Chapter 1

Modeling and Applications of Downstream Processing

A Survey of Innovative Strategies

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Downstream processing is playing an increasingly important role in the biochemical industry, especially since the advent of recombinant DNA technology. The use of recombinant DNA technology not only enables improvements in the production efficiency of therapeutic and industrial proteins, but it also permits the modification and improvement of protein structure and thus function. However, the commercial application of such technology was initially accompanied by concerns over product safety.

Quality criteria have been made especially stringent for products derived from genetically-modified microorganisms. The establishment of strict quality guidelines was the result of early concern about the oncogenic potential related to products contaminated by DNA sequences of the host mammalian cells (1). The quest for high quality has created a growing need for high-resolution techniques at the process scale as well as for novel strategies for the isolation and purification of bioproducts. Since the typical environment for producing biologicals is a complex one and quality criteria need to be strict, primary recovery techniques are typically implemented in a purification scheme prior to (or in conjunction with) high-resolution techniques. The most sophisticated and useful schemes take advantage of both the different physical and chemical properties of the components in complex mixtures and of the interactive nature of the downstream processing techniques (see Figure 1).

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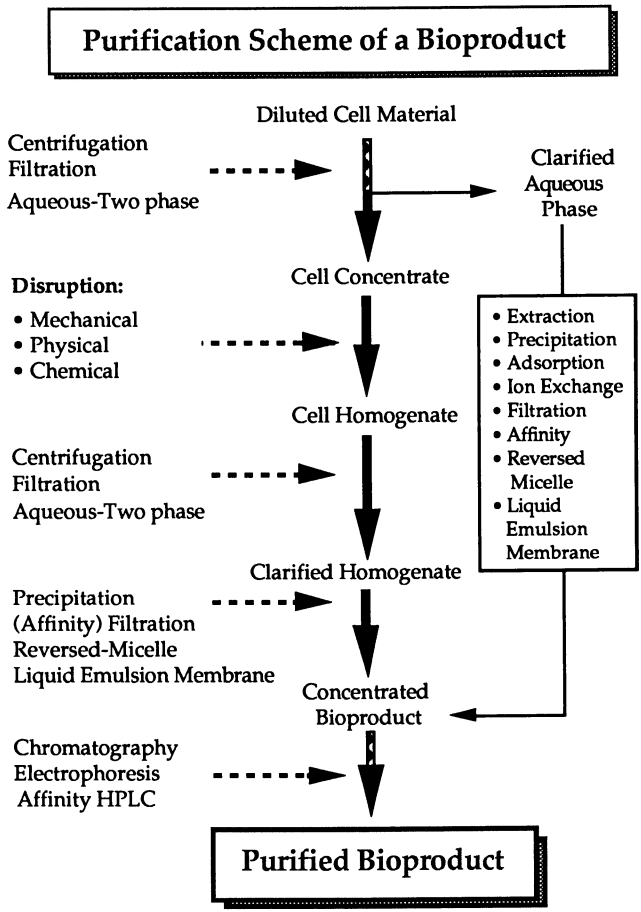

Figure 1. Bioproduct Purification Chart

Intracellular Bioproduct Route

Extracellular Bioproduct Route

Since proteins are polymers of amino acids, the chemical nature of the amino acid side chains and the order of the amino acids play an important role in establishing the biological properties of the active protein. Proteins may differ from each other according to size, charge density, shape and biological activity. Similarly, protein purification schemes require a similar diverse combination of separation techniques based on the various physicochemical properties of proteins.

Typically, protein is lost at every purification step and one normally wishes to reduce the number of steps. An added advantage of fewer steps for some unstable proteins is faster processing time and thus, improved quality of the desired protein when time is critical to maintain

stability. However, the number of physicochemical properties are limited; so are the number of purification techniques developed from them.

