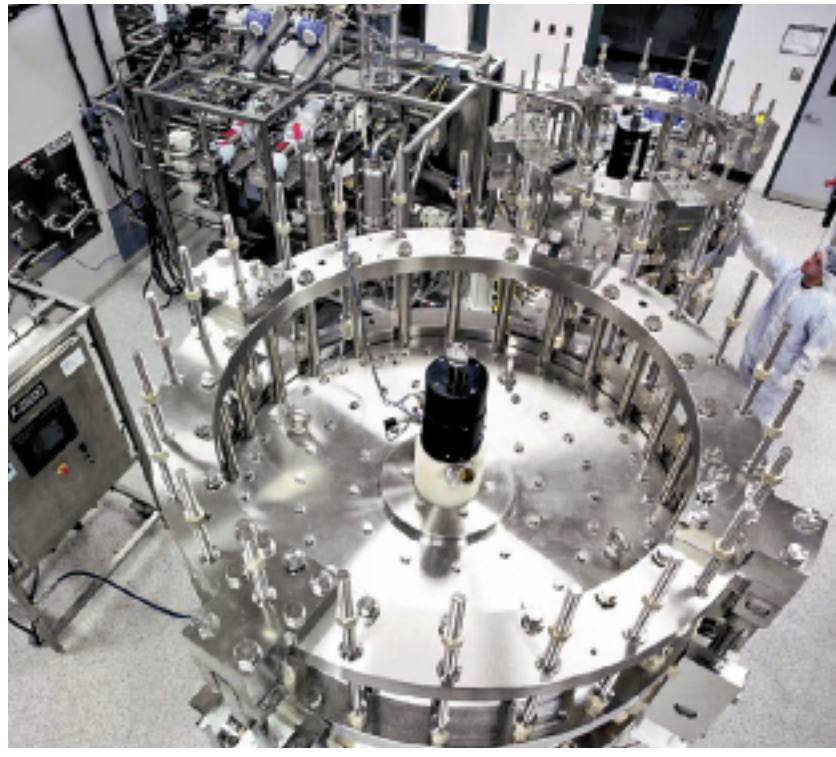


Courtesy of Lonza Biologics, plc

Process Chromatography: Five Decades of Innovation

Over the years, chromatography has become the central enabling technology in all biopharmaceutical downstream processing.

JOHN CURLING

ABSTRACT

This article explores the development of process chromatography. Process chromatography was first applied to the removal of low molecular weight solutes from whey by gel filtration about 50 years ago. An analytical method using size exclusion chromatography was scaled up for insulin production in the 1970s, when ion exchange became a viable technology for the same application. Ion exchange was adopted as the industry workhorse as robust resins became available and formed the backbone of chromatographic processing of blood plasma fractionation in alternatives to and extensions of ethanol precipitation. Cost restrictions kept affinity chromatography in the laboratory until the production of MAbs made efficient immunoaffinity indispensable in high purity coagulation factor production in the 1980s. Since then, spurred on by the advent of biotechnology, an extensive toolbox of chromatographic methods has been developed, and a process chromatographic capture–purify–polish regime is ubiquitous. Affinity capture of antibodies on Protein A adsorbents is used throughout the industry with widespread discussion of affinity versus ion exchange. The emerging debate pitches chromatography against membrane separations. Column technology has advanced, but not to the "plug-and-play" status of membrane technologies. Axial flow systems still dominate, but advances in engineering may make radial flow accessible and technologies such as expanded beds more attractive. Process chromatography stands at the threshold of industrialization.

JOHN CURLING is president of John Curling Consulting AB, Uppsala, Sweden, and is senior advisor to ProMetic BioTherapeutics, Inc., Gaithersburg, MD, USA, +46.18 290620, john@consultcurling.se. He is also a member of *BioPharm International's* Editorial Advisory Board.

Professor Arne Tiselius, who had earlier described adsorption and displacement chromatography[1] and later the use of hydroxyapatite, summed up the importance of partition chromatography in his presentation speech for the Nobel Prize in Chemistry (1952), awarded to Martin and Synge:

> "This tool has enabled research workers in chemistry, biology, and medicine to tackle and solve problems which earlier were considered almost hopelessly complicated."[2]

It is perhaps the inherent simplicity of the method which has made chromatography not just an analytical tool *par excellence* but the central enabling technology in all biopharmaceutical downstream processing.

