method” was used in which the culture is continued until autolysis of packaging 293 cells occurs (patent applied for) (4). After filtration and ultrafiltration steps the sample was applied to a column containing SOURCE 15Q. Optionally, a treatment with a nuclease (Benzonase) can be introduced before chromatography to provide an additional guarantee as to the removal of host cell nucleic acid.

One-step purification on SOURCE 15Q

Initial experiments used a 1 ml RESOURCE column on FPLC System. Process development studies were done on ÄKTAexplorer 100 using FineLINE Pilot 35 column. A salt gradient elution method was developed and optimised on ÄKTAexplorer 100. Conditions were optimised to meet set quality specifications. Note that information about operating conditions is proprietary. After the chromatography step, the purified virus was transferred to the final buffer using diafiltration.

In this process it was possible to obtain clinical grade material using a single chromatography step on SOURCE 15Q. The little bump which is in the tailing edge of the virus peak has been identified as empty particles (devoid of viral DNA). It is collected as a separate fraction and discarded.

* Trademark of Nycomed Pharma A/S.
Cleaning in place
After each run the column was cleaned with sodium chloride, sodium hydroxide and phosphoric acid/sodium chloride. Phosphoric acid was found to be more efficient than acetic acid in removing tightly bound nucleic acids. At least 20 purification cycles can be run without any reduction in column performance.

Comments
Due to the excellent performance of SOURCE 15Q it was possible to develop a one-step purification process for the clinical production of adenovirus for gene therapy. Particle recovery during purification was consistently between 75% and 90% irrespective of scale and met the specifications for purity as outlined in vector quality goals. Chromatography on SOURCE 15Q also allows for separation of infectious and non-infectious particles. Non-infectious particles are undetectable in final preparations. Residual Benzonase was not detectable in final product (< 0.2 ng / 2.5 x 1012 vp). The chromatography step takes less than three hours, CIP takes several hours and the whole process from harvest to filled product takes two days.

Enormous potential
These studies are a contribution to the efforts being made and to meeting the challenges in developing molecular medicine. There are still many hurdles to pass, ethical as well as technical, but the potential for gene therapy is a fantastic driving force.

Patents
Rhône-Poulenc Rorer has filed a patent application for the production/purification process described above (4).

References
4. RPR patent application number WO 98/00524.
The group of diseases collectively known as transmissible spongiform encephalopathies (TSEs) include several human diseases such as Creutzfeldt-Jakob Disease (CJD), Fatal Familial Insomnia (FFI) and Gerstmann-Strassler Syndrome (GSS). In addition, there are other TSEs known to infect animals, with sheep scrapie being the best characterised. Until the 1990s, TSEs had not been of great public concern. However, recent issues surrounding the “Mad Cow” phenomenon in the United Kingdom have increased public awareness of these diseases and recent evidence strongly suggests that bovine spongiform encephalopathy (BSE) may have entered into the human population (1, 2).

Although disputed, the causative agent for these diseases is generally believed to be the prion protein. In the diseased state, the prion protein is in an altered conformation that makes it more proteinase resistant, less soluble and is frequently used as a marker for disease (for more comprehensive reviews see references 3 or 4).

Regardless of the nature of infectivity, the TSE agent is tenacious and resistant to standard methods of pathogen inactivation. Indeed, methods commonly used to inactivate viral or bacterial pathogens such as autoclaving (5, 6), pH extremes (7, 8), detergent treatments (7), high temperatures (9) or even combinations of these methods (5), do not guarantee complete inactivation of TSE agents.

With recent discussions from the European Union and the FDA regarding the banning of pharmaceuticals containing bovine- or human-derived products, pharmaceutical companies around the world are attempting to understand and develop procedures for inactivation of TSE agent(s). Sweeping bans of these products could potentially include not only animal-derived products but also recombinant products which utilize materials obtained from animal tissues, products obtained from cells grown in the presence of animal sera and/or products which may use an animal product as a stabilising excipient (e.g. albumin). Unfortunately, efforts to inactivate the TSE agent are hindered not only by the resiliency of the infectious agent but also the cumbersome and lengthy bioassay required to assess infectivity.