In non-genetically engineered microorganisms or cells, the protein of interest often represents a small fraction of total cellular or extracellular protein. Several strategies have been developed, using the techniques of molecular biology (e.g. gene dosage, leader sequence), which permit the design of efficient and simple purification schemes. For example, the overexpression of cloned genes in Escherichia coli or animal cells is an increasingly used strategy to produce eukaryotic proteins. Overexpression in bacteria often results in the formation of insoluble protein aggregates which are usually not in an active form. In some cases, the desired protein is already relatively pure and may represent up to 25% of total cell protein. An initial isolation step involving a combination of a disruption and chemical/physical separation could therefore produce a relatively pure product. By comparison, if that same protein were produced as a soluble protein, its initial purity would likely be significantly lower. Thus, an integrated view of each process is of critical importance. Whether the protein produced is soluble or insoluble, the isolation of intracellular proteins typically requires the use of disruption techniques.

High-pressure homogenization is an effective technique to free intracellular products. The detailed mechanisms by which the cells are disrupted are not known, and the parameters for determining the degree of disruption can only be determined empirically (2). Then, such knowledge would be likely to impact the design of equipment. In the last ten years, for example, major efforts have been devoted to homogenizer valve design and to configurations permitting higher pressure (>600 bar) operation, with the rationale that such conditions produce more efficient disruption - in terms of amount of product released per pass. Since the relationships between pressure and particle size distribution are poorly understood, there is a possibility that increasing the homogenizer operating pressure produces decreasing particle size. Smaller particles, in turn, may have a negative impact further downstream, in that their removal during clarification operations may be more difficult.

Often, high-resolution techniques like electrophoresis or affinity chromatography cannot be used readily on a complex mixture. However, in most isolation/purification processes of proteins, chromatography will appear in one form or another. Affinity chromatography has received considerable attention in the last ten years since it is one of the most powerful tools for separating biological products. This technique has been largely researched at the small-scale and only recently have large-scale studies been detailed in the literature. For example, the use of a monoclonal antibody column was recently reported to have provided major purification in a single step of interferon α–2a from extracts of recombinant Escherichia coli cells (3). As a result, a process using affinity chromatography may permit the reduction of the number of steps

compared to processes based on other techniques like centrifugation and filtration.

Overall however, there are few published reports of large-scale processes based on affinity separations, and in this context aqueous two-phase systems and membrane technology have imposed themselves (4). This book includes detailed studies by **Cabezas et al.** (5), **Forciniti and Hall** (6), **Szlag et al.** (7), **Dall-Bauman and Ivory** (8), **Guzman et al.** (9) and **Sheehan et al.** (10) based on such technologies as well as many others still confined to the laboratory scale. The contributions are varied in that: 1) some are theoretical, some experimental and some are both, 2) the authors represent both the academic and the private sectors, 3) there are several attempts to describe large-scale processes.

The remainder of this introductory chapter focuses on downstream processing and bioseparation relevant to the chapters presented in this book. Thus, the following topics are covered: multi-phase systems, membrane separation, centrifugation and adsorption techniques, electrophoresis, chromatography, and affinity separations.

MULTI-PHASE SYSTEMS FOR THE RECOVERY OF PROTEINS

Aqueous Biphasic System

More than 70 years elapsed between the first report of aqueous two-phase systems (11) and their subsequent applications to biochemical systems (12). In the last ten years in particular, there have been several innovative applications of aqueous two-phase systems (13). Aqueous two-phase systems consist of two immiscible fluids in a bulk water solvent. In such systems, the percentage of water in both phases is high, i.e. between 75 and 95%. As a consequence, the surface tension between the two immiscible phases may be as low as 0.1 dyne/cm so that a gentle mixing is sufficient to produce and maintain an emulsion (14).

One of the best characterized systems involves mixtures of dextran and polyethyleneglycol (PEG). In such a system, biological substances ranging from soluble proteins to particulate materials (cells or organelles) will partition preferentially in one of the phases. In order to characterize the separation of a substance of interest in an aqueous two-phase system, it is convenient to define a partition coefficient as the ratio of this substance's relevant property in the top and bottom phases. For example, for a protein with biological activity:

$$K_{act} = ACT_{top}/ACT_{bottom}$$

where: K_{act} is the partition coefficient of the protein,
ACT_{top} is the activity in the top phase, and
ACT_{bottom} is the activity in the bottom phase.