The work of these mid-century laureates has its roots in the investigations of Mikhail Tswett, who, although he described the principles of his separation techniques applied to plant pigments in 1903, first used the term *chromatography* in 1906:

> "....the different components of a pigment mixture, obeying a law, are resolved on the calcium carbonate column and then can be qualitatively and quantitatively determined. I call such a preparation a *chromatogram* and the corresponding method *the chromatographic method.*"[3]

The early history and invention of chromatography are summarized by Ettre in two articles in *LCGC North America*. [3–4]

The 1960s: Matrices, Molecules, and Engineering

Following Tswett's experimentation with various adsorbents and mobile phases, researchers in the 1950s investigated protein chromatography on new matrices. Low porosity, hydrophobic styrene-divinyl benzene resins were readily available, but for protein separations, porous and hydrophilic supports were needed. The introduction of cellulose ion exchangers by Peterson and Sobers in 1956,[5] cross-linked dextrans (Sephadex) by Porath and Flodin in 1959,[6] and polyacrylamide (1961) and agarose (1964) by Hjertén,[7–8] initiated a revolution in protein chromatography. The first supports, generally referred to as "gels," were largely unsuitable for use in process chromatography: one gram of dry Sephadex G-100 adsorbs 100 mL of water and has therefore only 1% dry substance and 6% agarose media and 94% water.

Low-pressure process chromatography could not have developed without immense efforts to resolve scale-up issues in both column design and matrix stability.

Ligands and Matrices

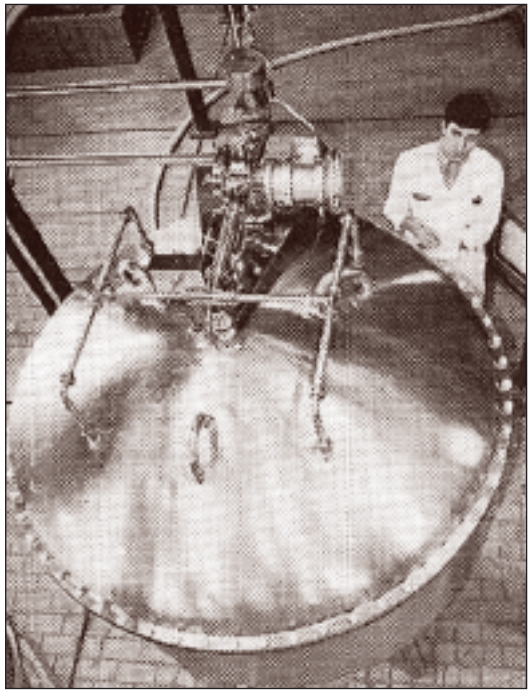
The commercial availability of a range of carbohydrate-based supports enabled the expansion of chromatographic techniques. At this point, the science largely bifurcated into ligand discovery and matrix improvement. Axén's[9] introduction of cyanogen bromide activation in 1967 allowed the development of affinity chromatography, the invention of which was attributed to Cuatrecasas et al. (1968).[10] Interactions between Protein A and immunoglobulins were under investigation by Sjöqvist's[11] group at Uppsala University in Sweden in the mid-1960s but IgG purification using Protein A adsorbents generally is ascribed to the Lund researchers Hjelm et al.[12] and Kronvall et al.[13] Uppsala, however, is intrinsically linked to bioseparations from the time of The Svedberg (Nobel Prize, 1926), through the activities of the Institute of Biochemistry (The Biomedical Centre) and the research and product development at Pharmacia Fine Chemicals, now part of GE Healthcare.

The drawbacks of hydrophobic Amberlite IRC-50[14] and Dowex resins for protein separations gave rise to the search in the 1950s for matrices that did not interfere with the separation on derivatized gels. In 1947, Boscott[15] had described the use of solvent-treated cellulose acetate as a "satisfactory stationary organic phase for chromatography," which Howard and Martin termed "reversed-phase partition chromatography" (RPC).[16] RPC has a continuous polar stationary phase and requires organic solvents, whereas hydrophobic interaction chromatography (HIC) has polar ligands substituted onto a neutral backbone and is run with an aqueous mobile phase. Although these related technologies of RPC and HIC were born in the 1950s, they did not come to commercial use until considerably later.

Neutral resins for HIC were developed as a result of the work of Porath[17] and Hjertén,[18] who also introduced the accepted name of the technique, and products became available as late as 1977. Also, in 1972–73, hydrocarbon-coated Sepharose derivatives were developed at the Weizman Institute.[19] Reverse-phase separations took a development path through high-performance liquid chromatography (HPLC), driven by Horvath's work starting in 1966 and his invention of the HPLC instrument.[20] Largely due to the work of Kirkland[21] at Dupont, bonded-phase silica became the matrix of choice for RP–HPLC; new stationary phases were developed for biomedical applications in the 1980s. HPLC is now one of the most accepted techniques.