The purpose of this review is to compare some of the chemical and physical methods that have been reported to affect the agent and to assess the potential application of these methods to the pharmaceutical industry. It is important to emphasise that while no method has been shown to totally inactivate the TSE agent, some methods have demonstrated effective diminution of infectivity. The data reported within reflects bioassay data obtained from animal studies using samples treated in idealised systems (e.g. purified or dilute infective material). For any given process step where TSE infectivity is to be assessed, infectivity inactivation and/or removal would need to be determined under the specific set of conditions from which the process solution is derived.

Finally, conclusions extrapolated from bioassay studies must always take into account the specific TSE strain utilised in the study. For example, BSE may have a different resistance profile to a given inactivation method compared to a particular rodent-adapted sheep scrapie. It is always in an investigator’s best interest to use the most relevant strain of TSE and/or the most resilient strain to insure maximum efficacy against an unknown TSE burden (10).

Chemical methods of inactivation

Table 1 lists several chemical agents which may be of utility to inactivate TSE infectivity. In general, most organic chemicals necessary to quantitatively inactivate TSEs are harsh on most pharmaceutical process equipment. For
example, acidified aqueous phenol which has a high probability of success to kill the agent (8), would be impractical given the hazardous nature of the material and equipment corrosion issues that surround the use of such a solution on the manufacturing floor. Other phenol and phenol combinations demonstrated some effectiveness as well (7). Finally, the formaldehyde combinations typically used to fix tissues for histology purposes generally failed to effectively inactivate the TSE agent (5).

Other possible inactivating chemicals varied in their efficacy to reduce TSE titers. For example, although chaotropic chemicals (guanidine) at higher concentrations showed some inactivation of the TSE infectivity (7), detergents (SDS, N-lauryl sarcosine) proved to have no significant effect (7, 11). Acids at very high concentrations proved to be effective, but their effectiveness also diminished at lower concentrations (7).

Sodium hydroxide, in most cases, is an excellent sanitising agent for viral pathogens and is compatible with many GMP cleaning regimes, including many chromatography media. However, in the TSE literature there are some discrepancies in data reporting its effectiveness. In general, sodium hydroxide at 1–2 M concentrations does decrease infectivity of some of the TSE agents tested, but in no case that has been reported has sodium hydroxide (or any other chemical) totally and consistently removed infectivity. Indeed, as shown in Table 1, its effectiveness varies significantly from study to study.

### Physical methods of TSE inactivation

Table 2 is a brief compilation of various physical methods that have been used to inactivate the infectious entity. Although not practical for use directly on process solutions, it is possible that some of these techniques may be used to treat chromatography media and/or process equipment.

Irradiation using g-rays, proved to be ineffective on TSEs of human origin (12). Likewise, UV irradiation at varying doses and wavelengths also proved to be ineffective (13). However, it should be noted that many of the irradiation experiments in the TSE literature are difficult to interpret given variations in dosing parameters such as time, sample size (mass and volume), effectiveness of permeation, etc.

While autoclaving has been demonstrated to insure sterility against viral and bacterial pathogens, the effectiveness of autoclaving against TSE agents is less certain. This observation is complicated by the number of configurations available to perform an autoclaving experiment. While no autoclaving technique can guarantee a complete kill of the TSE agent, gravity displacement at 135 °C for 1 hour or longer is generally considered effective at inactivating over 5 logs of infectivity (8).

Boiling infected brain homogenate for short time periods has proven moderately effective, removing less than 3 logs of infectivity (9). Dry heat at temperatures of 160 °C has shown to remove 6 to 7 logs of infectivity (9). Ashing materials at 360 °C for 1 hour or incineration of material is also an effective method to inactivate the TSE agent (5) although not a viable option for a process step for pharmaceutical products.

### Chromatography resin considerations

Cleaning of chromatography resins and supporting process equipment provides an additional challenge in process development. For any contaminated product that contacts resin, it is generally assumed that some residual protein remains on the resin. For this reason it would be useful if some level of inactivation of TSE by either chemical or physical methods could be employed.

Of the chemicals listed in Table 1, NaOH is probably the most commonly used cleaning agent in manufacturing processes and most non-silica resins will withstand up to 2 M NaOH, within certain time constraints. Likewise, 7 M Guanidine-HCl, 3 M Guanidine-thiocyanate, and methanol/sodium hydroxide are possibilities, but they are more difficult to use at a larger scale. The majority of the other agents listed would either damage the resins or present handling dangers in the manufacturing setting.