For cells or organelles, the partition coefficient is defined in terms of concentrations. The ability of a given substance to partition in an aqueous two-phase system is the result of several types of interactions (i.e. hydrophobic, electrostatic, and conformational) between this substance and the polymers. Thus, the behavior of homogenized cell material with a wide size distribution is a complicated system to characterize mechanistically. Proteins provide somewhat simpler systems, in that their partitioning can be understood by changing the nature and the concentration of the ions present in the system (15, 16). Several applications of different nature are worth mentioning.

As indicated in the flowchart presented earlier in this chapter (Figure 1), aqueous two-phase systems are especially useful during early primary recovery steps. One of the attractions of this system for extraction and purification of intracellular proteins is its ability to remove cell debris. Since the first report in 1976 describing the use of aqueous two-phase systems for cell debris removal (17), several investigators have demonstrated the generality of the technique. Using dextran/PEG, extractive cell debris removal experiments were carried out with Bacillus sphaericus for the extraction of leucine dehydrogenase (18), with Candida boidinii for the extraction of catalase, formaldehyde dehydrogenase and formate dehydrogenase (19), and with Klebsiella pneumoniae for the extraction of pullulanase (20) to cite just a few examples of enzyme extractions; in these cases yields were above 90% and partition coefficients between 3 and 10. Other original processes exploited the biocompatibility of dextran/PEG systems. In a process of extractive bioconversion, where bioconversion of a substrate is combined with removal of an inhibitory product, Clostridium tetani cells partitioned preferentially in a dextran-rich bottom phase, while the proteolytic toxin they produced remained more evenly distributed between the dextran and the PEG phases. As a result, the degradation of the cell walls of the bacteria was significantly less than compared to a simple aqueous phase system (21). Extractive bioconversion has been successfully demonstrated more recently for glucose fermentation and in the bioconversion of cellulose to ethanol (22). Besides being biocompatible, the dextran/PEG system is flexible in that coupling of this technique with other purification procedures is feasible; for example, it has been successfully integrated in a process using a separator, a settling tank and concentration and ultrafiltration equipment for the purification of leucine dehydrogenase (18).

Most of the research conducted with aqueous two-phase systems has been experimental and empirical; few studies of the fundamental thermodynamic mechanisms of phase separation and partitioning have been conducted (5, 23, 24). Furthermore, the systems which have been described use highly purified, expensive polymers, for model laboratory-scale applications. Novel bioseparation research based on aqueous two-phase systems needs to focus more on fundamental aspects needed to design phase diagrams and calculate partition coefficients. This

knowledge will, in turn, provide the basis for the design of industrial processes. The high cost of the dextran/PEG creates opportunities to design less expensive polymer systems (25). Such an approach has already proved to be fruitful and hydroxypropylstarch was used in combination with polyethyleneglycol for the partitioning of catalase and ß-galactosidase (26). In this book, several chapters by **Cabezas et al.** (5), **Forticini and Hall** (6), **Szlag et al.** (7), are devoted to both fundamental and practical aspects of research based on aqueous two-phase systems.

Reversed - Micellar Systems

Reversed micelles result from the formation of aggregates of surfactants that form in an organic/aqueous environment. The surfactants used in such systems have an hydrophilic headgroup and an hydrophobic tail. When placed in an organic/aqueous environment, the hydrophilic headgroups of the surfactant form a polar core containing water, while the hydrophobic tails remain in contact with the bulk organic phase (See Figure 2).