Hancock notes that as an analytical method,"RP–HPLC played a key

Figure 1. A 2,500-L "Sephamatic Gel Filter" packed with Sephadex G-25 used for the production of a desalted whey protein concentrate (free from lactose and salts) in 1968.



Courtesy of GE Healthcare

role at Genentech in the development of rhHGH as a pharmaceutical,"[22] and RP–HPLC has continued to play that key role in product development and control in biopharmaceutical laboratories around the world. For protein separation at an industrial scale, however, HPLC is more limited in its applicability because of the need for organic solvents and because of the pressure demands in an industry that otherwise operates below 3 bar. The notable exception is Eli Lilly's use of the technology for purifying biosynthetic human insulin (as it was called at the time).[23]

Scale-Up

Low-pressure process chromatography could not have developed without immense efforts to resolve scale-up issues in both column design and matrix stability. Early work in scale-up was thus restricted to the use of rigid gels such as Sephadex G-25 in stainless steel columns or "Gel Filters," which were developed and introduced in 1968 by Pharmacia Fine Chemicals (Figure 1). Efforts were being made to overcome the pressure-flow restrictions of soft gels, and work by Janson[24] led to the commercialization of the "Stack" or sectional column. The column dimensions were 16 cm bed height by 37 cm diameter, only because this was the largest polypropylene mold size that could be made at the time. These early columns had fixed bed heights and the gel filters could be pump packed, predating today's packing methods by several decades.

Improving Media

Since the late 1960s, manufacturers of chromatographic resins have developed increasingly robust media for process scale chromatography. They continue to search for improvements in stationary phases to keep pace with the increasing demands of the biotechnology industry for improved product throughput. The development battleground was and still is overcoming mass transfer limitations due to diffusion, in turn limited by residence time, bead porosity, bead size, and matrix morphology in the case of continuous stationary phases.

The Age of Purification

According to PubMed, over 10,000 articles with *purification* in the title were published in the decade between 1970 and 1980. This was the age of purification, enabled by an ever expanding toolbox. A plethora of adsorbent alternatives for chromatography was developed, mostly based on dextran and agarose, but also using cellulose, polyacrylamide, and methacrylates.[25] In a 1990 review, Low[26] discussed the methodologies available for scale-up, comparing their characteristics; these generally hold true today. Industry pressure on high-volume biopharmaceutical manufacturing is causing vendors to look for significant throughput and safety improvements.

It is significant that the number of biopharmaceutical products (biologics) was small before 1982, when recombinant human insulin was approved. Products were generally purified from natural sources: human and animal blood, urine, pancreas, lung, etc. Other than antibiotics, vaccines were essentially the only products of microbiology, which was in its infancy as a source of product because only endogenously expressed proteins could be isolated. Biologics companies (and the regulatory agencies) were generally focused on vaccines for protection against childhood infectious diseases and polio.[28] Blood plasma products had been developed and introduced in response to a wartime need in early 1940s. Although there was a renaissance of "industrial" methods and a search for simplicity in processing while maintaining safety, none of these biotherapeutics were purified using the technologies commonly applied to biopharmaceutical production today. Early biologics differ significantly from their microbiologically expressed successors as they were generally present at low concentration among many other proteins from the same source. Thus purification problems were different from those of today, except where, for example, blood plasma was used as source material from which multiple products are obtained.

Marketed biologics in use before 1982 are shown in Table 2. Most, if not all, of these products have been withdrawn, substituted by other products, or have been changed dramati-

cally (and newly licensed). The states of purity and the formulations in which they were first made available, not to mention the sources from which they are derived and the methods by which they are manufactured, are significantly different today. For example, none of the products before 1960 were subject to purification schedules using process chromatography. However, some products were made by local institutions or blood banks, which may have used rudimentary purification on cellulose ion exchangers.

Table 1. Characteristics of chromatographic methods of protein purification. Adapted from Low[26] and Jungbauer.[27]

Method characteristics					
Protein property	**Separation method**	**Action principle**	**Resolution**	**Speed**	**Capacity**
Molecular size and shape	Gel filtration	Size exclusion	Moderate in fractionation	Low for fractionation, high for desalting	Limited by sample volume
Surface charge	Ion exchange	Ionic binding	Usually high	High	Very high. Sample volume not limiting
Hydrophobicity and hydrophobic patches	HIC	Hydrophobic complex formation	Good	Fast	High
	RPC		Very high	Very fast	High
Molecular structure	Affinity chromatography	Biospecific adsorption/desorption	Excellent	Very fast	Very high

The introduction of chromatography in the early 1960s—mainly ion exchange and gel filtration—provided new opportunities for purification.