Sodium hypochlorite may be a practical alternative to NaOH at the manufacturing scale. In the literature, sodium hypochlorite is described as a good inactivation agent for TSEs if chlorine concentrations are maintained at suitable levels (6, 10). If handled properly, at ambient temperature, sodium hypochlorite is less hazardous and as effective as heated NaOH. However, process equipment must be modified in such a manner that it is able to tolerate the corrosive nature of the hypochlorite, and chlorine concentrations should be carefully monitored to insure proper levels of chloride are maintained.

Most of the physical methods listed would not be practical on the manufacturing floor. For example, for irradiation to be effective it would be required that adequate and consistent energy levels are provided to insure complete and overall effectiveness of the technique. This would be difficult for chromatography resins used in either column or batch formats. Heating may be useful, although the extreme conditions required for effective inactivation would be difficult to maintain and validate. A compromise solution might include a combination of physical (e.g. heating) and chemical (e.g. NaOH) methods. For either heating or a combination techniques, the resin must be able to withstand the method ascribed.

Finally, it should be noted that the resin manufacturing procedure itself should be certified as TSE free.

### Other considerations

Additional considerations should include the physical state of the potentially infectious material. For instance, it is...
Table 1. Comparison of various chemical methods for inactivation of TSE agents. Asterisks (*) indicate that the data was obtained from a graph. Depending upon how the bioassayed was performed, actual log_{10} decreases may not be available (NA). Numbers in parentheses indicate increases in infectivity. Effectiveness is classified as follows: ≤1.5 log_{10} decrease, ineffective; 1.5–3.0 moderately effective; ≥3.0 effective. Unless otherwise stated, all assays were performed by rodent bioassay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time/Temperature</th>
<th>Agent tested</th>
<th>Log ID_{50} change</th>
<th>Comment(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% Acidified aqueous phenol</td>
<td>0.5 hr/RT</td>
<td>Hamster Scrapie</td>
<td>5*</td>
<td>Effective; compound is aqueous acidic phenol</td>
<td>(8)</td>
</tr>
<tr>
<td>0.9% Acidified aqueous phenol</td>
<td>16 hr/RT</td>
<td>Hamster Scrapie</td>
<td>≥7.4</td>
<td>Effective; compound is aqueous acidic phenol</td>
<td>(8)</td>
</tr>
<tr>
<td>0.5% Acidified aqueous phenol</td>
<td>0.5 hr/RT</td>
<td>Hamster Scrapie</td>
<td>≥7.0</td>
<td>Effective; compound is aqueous acidic phenol</td>
<td>(8)</td>
</tr>
<tr>
<td>0.9% Acidified aqueous phenol</td>
<td>0.5 hr/RT</td>
<td>Hamster Scrapie</td>
<td>≥7.0</td>
<td>Effective; compound is aqueous acidic phenol</td>
<td>(8)</td>
</tr>
<tr>
<td>0% phenol</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>80% phenol</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>1 M Guanidine-HCl</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>7 M Guanidine-HCl</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>1 M Guanidine-thiocyanate</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>3 M Guanidine-thiocyanate</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>10 M formaldehyde</td>
<td>48 hr/RT</td>
<td>Hamster Scrapie</td>
<td>1.5</td>
<td>Ineffective</td>
<td>(5)</td>
</tr>
<tr>
<td>10 M formaldehyde + autoclave</td>
<td>48 hr/RT, 0.5 hr/134 °C</td>
<td>Hamster Scrapie</td>
<td>1.8</td>
<td>Moderately effective</td>
<td>(5)</td>
</tr>
<tr>
<td>Autoclave /10 M formaldehyde</td>
<td>0.5 hr/134 °C, 48 hr/RT</td>
<td>Hamster Scrapie</td>
<td>6.8</td>
<td>Effective</td>
<td>(5)</td>
</tr>
<tr>
<td>10 M formaldehyde</td>
<td>48 hr/RT</td>
<td>Hamster Scrapie</td>
<td>(0.3)</td>
<td>Ineffective</td>
<td>(5)</td>
</tr>
<tr>
<td>15% formalin</td>
<td>96 hr/RT</td>
<td>CJD</td>
<td>0</td>