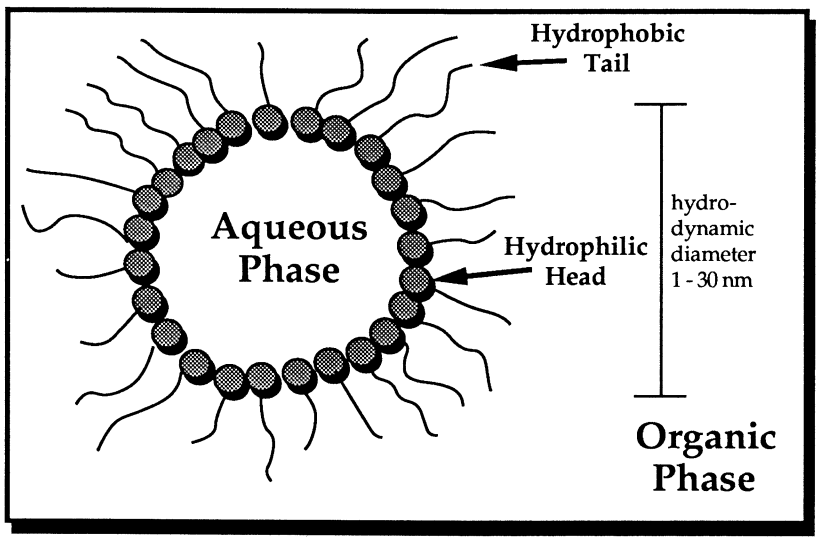

Figure 2. Diagram of a Reversed-Micellar System

Such a system was used successfully to solubilize enzymes within the inner core of reversed micelles without significant loss of activity (27). Besides its use to study enzymatic reactions in organic solvents with poorly water-soluble substrates (27), reversed-micellar systems have also been developed for the isolation and recovery of solubilized proteins

(28), and recently for the refolding of denatured proteins (29). For example, extraction experiments at the small-scale have been reported where α-amylase was extracted from a water phase into an oil phase (trioctylmethylammoniumchloride in isooctane) with reversed micelles, followed by the extraction of α-amylase from the oil phase to another water phase (30). By careful manipulation of pH and salt concentration, significant α–amylase activity could thus be recovered (30).

Novel aspects of protein extraction with reversed-micelles include both fundamental studies and process design studies/approaches. Fundamental studies are essential in order to design a reversed-micelles based extraction process in a rational manner. Such theoretical programs have been initiated and are providing a better understanding of the partitioning and transport phenomena in such systems (31). In this book, **Jolivalt et al.** (32) review the modeling aspects and the applications of reversed micelles for protein separations.

Furthermore, the results obtained with several experimental models are encouraging and suggest that the recovery of a single protein from a complex mixture, like a cell culture supernatant or a fermentation homogenate, may be feasible.

Liquid Membranes

Liquid membranes consist of an emulsion of two immiscible phases dispersed in an external, continuous phase (33) (Figure 3).

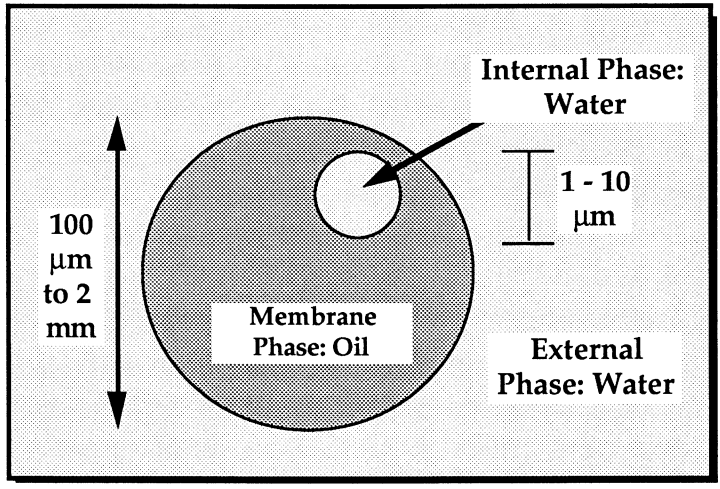

Figure 3. Diagram of a Liquid Membrane System

In such a system the internal and external phases are separated by an oil phase often called the membrane phase. As a result, the internal and external phases cannot come into direct contact.

Liquid membrane systems were first introduced in 1968 (34), and since then they have been evaluated for various chemical and biochemical applications (35). Some of the applications include: the selective extraction of hydrocarbons (36), the recovery of rare earths from process streams (37), the extraction of organic contaminants like phenol from water streams (38), and amino acid recovery (39).

These applications demonstrate the versatility of the liquid membranes, which can be adapted to obtain desired properties, such as stability and selectivity. Liquid membranes offer several advantages, including: 1) the ease to maintain them in suspension by agitation, 2) their relative large surface area per unit volume, facilitating mass transfer between the external and the internal phases, 3) their simple recovery, since upon interruption of agitation, the droplets coalesce to form an emulsion layer which can be separated from the external phase by gravity and, 4) the possibility to achieve recovery and concentration in a single step.