The introduction of chromatography in the early 1960s—mainly ion exchange and gel filtration—provided new opportunities for purification, but the sources remained largely animal and human tissues (including blood) until the 1970s. During this period, the focus in biochemistry was on purification as an enabling technology to improve the accuracy of structure and function studies. Chromatography scale-up often was performed by a simple increase of column volume, with little regard to the maintenance of column aspect ratios or residence time, and often restricted by the physical characteristics of the gels.

The use of zinc-initiated crystallization had dramatically improved insulin purity by the 1960s. Research into the causes of antibody generation in response to insulin and allergenic reactions led both Eli Lilly & Co. and Novo Nordisk (Novo and Nordisk were two separate companies at the time) to investigate new methods of purification: proinsulin, glucagon, somatostatin, and modified forms of insulin such as desamido insulin were identified as the root cause of immunogenicity of bovine- and porcine-derived products.[30] Enabled by the introduction of columns for large-scale chromatography using "soft" gels and scale-up of insulin purification on Sephadex G-50, Eli Lilly introduced "single peak insulin."[31] This was termed so because it gave a single peak in analytical gel filtration. Novo introduced a "monocomponent" or "MC" insulin in 1973[32] purified by ion exchange chromatography, which gave a single band in electrophoresis.

Throughout this period, Pharmacia Fine Chemicals dominated the chromatographic separations industry, launching Sepharose in 1966, Protein A Sepharose in 1975, HIC products in 1977, and IMAC in 1979. IBF (Industrie Biologique Française), a Rhône-Poulenc company (now BioSepra, part of Pall Corp.), was also active, as were Whatman and Bio-Rad Laboratories. Tosoh (Toyo Soda), in alliance with Rohm & Haas, focused on methacrylate supports and became known for its products for HIC and size exclusion.

Biologics research had a significant base in academia rather than the pharmaceutical industry.[33] Some products were in the domain of government defense laboratories, partly for reasons of national security and because specialized microbiological competence was located in such institutions. At this time, the focus of the pharmaceutical industry was on the development of new chemical entities (NCEs), but that changed significantly with the molecular biology revolution of the 1980s.

The Age of Downstream Processing

The Asilomar conference of 1975 has been called the "Woodstock of molecular biology"[34] and has served as a questioning reminder of the power of recombinant DNA technology. The conference triggered the first guidelines for research on rDNA[35] and marked the beginning of biopharmaceutical regulation in the United States and elsewhere. From 1980 to 1994, 29 new biologic

entities (NBEs), including 10 new recombinant entities, were approved, with an average time of 61 months from investigational new drug (IND) to licensure, 38.9 months shorter than for NCEs during the same 15 year period.[36] This was the era of molecular biology. Transiently, chromatography became a tool to expedite analysis and product purification of what could be termed "new age" biologics. However, a new discipline of downstream processing was minted, and now biopharmaceutical manufacturing divided into upstream (bacterial and yeast fermentation or mammalian cell culture) and downstream.

In contrast to the early years, new biopharmaceutical approvals currently run at about 40 per year in the US. The Biotechnology Industry Organization cites 254 drugs approved for 385 indications from 1982 to 2005.[37] In 2006, CDER approved only four new biological products and CBER approved nine new biological products. The number of products entering clinical trials also has tapered off since 1980. Clinical and approval phase lengths vary widely, with a trend to longer clinical phases. In 2003, the Tufts Center for the Study of Drug Development conservatively projected that more than 30 new biotherapeutics would be successful in the next six to seven years.[38]

In the European Union, 88 recombinant products and MAbs have been approved by 2002, representing 36% of all new approvals since 1995 under the centralized European drug approval system.[39] The success rate for biologics is significantly higher than for small-molecule NCEs,[36] partly because of the way they are developed. A key area of focus for the safety of small molecules is their side effects, whereas the concern for biologics is immunogenicity.[40]

Biopharmaceutical products are subject to downstream processes that are built on process chromatography as the main purification agent and with membrane technologies providing clean feed streams, buffer exchange, product concentration, virus removal, and sterile filtration. As Jungbauer notes: "Bioseparation processes are dominated by chromatographic steps. Even primary recovery is sometimes accomplished by chromatographic separation, using a fluidized bed instead of a fixed bed."[27] The expansion of chromatography as the prime tool of downstream processing is manifest in the increase of bioprocess revenues at GE Healthcare's Life Science Division from approximately $36 million in 1986[41] to $461 million in 2006.[42]

The standardization of feed stocks allowed a more systematic approach to process development and the introduction of the now ubiquitous capture–purify–polish paradigm .