<td>Ineffective; typical tissue fixation technique</td>
<td>(14)</td>
</tr>
<tr>
<td>sat’d phenol-formalin, formalin</td>
<td>24 hr, 72 hr/RT</td>
<td>CJD</td>
<td>5</td>
<td>Effective</td>
<td>(14)</td>
</tr>
<tr>
<td>96% formic acid'n 96% formic acid</td>
<td>24 hr, 72 hr/RT</td>
<td>CJD</td>
<td>&gt;6</td>
<td>Effective</td>
<td>(14)</td>
</tr>
<tr>
<td>1 N hydrochloric acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>&gt;8</td>
<td>Effective</td>
<td>(14)</td>
</tr>
<tr>
<td>20% formic acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>0.1</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>60% formic acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>80% formic acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>0.2 M trichloroacetic acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>3 M trichloroacetic acid</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>≥7.0</td>
<td>Effective</td>
<td>(7)</td>
</tr>
<tr>
<td>Hypochlorite (1000 ppm)</td>
<td>0.5–14 hr/RT</td>
<td>Mouse scrapie</td>
<td>3.8–5.9</td>
<td>Effective at longer incubation times and dependent on TSE stain</td>
<td>(10)</td>
</tr>
<tr>
<td>Hypochlorite (10,000 ppm)</td>
<td>0.5–14 hr/RT</td>
<td>Mouse scrapie</td>
<td>≥4.6</td>
<td>Effective</td>
<td>(10)</td>
</tr>
<tr>
<td>Sodium hypochlorite (8000–16500 ppm chlorine)</td>
<td>0.5–2 hr/RT</td>
<td>BSE</td>
<td>NA</td>
<td>Effective</td>
<td>(6)</td>
</tr>
<tr>
<td>Sodium dichloroisocyanurate (8000–16500 ppm chlorine)</td>
<td>0.5–2 hr/RT</td>
<td>BSE</td>
<td>NA</td>
<td>Moderately effective</td>
<td>(6)</td>
</tr>
<tr>
<td>1 N sodium hydroxide</td>
<td>1 hr/RT</td>
<td>Hamster Scrapie</td>
<td>≥5.5</td>
<td>Effective</td>
<td>(10)</td>
</tr>
<tr>
<td>2 N sodium hydroxide</td>
<td>2 hr/RT</td>
<td>Hamster Scrapie</td>
<td>≥5.1</td>
<td>Effective</td>
<td>(10)</td>
</tr>
<tr>
<td>0.09 N sodium hydroxide</td>
<td>2 hr/RT/121 °C/1 hr</td>
<td>Hamster Scrapie</td>
<td>≥7.4</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>0.9 N sodium hydroxide</td>
<td>2 hr/RT/121 °C/1 hr</td>
<td>Hamster Scrapie</td>
<td>≥7.4</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>1 N sodium hydroxide</td>
<td>1 hr/RT</td>
<td>Hamster Scrapie</td>
<td>6.0</td>
<td>Effective</td>
<td>(15)</td>
</tr>
<tr>
<td>0.25 N sodium hydroxide</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>–1</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>1 N sodium hydroxide</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>2.9</td>
<td>Moderately effective</td>
<td>(7)</td>
</tr>
<tr>
<td>2 N sodium hydroxide</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>1.8</td>
<td>Moderately effective</td>
<td>(7)</td>
</tr>
<tr>
<td>Methanol/sodium hydroxide</td>
<td>4 hr/40 °C</td>
<td>Hamster Scrapie</td>
<td>≥7.8</td>
<td>Effective</td>
<td>(15)</td>
</tr>
<tr>
<td>1Room temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 M N-lauryl sarcosine</td>
<td>Added to bioassay diluent</td>
<td>Mouse Scrape</td>
<td>(2)</td>
<td>Ineffective</td>
<td>(11)</td>
</tr>
<tr>
<td>1% 3 SDS</td>
<td>2 hr/RT</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>3% 3 SDS</td>
<td>2 hr/60 °C</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>3% 3 SDS</td>
<td>2 hr/100 °C</td>
<td>Mouse CJD</td>
<td>0</td>
<td>Ineffective</td>
<td>(7)</td>
</tr>
<tr>
<td>50% ethanol</td>
<td>Overnight/4 °C</td>
<td>Mouse Scrape</td>
<td>NA</td>
<td>Ineffective</td>
<td>(16)</td>
</tr>
<tr>
<td>0.2%–1.0% b-propiolactone</td>
<td>2 hr/37 °C</td>
<td>Mouse Scrape</td>
<td>0.6–1.9</td>
<td>Ineffective</td>
<td>(17)</td>
</tr>
<tr>
<td>Ether extraction (equal volume)</td>
<td>10 min/6 °C</td>
<td>Mouse Scrape</td>
<td>–2</td>
<td>Ineffective</td>
<td>(18)</td>
</tr>
<tr>
<td>Chloroform extraction (equal volume)</td>
<td>10 min/4–6 °C</td>
<td>Mouse Scrape</td>
<td>0.7</td>
<td>Ineffective</td>
<td>(18)</td>
</tr>
<tr>
<td>flourocarbon (Freon 113)</td>
<td>10 min/4–6 °C</td>
<td>Mouse Scrape</td>
<td>0.6</td>
<td>Ineffective</td>
<td>(18)</td>
</tr>
</tbody>
</table>