Pilot plant feasibility studies have been encouraging, and economic evaluations have indicated that liquid membranes can compete with other conventional ion exchange or solvent extraction techniques (40, 41). While the initial work with liquid emulsion membranes involved chemical systems, the first biomedical application was demonstrated with the use of liquid emulsion membranes for drug delivery and drug overdose prevention systems (42). In the biochemical field, an early study describes a liquid emulsion membrane-encapsulated bacterial cell-free homogenate able to carry out the reduction of nitrate and nitrite (43). Since this early study in 1974, many other biochemical applications have been reported which describe more complex enzyme/liquid emulsion membrane systems. They detail the critical role of membrane formulation in minimizing membrane breakage and protein inactivation (44). Membrane breakage can be affected by emulsion composition or by hydrodynamic shear, and translates into the leakage of the internal phase through the emulsion (45, 46, 47, 48).

In the future, novel developments of liquid membranes for biochemical processes should arise. There are several opportunities in the area of fermentation or cell culture, for the in situ recovery of inhibitory products, for example. Another exciting research direction is the use of liquid membrane for enzyme encapsulation so that enzymatic reaction and separation can be combined in a single step. Chapter 6 by **Simmons et al.** (49) is devoted to this technique. The elucidation of fundamental mechanisms behind the liquid membrane stability is essential, and models should be developed for the leakage rate in various flow conditions. Such models will be useful to address the effect of parameters such as flow regime, agitation rate, and microdroplet volume

on leakage of the internal phase into the external phase. Furthermore, such knowledge would form a basis for the design of recovery processes.

MEMBRANE SEPARATION

In the last few years, there has been an increasing interest in the use of membranes for the pharmaceutical and the food industries due to the limitations and drawbacks of competing technologies. Membranes are effectively used for air and aqueous feed stream sterilization (50), for recovery and purification of bioproducts or treatment of wastes (51) and for extractive fermentation processes (52), as support to immobilize biocatalysts (50), or in an affinity cross-flow filtration design (53). A large choice of membranes is available depending on their hydrophobic character, on their chemical structure (ceramic or polymeric), on the geometry of their pores, on their performance and on their cost. They offer ease of operation and great flexibility, and do not require addition of chemical agents. Thus, they are found in a multitude of process configurations, including cross-flow (also called tangential-flow) filtration, reverse osmosis, electrodialysis, affinity filtration, pervaporation and membrane distillation. Furthermore, for a given process the membranes can be packed in several configurations. For example, reverse osmosis membranes may be in one of the following classes: tubular, spiral wrap, fiber, flat plate (54).

Membranes are particularly suited for bioprocesses involving the cultivation of microorganisms or cells as biocatalysts, in which the product of interest is produced extracellularly. Such processes are becoming increasingly attractive when compared to those in which the products accumulate intracellularly. Some of the reasons for this include the use of novel expression systems which favor higher product concentrations, and the ease of purification as compared to an intracellular bioproduct route. One of the drawbacks remains that extracellular protein products are produced in dilute concentration. Extracellular-product based-processes require cell separation, product recovery and concentration. The use of ultrafiltration and microfiltration membranes has become a method of choice in such process schemes.

Microfiltration applies when particulate materials above 50 nm diameter are to be separated from an aqueous phase or from macromolecules. Thus, microfiltration can be used for cell concentration. On the other hand, the same unit operation can be viewed as a fractionation procedure in processes where products are produced extracellularly. Although the first research on microfiltration was carried out in Germany in the early 1900s (55, 56), the technology didn't find major application until after World War II when it was used for the analysis of waste aqueous streams (57). In the late 1970s, applications for cell separation appeared as a substitute for centrifugation in the separation of plasma from whole blood (58). Thus, a significant data base

has been produced over the years. Ample literature exists on both the development of flux models (59) and on hemolysis (60) and such studies should now be useful for biotechnology applications involving non-rigid cells. While microfiltration is a very common technique for sterilization (of air and wastewater streams), it is less used in separation schemes (57).