Modernization of Process Chromatography

With the development of bacterial fermentation and mammalian cell culture as the sources for new recombinant products came a standardization of raw feed stocks with manufacturers sharing the same types of problems. The reduction of endotoxin levels from *E. coli* fermentation or the reduction of host cell proteins and DNA from CHO cell culture products are prime examples. This standardization allowed a more systematic approach to process development and is the underlying reason for the introduction of the capture–purify–polish paradigm, now ubiquitous in downstream process design. Industry developed a new, systematic approach integrating process design, engineering and control, process economics, hygiene, and regulatory issues, summarized by Sofer and Nyström[43] in 1989 and followed by a text on validation[44] in 1991. Bioprocessing systems were introduced and computerized control took over from technologies that were previously dominated by manual operation and therefore subject to operator error.

Focus on Viral Clearance

The transmission to hemophiliacs of

Courtesy of GE Healthcare, originally from CSL Ltd, Melbourne, Australia.

Figure 2. The "Stack" column used for insulin purification in the 1970s. Single sections of this column became standard in the pilot scale use of ion exchangers for, for example, plasma protein purification. The 16-cm bed height was a driving force in the move to short bed columns and the scale up to a 30 cm x 150 cm bed column as standard in the 1970s.

HIV by human plasma-derived Factor VIII renewed the focus on viral clearance and methods of virus kill in the plasma fractionation industry. In the recombinant industry, cell cultures need to be protected from adventitious viral contamination by viruses such as virus of mouse (MVM), epizootic hemorrhagic disease virus (EHDV), and reovirus,[45] which may influence expression of the product by the host machinery. This need led to efforts to eliminate animal-derived raw materials from the process chain and thus improved safety.

Robustness, tolerance to alkaline cleaning agents, validated viral clearance, and long-term performance over many cycles became a focal point of adsorbents for process chromatography.[46] However, the 1990s were perhaps an age of process engineering with little attention paid to improving separation media, with the exception of the introduction of expanded bed adsorption chromatography.[47] This technology, which integrates unit operations of solid–liquid separation, clarification, and recovery of the target protein by adsorption, has met with limited success in the biopharmaceutical industry but has found large-scale application in the dairy industry.[48] Now the industry has moved to the development of platform technologies, which can be applied to monoclonal antibody (MAb) products,[49–50] but case-by-case development still remains a challenge for manufacturers with diverse product types.

Table 2. Biologics in the pre-recombinant DNA era. Adapted from tables compiled by Builder et al.[29] * Note: most live attenuated vaccines in use today are derived from serial passage in cultured cells, including human diploid cells (e.g., fetal lung tissue, other fibroblasts), monkey kidney cells, and chick embryos, among others. DPT = Diphtheria, pertussis, tetanus; MMR = Measles, mumps, rubella.

Year introduced	Product	Source	Use
1913	Antitoxins and antivenins	Equine blood	Against specific toxins (Passive immunization)
1939	Pepsin	Animal pancreas	Digestive aid
1939	Insulin	Bovine/porcine pancreas	Diabetes
1941	Albumin	Human blood plasma	Shock, burns
1943	Immunoglobulin	Human blood plasma	Immune insufficiency
1944	Thrombin	Bovine blood plasma	Topical bleeding control
	Plasmin	Bovine blood plasma	Wound debridement
1951	Trypsin (and chymotrypsin)	Pancreas	Cataract surgery
1957–1958	Factor VIII as concentrate	Human blood plasma	Hemophilia A
1957–1963	Whole viral vaccines	DPT, MMR, polio, rabies, yellow fever, etc.*	Confer specific immunity
1958	Hyaluronidase	Bovine testicle	Facilitation of subcutaneous administration of drugs
1958	Penicillinase	Bacteria	Anaphylaxis after penicillin
1959	Fibrinolysin	Human blood plasma	Wound debridement
1959	Trypsin inhibitor (Aprotinin)	Bovine lung	Pancreatitits, etc.
1959	Factor IX as concentrate	Human blood plasma	Hemophilia B
1960	Lipase/amylase	Porcine pancreas	Pancreatitis etc.
1960	Glucagon	Pancreas	Hypoglycemia
1962	Papain	Papaya latex	Reduction of soft tissue inflammation
1963	Bromelain	Pineapple	Reduction of soft tissue inflammation
1963	Amylase	Bacteria	Digestive aid
1968	Rh immunoglobulin	Human blood plasma	Prevention of Rh immunization
1968	DNAse	Bovine pancreas	Wound debridement
1969	Bacillus proteases	Bacteria	Wound debridement
1971	FSH/LH (Hormones)	Postmenopausal urine	Induction of ovulation, etc.
1971	Chorionic gonadotropin	Pregnancy urine	Cryptoorchidism. Stimulation of spermatogenesis
~1974	Collagen	Bovine tissue	Control of bleeding, cosmetic surgery
1976	Human growth hormone	Human cadaver pituitaries	Hypopituitary dwarfism
1978	Asparaginase	Bacteria	Cancer therapy
1979	Urokinase	Mammalian cells	Blood clot lysis
1980	Streptokinase	Bacteria	Blood clot lysis