* Room temperature
generally contended that if a TSE agent ever “dries” onto a given surface, then it becomes more difficult to inactivate. Therefore, it is recommended that any given surface that is possibly infectious not be allowed to stand in a dried state until thoroughly cleaned, preferably using GMP protocols.

Selection of the TSE agent is important in the evaluation of any of these treatments. Different forms of these diseases may have varying degrees of resistance to certain inactivation treatments (10). Since inactivation experiments typically do not show a linear response to any given inactivation procedure, a heterogeneity probably exists within any given population of infectivity that could display varying degrees of resistance. This would imply that some methods would readily inactivate a certain, specific amount of infectivity resulting in a residual amount of infectivity that is unaffected by the treatment. It is important to consider that the majority of the experiments in the literature represent an idealised situation for the study of the inactivation of the pathogen. In other words, these experiments are typically performed using simple brain homogenates or the purified pathogen in a simple system. The results obtained from these types of experiments may be different if other proteins are present (e.g. those in a process solution) which may stabilise the infectious agent, thus reducing the effectiveness of the treatment. Therefore, any given claims of TSE removal/inactivation would require some form of validation/verification.

Conclusions

We have compiled many of the reagents and processes that have been tested to ascertain any reduction and/or removal of TSE infectivity. Although, most chromatography resins cannot withstand many of the treatments listed, some are certainly feasible if not completely practical. Collaborations between both resin and pharmaceutical manufacturers are required to determine the most effective as well as the most practical methods to achieve this goal.