Ultrafiltration employs membranes of smaller pore size, able to retain proteins and other macromolecules (M.W. of 10^3 to 10^6). Ultrafiltration can be used strategically for separation of macromolecules and microorganisms from water and low molecular weight solutes. Unlike microfiltration, separation by ultrafiltration occurs at the molecular level, and thus is mostly suited for soluble substances. Shortly after the initial demonstrations of ultrafiltration applied to bioprocesses in the early 1960s, the first laboratory-scale ultrafiltration membranes became available (61). Since the first report, in 1965, on protein concentration by ultrafiltration (62), this technology has been tested in various configurations on a multitude of biological models, including the ultrafiltration of a cell suspension with proteins in solution (63), the concentration of human albumin using hollow-fiber ultrafiltration (64), the ultrafiltration of skim milk in a rotating module (65), the concentration of S49 lymphoma cells by cross-flow ultrafiltration (66), the concentration and purification of antibiotics and enzymes (67), the production of soybean and peanut protein isolates in a hollow-fiber membrane system operated in an ultrafiltration or a diafiltration mode (68), the recovery by ultrafiltration and diafiltration of high molecular weight products (e.g. polypeptides or enzymes) obtained in dilute aqueous solutions in bioreactors (69), the concentration of soya protein precipitate (70), the recovery of steroids from biotransformation medium by tangential-flow filtration used in combination with microsized polymeric particles (71). From a practical standpoint, cross-flow ultrafiltration and cross-flow microfiltration have a lot in common. However, in cross-flow microfiltration, parameters like "deformability" (for cells), adsorption (for colloids) and transmembrane flux are critical (57).

Over the last 15 years, there has been an increasing interest in the use of cross-flow filtration for processing cell suspensions. In spite of this, little engineering performance data useful in design or in elucidating fundamental mechanisms is available in the literature. There are few reports of industrial-scale experiments. One of the earlier reports on industrial-scale cross-flow filtration, describes cell harvest data for eight different organisms in high-velocity filters (72). The chapter by **Sheehan et al.** (10) extends our knowledge of cross-flow filtration systems applied to cell separation and product recovery, in their comparative evaluation of the performance of centrifugation and filtration operations at the pilot-scale. Part of the experimental work was carried out at the pilot-scale level, and the study reports a comparative evaluation on the performance of centrifugation and filtration unit operations.

There are several research opportunities in membrane filtration including: cross-flow filtration for processing shear-sensitive animal cell

suspensions, pilot-scale cross-flow filtration for cell separation and macromolecule concentration, correlations between microfiltration flux data and theoretical models, predictive models for ultrafiltration performance in multicomponent systems, mechanisms of flux reduction in multicomponent protein solutions, and effects of concentration polarization on experimental rejection coefficients.

ANALYTICAL and ISOLATION TECHNIQUES

Ultracentrifugation

About 50 years ago, the advent of the analytical ultracentrifuge offered to researchers an alternative tool to fractionate and characterize proteins (73, 74). It thus permitted 1) to push further the detection limits of the previous techniques, mostly based on the solubilities of proteins, and 2) to characterize individual proteins in complex solutions. This old and respected technique has nearly been displaced by electrophoresis and HPLC but it deserves another look. Ultracentrifugation is a powerful tool to determine size, composition and concentration of a macromolecule; however, the equipment involved is expensive which explains, in part, why it remains essentially a small-scale laboratory technique. In this book, **Phillips and Brogden** (75) revisit CsCl gradient ultracentrifugation as a tool for the isolation of lipopolysaccharides (LPS) from gram-negative microorganisms. Its potential use for the isolation of LPS produced by recombinant organisms is also discussed in that chapter.