Figure 3. Process chromatography in 2006

Courtesy of Genentech, Inc.

Innovation and the Search for Improvement: 2000 and Beyond

Since the new millennium, the purification of MAbs—with their improved expression levels—has dominated the development of process chromatography. Process affinity chromatography using Protein A adsorbents has received much attention with the introduction of new products manufactured without using animal-derived raw materials, improved robustness and resistance to alkaline cleaning, and binding capacity in the 20–30g/L range with short residence times and at flow rates between 100 and 500 cm/hr.

However, driven by increasing product titers[51] the biopharmaceutical bottleneck has moved to downstream processing[52] and will require even more innovation and improvement. Sofer and Chirica project the development of high flow ion exchangers running at over 700 cm/hr in 20 cm columns and capacities of ~100 g/L at residence times of 2–6 minutes to cope with 40-kg bioreactor batches and a product output of 1,000 kgs/year.[53] Discussing the future of antibody purification, Low et al. conclude that the "true bottleneck in recovery processes is the first adsorptive column."[54]

Beyond Chromatography?

Although current unit operations are likely to remain as is in the near future, another school of thought projects that future processes will be based entirely on membrane chromatography instead of fixed bed operations.[55]

It has been noted that "Process chromatography has the notoriety of being the single largest cost center in downstream processing" and Przybycien et al. have asked, "Is there life beyond packed bed chromatography?"[56] Of the current alternatives, few technologies are likely to have a major impact on downstream processes, and process chromatography will remain the workhorse of the industry. However, protein precipitation with concomitant virus kill[57] is a likely complement or alternative in protein purification, and plasmid DNA may be produced most efficiently by differential precipitation.[58] Perfusion chromatography[59] perhaps never lived up to its expectations, but the struggle to overcome mass transfer limitations in chromatography is currently being addressed by monoliths,[60] which still suffer from low capacities for bulk protein purification. Membrane chromatography[61] is becoming a more widespread technique for removing impurities. Displacement chromatography[62] using low molecular weight displacers is of increasing interest now that commercial displacers are available. Because of its selectivity, affinity chromatography still shows promise for the future, particularly when alkaline-resistant, synthetic ligands are used instead of protein or peptide ligands.[63]

Various engineering solutions have been attempted over the years to address various challenges of unit operations. Examples include continuous (annular) chromatography and fluidized, rather than expanded beds, and integrating process steps into continuous processes. With improved engineering, radial flow columns provide an interesting opportunity to maximize adsorbent performance and reduce dilution during elution.[64]

A vision for the future of process chromatography is expressed by Jan-Christer Janson:

> "In a world where not only the pure technical problems are important to the biochemical engineer but where regulatory constraints have become more and more an issue, a relevant vision for the future would be that systems—columns and media integrated—will be available that allow continuous scaling up for production and scale down for various process validation reasons, such as virus clearance studies and trouble shooting".[65] ★