<table>
<thead>
<tr>
<th>Method</th>
<th>Duration/Conditions</th>
<th>Agent Tested</th>
<th>Log Decrease</th>
<th>Comments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>g-irradiation</td>
<td>times varied/doses varied</td>
<td>Kuru, CJD</td>
<td>NA</td>
<td>Ineffective, assayed in primates, much variability was observed.</td>
<td>(12)</td>
</tr>
<tr>
<td>237 nm UV-irradiation</td>
<td>2 °C</td>
<td>Sheep Scrapie</td>
<td>1.8-4.1</td>
<td>Effective at the higher dose tested (2.9x10^6 ergs/mm²)</td>
<td>(13)</td>
</tr>
<tr>
<td>250 nm UV-irradiation</td>
<td>2 °C</td>
<td>Sheep Scrapie</td>
<td>0.8-1.9</td>
<td>Moderately effective at the higher dose tested (6.5x10^5 ergs/mm²)</td>
<td>(13)</td>
</tr>
<tr>
<td>254 nm UV-irradiation</td>
<td>2 °C</td>
<td>Sheep Scrapie</td>
<td>1.1-3.1</td>
<td>Moderately effective at the higher dose tested (14.3x10^5 ergs/mm²)</td>
<td>(13)</td>
</tr>
<tr>
<td>280 nm UV-irradiation</td>
<td>2 °C</td>
<td>Sheep Scrapie</td>
<td>1.1-2.3</td>
<td>Moderately effective at the higher dose tested (10^8 ergs/mm²)</td>
<td>(13)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>0.5 hr/134 °C, GD</td>
<td>Hamster Scrapie</td>
<td>5.3</td>
<td>Effective</td>
<td>(5)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>0.5 hr/121 °C, PL</td>
<td>Hamster Scrapie</td>
<td>3.8</td>
<td>Moderately effective</td>
<td>(19)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>18 min or 30 min/134 °C, PL</td>
<td>Hamster Scrapie</td>
<td>7</td>
<td>Effective</td>
<td>(6)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>18 min/134-138 °C, PL</td>
<td>BSE</td>
<td>2.5</td>
<td>Moderately effective, however low starting titer of spiking material (10^4 ID₅₀/g), assayed in rodents</td>
<td>(6)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>1 hr/121 °C, GD</td>
<td>Hamster Scrapie</td>
<td>5.4*</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>1.5 hr/121 °C, GD</td>
<td>Hamster Scrapie</td>
<td>5.6*</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>1 hr/135 °C, GD</td>
<td>Hamster Scrapie</td>
<td>6.6*</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>autoclaving</td>
<td>1.5 hr/135 °C, GD</td>
<td>Hamster Scrapie</td>
<td>≥7.4</td>
<td>Effective</td>
<td>(8)</td>
</tr>
<tr>
<td>heat</td>
<td>10-60 min/70 °C</td>
<td>Sheep Scrapie</td>
<td>&lt;1</td>
<td>Ineffective, samples were heated as a homogenate in a water bath.</td>
<td>(9)</td>
</tr>
<tr>
<td>heat</td>
<td>10-60 min/85 °C</td>
<td>Sheep Scrapie</td>
<td>&lt;1</td>
<td>Ineffective, samples were heated as a homogenate in a water bath.</td>
<td>(9)</td>
</tr>
<tr>
<td>heat</td>
<td>10-60 min/100 °C</td>
<td>Sheep Scrapie</td>
<td>1.6-2.8</td>
<td>Moderately effective, samples were heated as a homogenate in a water bath.</td>
<td>(9)</td>
</tr>
<tr>
<td>heat</td>
<td>10 min/124 °C</td>
<td>Sheep Scrapie</td>
<td>4.3</td>
<td>Effective, samples were heated as a homogenate in a water bath.</td>
<td>(9)</td>
</tr>
<tr>
<td>dry heat</td>
<td>10.60 min/160 °C</td>
<td>Hamster Scrapie</td>
<td>6, 7</td>
<td>Effective, however, there was ≥3.6, ≥2.2 log₁₀ remaining in the treated samples respectively.</td>
<td>(5)</td>
</tr>
<tr>
<td>dry heat</td>
<td>60 min/360 °C</td>
<td>Hamster Scrapie</td>
<td>8</td>
<td>Effective</td>
<td>(5)</td>
</tr>
</tbody>
</table>
References


Conference on affinity chromatography
San Diego, California USA
April 16-17, 1998

The general theme of this meeting was the rethinking of affinity chromatography as a capture step. The advantages of single-step purification and concentration versus the seeming disadvantages of ligand availability, leakage and cost were discussed in depth.

As a starter for the conference, a workshop on the current state of affinity chromatography looked at the possibilities of combinatorial chemistry for production of small synthetic ligands aimed at overcoming the availability, regulatory and economic barriers to affinity chromatography used as a first step.

The main part of the conference could generally be divided into two parts: the development of new affinity chromatography media and the purification of monoclonal antibodies using Protein A.

J. Maclennan and T. Ransohoff (Dyax) went over the basics of phage display and combinatorial chemistry for generating highly specific ligands. Opportunities for these technologies lie in, not only affinity chromatography, but also drug discovery and gene therapy.

L. Lagerlund (Amersham Pharmacia Biotech) spoke on the importance of having not only an appropriate base matrix but the correct chemistry of attachment (ligand coupling, spacer arms, ligand density and orientation) for coupling these highly specific ligands generated by these new technologies.

A number of case studies were presented looking at the process development considerations for affinity steps used at large scale. Several speakers, including R. Bigelow (Genzyme) showed their work on fluidized affinity resins for both recovery and initial purification.

P.-Å. Nygren, (Royal Institute of Technology, Stockholm) showed their work on “Affibodies.” These are ligands made from one of the IgG binding domains of Protein A which can be re-engineered through phage display methods to be specific for any target molecule chosen. He showed work done on making affinity ligands for Taq polymerase and apolipoprotein-A from the same starting point.