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References

1. Tiselius A. Nobel Lecture, 1948; http://nobelprize.org, accessed 2006, Nov.
2. Tiselius A. Presentation Speech,

1952; http://nobelprize.org, accessed 2006, Nov.
3. Ettre LS, MS Tswett and the invention of chromatography. LCGC North America. 2003;(5)21:5.
4. Ettre LS. The centenary of chromatography. LCGC North America. 2006;(7)1.
5. Peterson EA, Sober HA. Chromatography of proteins. I Cellulose ion-exchange adsorbents. J Am Chem Soc. 1954;76:751–755.
6. Porath J, Flodin P. Gel filtration: A method for desalting and group separation, Nature. 1959;183:1657.
7. Hjertén S. Agarose as an anticonvection agent in zone electrophoresis. Biochim Biophys Acta. 1961;53(3):514–517.
8. Hjertén S. The preparation of agarose spheres for the chromatography of molecules and particles. Biochim Biophys Acta. 1964;79:393–398.
9. Axén R, Porath J, Ernback S. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. Nature. 1967;214:1302–4.
10. Cuatrecasas P, Wilchek M, Anfinsen CB. Selective enzyme purification by affinity chromatography. Proc Natl Acad Sci USA. 1968;61(2):636–43.
11. Forsgren A, Sjöquist J. Protein A from S aureus I Pseudo-immune reaction with human gamma-globulin. J Immunol. 1966;97(6):822–7.
12. Hjelm H, Hjelm K, Sjoquist J. Protein A from Staphylococcus aureus. Its isolation by affinity chromatography and its use as an immunosorbent for isolation of immunoglobulins. FEBS Lett. 1972;28(1):73–6.
13. Kronvall, G. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin. G J Immunol,.1973; 111(5):1401–6.
14. Paléus S, Neilands JB. Preparation of cytochrome c with the aid of ion-exchange resin. Acta Chem Scand. 1950;4:1024–1030.
15. Boscott RJ. Solvent-treated cellulose acetate as the stationary phase in partition chromatography. Nature. 1947;159;342.
16. Howard GA, Martin AJP. Separation of the C12-C18 fatty acids by reversed phase partition chromatography. Biochem J, 1950; 46;532–538.
17. Porath J, Sundberg L, Fornstedt N, Olsson I. Salting-out in amphiphilic gels as a new approach to hydrophobia adsorption. Nature. 1973; 245:465–466.
18. Hjertén S. Some general aspects of hydrophobic interaction chromatography. J Chrom A. 1973; 87:2:325–331.
19. Er-El Z, Zaidenzaig Y, Shaltiel S. Hydrocarbon-coated sepharoses—use in the purification of glycogen phosphorylase. Biochem Biophys Res Comm. 1972; 49:383–390.
20. Ettre LS. Csaba Horvath and the development of the first modern high performance liquid chromatograph. LCGC Magazine North America. 2005; May 1.
21. Kirkland JJ. Development of some stationary phases for reversed-phase high performance liquid chromatography. J Chrom A. 2004; 1060:9–21.
22. Hancock WS. High performance liquid chromatography in biotechnology. New York: J Wiley & Sons; 1990, p. 2.
23. Kroeff EP, Owens RA, Campbell EL, Johnson RD, Marks HI. Production scale purification of biosynthetic human insulin by reversed-phase high performance liquid chromatography. J Chrom A. 1989;461:45–61.
24. Janson JC. Columns for large-scale gel filtration on porous gels. Fractionation of rape seed proteins and insulin. J Agr Food Chem.1971; 19(4):581–588.
25. Janson JC, Hedman P. Large-scale chromatography of proteins. Adv Biochem Eng. 1982; 25:43–99.
26. Low D. Scale-up of protein chromatographic separations. In Hancock WS (Ed). High performance liquid lhromatography in biotechnology. New York: J Wiley & Sons; 1990. pp. 117–169.
27. Jungbauer A. Chromatographic media for bioseparation. J Chrom A, 2005; 1065:3–12.
28. Bren L. The Road to the biotech Revolution; Highlights of 100 years of biologics regulation. FDA Consumer Magazine, Centennial Edition, 2006; Jan–Feb.
29. Builder SE, Garnick RL, Hodgdon JC, Ogez JR. Proteins and peptides as drugs: Sources and methods of purification. In Rehm HJ and Reed G (Eds) Biotechnology, Vol 3; Bioprocessing (Stephanopoulos, G, (Ed) Weinheim, Germany: VCH. 1989, pp. 58–73.
30. Root MA, Chance RE, Galloway, JA. Immunogenicity of insulin. Diabetes, 1972;21(2):657–660.
31. Walsh G. Therapeutic insulins and their large-scale manufacture. Appl Microbiol Biotechnol, 2005;67: 151–159.
32. Novo Nordisk History, pp 8–9, www.novo.dk, accessed Nov 2006.
33. Lasagna L. The biological revolution: Commercialization of the molecule. Research Mgmt Rev. 1987;1:5–13.
34. Barinaga M. Asilomar revisited: Lessons for today? Science 2000;287:1584–1585.
35. US Food and Drug Administration. Guidance for industry. Research involving recombinant DNA molecules. Rockville, MD;1976.
36. Gosse ME, Manocchia M. The first biopharmaceuticals approved in the United States: 1980–1994. Drug Info J, 1996;30:991–1001.
37. Approved Biotechnology Drugs, www.bio.org (accessed Nov 2006).
38. Reichert JM, Paquette C. Therapeutic recombinant proteins: Trends in US approvals 1982–2002. Curr Op Mol Therapeutics. 2003;5(2):139–147.
39. Walsh G. Pharmaceutical biotechnology products approved within the European Union. Eur J Pharm Biopharm. 2003;55:3–10.
40. Shankar G, Shores E, Wagner C, Mire-Sluis A. Scientific and regulatory considerations on the immunogenicity of biologics. Trends Biotechnol. 2006; 24(6):274–80.
41. Pfund NE. The wheat from the chaff: The separations industry comes of age. Hambrecht & Quist, 1987.
42. Ehrenheim P. GE Healthcare presentation at bioLOGIC Asia, Mumbai, India, 2006;Nov 28–29.
43. Sofer GK, Nyström LE. Process chromatography. A practical guide. London: Academic Press; 1989.
44. Sofer GK, Nyström LE. Process chromatography. A guide to validation. London: Academic press; 1991.
45. Feldman F. Production of proteins for replacement therapy: plasma or recombinant? IBC Conference: Biological safety and Production, Boston, 1999.
46. Jungbauer A, Boschetti E. Manufacture of recombinant proteins with safe and validated chromatographic sorbents. J Chrom B. 1994;662:143–179.
47. Chase HA. Purification of proteins by adsorption chromatography in expanded beds. Trends Biotechnol. 1994;12(8):296–303.
48. Noel R. EBA for industrial scale protein isolation. Recovery of biological products XII. Arizona, 2006;Apr 2–7.
49. Fahrner RL, Knudsen HL, Basey CD, Galan W, Feuerheim D, Vanderlaan M, Blank GS. Industrial purification of pharmaceutical antibodies: development, operation, and validation of chromatography processes. Biotechnol Genet Eng Rev. 2001;18:301–327.
50. Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies —application of platform technologies. J Chrom B. In press.
51. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nature Biotechnol.