Presentations on monoclonal antibody purification using Protein A mainly concerned the productivity, and the ability to use high flow rates to obtain high productivity using Protein A for the purification of monoclonal antibodies.

G. Blank (Genentech) gave a clear and structured presentation of “Scale Up Strategies For Protein A and Monoclonal Antibody Production”, while S. Sommers (Cellex Biosciences) presented results from a comparison study in which the binding of murine IgG1 to 7 different IgG affinity resins was evaluated. The highest binding capacity was found with rProtein A Sepharose Fast Flow and also the lowest resin cost per mg IgG.

The conference, though small, was focused and well attended. About 70 attendees were present at the conference and 40 at the workshop.
Amersham Pharmacia Biotech chosen to supply equipment for recombinant human serum albumin production

Amersham Pharmacia Biotech will supply its STREAMLINE technology to Yoshitomi, as well as downstream equipment and chromatography media. According to Amersham Pharmacia Biotech, the venture will likely result in the largest biopharmaceutical approved using Amersham Pharmacia Biotech’s STREAMLINE technology in the production process. STREAMLINE expanded bed technology was chosen for the initial capture step because it is more efficient, resulting in faster processing times and higher yields when compared to conventional technology.

“We are pleased to be the leading supplier of downstream technology for this exciting production” said Ingvar Wiberger, vice president business unit separations, Amersham Pharmacia Biotech.

The facility, which will be built in Chitose, Hokkaido, Japan, will include the world’s largest STREAMLINE (3x 1,000 mm id) and CHROMAFLOW (1,600 mm id) columns to date. Construction will be handled by Niigata Engineering Corp. Ltd., one of Japan’s foremost pharmaceutical industry engineering companies.

Upon completion of the first stage, production capacity will be 12.5 metric tons of pure rHSA per year. Company officials expect commercial production of rHSA, which will be marketed as “Albrec”, to begin in 2000. Plans for the 2nd and 3rd stages are already on the drawing board which will take production capacity initially up to 18 metric tons and finally up to around 40 metric tons per annum in the new millennium.

Kazuo Takeuchi, director of the industrial division of Amersham Pharmacia Biotech K.K. Tokyo, said, “the choice to use STREAMLINE over conventional purification technology will help GCC to maintain their leadership position in the development of recombinant human serum albumin.

Uppsala, Sweden (March 23, 1998) – Amersham Pharmacia Biotech and Yoshitomi (formerly Green Cross Corporation) today announced that their Bioprocess Group has been selected to provide equipment for the world’s first commercial production facility of recombinant human serum albumin (rHSA). Due to the risk of cross infection by HIV, or yet unknown viruses, and the associated screening costs there is enormous pressure worldwide to develop alternatives to using donor blood to obtain pure human serum albumin (HSA).

Clinical uses of HSA include blood volume replacement in cases of shock, burns and during surgery. Worldwide, HSA is the most widely used protein for clinical applications.
The filing of the NDA triggers a significant milestone payment from CIBA Vision to Isis. Under the exclusive worldwide distribution agreement established between Isis and CIBA Vision in July 1997, Isis will receive $20 million in precommercial fees and milestones.

Results from the Phase III trials of fomivirsen demonstrated that in both newly-diagnosed patients with CMV retinitis and in patients with advanced refractory disease, fomivirsen produced prolonged delay in disease progression.

“The filing of the world’s first NDA for an antisense drug is a seminal event for Isis, our stockholders and our partner, CIBA Vision,” said Stanley T. Crooke, M.D., Ph.D., Chairman and Chief Executive Officer, Isis Pharmaceuticals. “We are enthusiastic about our first commercial opportunity and look forward to working with CIBA Vision to ensure a successful launch of fomivirsen in the near-term.”

“We are very pleased with the Phase III safety and efficacy results demonstrated by fomivirsen and believe that the drug, assuming a favorable FDA review, will be an important addition to the treatment of CMV retinitis for patients in need of safe and effective therapeutic options,” said Daniel L. Kisner, M.D., President and Chief Operating Officer, Isis Pharmaceuticals.

“With this filing, we move one step closer to bringing this new, potentially more effective, treatment option to AIDS patients with CMV retinitis,” added Luzi von Bidder, President, Ophthalmics Business Unit, CIBA Vision.
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1998 Courses at Fast Trak Center USA

Course dates
DEV 1: September 16–18
COL 1: September 21–23

1998 Courses at Fast Trak Center Europe

Course dates
DEV 1: November 17–19
DEV 2: November 30–December 4
DEV 4: October 6–8
VAL 1: September 30–October 1
COL 1: November 10–12

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Standard courses:

VAL 1
Validation Issues in Chromatographic Downstream Processing. One and a half day seminar addressing key issues in validation of chromatographic units.
Course code number: 44-8000-08

DEV 1
Process Development: Basic Practical Course on Chromatographic Methods. Three day hands-on course which gives an overview of chromatographic techniques.
Course code number: 44-8000-01

DEV 2
Process Development: Practical Course on Chromatographic Methods. Four day hands-on course in the design and optimisation of a chromatographic process.
Course code number: 44-8000-02

DEV 4
Process Development: Practical Course on Optimisation and Scaling up to Full-Scale Production. Three day hands-on course.
Course code number: 44-8000-14

COL 1
Packing, Testing and Maintenance of Production Scale Columns and Standard Operation Procedures. Three day hands-on course.
Course code number: 44-8000-06

Customised courses:

We can also arrange customised training courses to deal with specific practical and theoretical topics associated with chromatography and downstream processing. Customised courses can be given at an Amersham Pharmacia Biotech training center or at your facilities, depending on the equipment involved. Our aim is to make information and expertise available in the most efficient way for your company.

English is the course language for most of our standard courses but we can offer customised courses in French, German, and Swedish.
This meeting, jointly sponsored by the European Commission and the FDA was well attended, with nearly 250 participants. The main objectives of the meeting were to discuss key issues related to the scientific development and design of vectors, their clinical-grade production, their use in clinical trial, the review process and associated regulations.

Olivier Danos’s (Généthon II & EWGT,CNRS) opening lecture provided the audience with a concise overview of the challenges in the development of new vectors for gene therapy. Vectors are experimental agents barely acceptable as drugs and their efficiency is too low. There is a trend towards in-vivo gene transfer which creates more problems since the regulatory requirements are more stringent. Tools are not available to evaluate all the safety risks.

Jörn Keck (European Commission) argued that the gene therapy industry in Europe is about 5 years behind that in the US, and of 300 clinical trials, two thirds are in the US. He suggested that one reason for this is that the financial climate in the US is more beneficial to start-up biotech firms. The regulatory situation in Europe and the US was explored by various speakers. Harmonisation within Europe still seems to be some way off.

The subject of intellectual property issues was introduced by lawyer Alex Houtart who distributed a useful checklist to follow from before the R&D stage to commercialisation. The patentability of inventions relating to gene therapy was presented in greater depth by S. Hoekstra from the European Patent Office.

One day was devoted to presentation of basic research on retroviral, adenoviral and other vectors and control of gene expression.

A solution to the problem of Replication-Competent Adenovirus contamination in the large scale production of recombinant adenoviral vectors was presented by Dinko Valerio (Introgene BV). J. Crouzet (Rhône-Poulenc Rorer Gencell) described the construction of DNA “minicircles” for improved transfection efficiency in non-viral gene transfer.

Several speakers described commercial or state-funded facilities for GMP production of vectors for gene therapy. The NIH provision of free vector to academics in the US was discussed by delegates: there are three NIH centres in Michigan, Indiana and Pennsylvania and 35 protocols have been approved for free vector provision since 1995. There were calls for a similar system to be set up in Europe to give gene therapy a much-needed boost in the academic sector. The last session of the meeting dealt with the validation and testing of vectors with subjects ranging from process validation, cell line and vector safety testing to removal of residual DNA in final product and aspects of biosafety in the clinic.

There was also a lively poster session covering a wide span of interests such as vector development, vector production, cationic lipid delivery, plasmid DNA analysis, vector biodistribution analysis and cancer therapy. In addition there were several posters describing methods for preparative purification of plasmid DNA in expanded and packed beds (Therexys Ltd., Instituto Superior Técnico, Portugal/MIT., MAGENTA Corporation, Amersham Pharmacia Biotech -see article on page 12).