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2004;22(11):1393–1398.
52. Langer E, Ranck J. Capacity bottleneck squeezed by downstream processes. BioProcess Int. 2006;4(3):14–18.
53. Sofer GK, Chirica LC. Improving productivity in downstream processing. BioPharm Int. 2006;19(11):48–55.
54. Low D, O'Leary R, Pujar NS. Future of antibody purification. J Chrom B. In press.
55. Van Reis R, Lébréton B, Fontes N, Lin AP, Banerjee D, Mehta A. Future trends in bioseparations. Recovery of biological products XII. Arizona, 2006;Apr 2–7.
56. Przybycien TM, Pujar NS, Steele LM. Alternative bioseparations: life beyond packed bed chromatography. Curr Opin Biotechnol. 2004;15:469–478.
57. Buchacher A, Iberer G. Purification of intravenous immunoglobulin G from human plasma – aspects of yield and virus safety. Biotechnol J. 2006;2: 148–163.
58. Lander RJ, Winters MA, Meacle FJ, Buckland BC, Lee AL. Fractional precipitation of plasmid DNA from lysate by CTAB. Biotechnol Bioeng. 2002;79(7):776–84.
59. Fulton SP, Shahidi AJ, Gordon NF, Afeyan NB. Large-scale processing and high-throughput perfusion chromatography. Biotechnol. 1992;10(6):635–639.
60. Strancar A, Podgornik A, Barut M, Necina R. Short monolithic columns as stationary phases for biochromatography. Adv Biochem Eng Biotechnol. 2002;76:49–85.
61. Zhou JX, Tressel T. Membrane chromatography as a robust purification system for large-scale antibody production. BioProcess Int. 2005;3:32–37.
62. Guhan J, Li YF, Moore JA, Cramer SM. Ion-exchange displacement chromatography of proteins. Dendritic polymers as novel displacers. J Chrom A. 1995;702:143–155.
63. Curling JM. Affinity chromatography: from textile dyes to synthetic ligands by design. BioPharm Int. 2004;17(7): 34–42 and 17(8):60–66.
64. Cabanne C, Raedts M, Zavadsky E, Santarelli X. Evaluation of radial chromatography versus axial chromatography, a practical approach. J Chrom B. In press.
65. Janson JC. The development of gel media and columns for large scale chromatography of proteins. A historical review and visions for the future. Recovery of biological products X, Cancún, Mexico;2001, Jun 3–8.