Isoelectric Precipitation

Proteins have historically been recovered by isoelectric precipitation and by salting-out with inorganic salts, usually ammonium sulfate. Polyelectrolytes such as carboxymethylcellulose (CMC) and polyacrylic acid (PAA) are also effective precipitants for proteins, and offer an operationally simple method for protein recovery which is easily scalable, produces a high purity and concentrated product stream, and does not denature the target protein (76). Unlike salt precipitation, only small quantities of precipitant are used, from 5 to 25% of the protein by weight. In this book, a chapter by **Clark and Glatz** (77) demonstrates the power of this method in recovery of lysozyme from a 1:1 mixture with ovalbumin. For example, at a dosage of 0.1 g/g protein, over 70% of the lysozyme was recovered essentially free of albumin. Precipitation occurs when polymer chains and proteins combine by electrostatic interactions to produce "primary particles" which aggregate into flocs upon aging (78). Key parameters are the pH and ionic strength, which govern the protein/polyelectrolyte interactions, and fluid turbulence, which disperses the polymer feed but may shear the flocs apart.

HIGH-RESOLUTION PURIFICATION TECHNIQUES

Electrophoresis

Electrophoresis, the migration of charged molecules under the influence of an electrical field, is an efficient and inherently mild technique which has found widespread use in both analytical and small-scale preparative purification of proteins and nucleic acids. Of four basic techniques - zone electrophoresis, moving boundary electrophoresis, isotachophoresis and isoelectric focusing (79) - only zone electrophoresis and isoelectric focusing are widely applied. Zone electrophoresis (ZE) resolves the components of a sample on the basis of their relative electrophoretic mobilities. The mobility is a function of charge and molecular weight for soluble species and of zeta potential for colloids and particles. Isoelectric focusing (IEF) separates proteins on the basis of their isoelectric point. A sample is placed into a support medium, usually a gel, containing a stable pH gradient decreasing from the cathode to the anode. When an electrical field is applied to the system, each protein migrates towards the position corresponding to its isoelectric point. When the protein reaches this position, its net charge falls to zero and its motion stops because the electrical field no longer exerts a force on it. Zone electrophoresis is a dynamic separation, as it is based on relative rates of movement, while IEF is an equilibrium separation which reaches a steady state.

Recent developments in electrophoresis have focused on two areas:

- extension of the scale of electrophoretic methods from the conventional sample size range of 10^{-3} to 10^{-6} g protein to extremely small (10^{-12} g) and large (1 to 10^{3} g per hour) scale operation.
- development of hybrid methods which combine electrophoresis with other separation techniques.

• Nanoscale separation

Capillary electrophoretic methods including open-column zone electrophoresis, disc electrophoresis in gels, isotachophoresis and isoelectric focusing have received considerable attention from the analytical community over the last three or four years (80, 81, 82). In capillary zone electrophoresis (CZE), nanogram quantities of sample are placed in a silica capillary, 50 to 300 microns in diameter and 50 to 100 cm long. Since the small dimensions of the capillary allow for efficient removal of Joule heat, electrical fields up to 350 V/cm can be applied. Under the influence of the field, sample components separate by zone electrophoresis while they are carried downstream by electro-osmosis.

Efficiencies on the order of 10^6 theoretical plates are achievable. Separated components may be detected by fluorescence, electrochemical detection or by interfacing to a quadrupole mass spectrometer via electrospray ionization (83). Mass spectrometry can provide extremely sensitive detection, in the attomole range. Moreover, the mass/charge spectrum of each product yields a precise measurement of its molecular weight, to the nearest dalton. Peptide analytes in the range of 500 to 2500 daltons have been separated and identified by CZE/MS (84, 85), and the technique can be extended to molecular weights on the order of 100,000 (86). Capillary zone electrophoresis/mass spectrometry may eventually compete with SDS/PAGE for molecular weight determinations. Current CZE research focuses on modeling column/solute interactions and other band-broadening phenomena (87), improvement of sample introduction, and development of more sensitive detectors.

Pulsed and crossed-field electrophoresis have recently become popular for separation of chromosome-sized DNA segments on agarose gels. In these techniques, the electric field in the gel is periodically shifted or reversed. At each shift, the macromolecules' migration is retarded while they change conformation and realign with the field. Relatively smaller molecules relax faster and move farther per cycle, resulting in much improved resolution. The development of pulsed-field electrophoresis has been driven largely by the human genome project and related studies. Though well-accepted for analytical separations, it is difficult to envision any process-scale applications for this technology.

• Process-scale separation

Three devices for free-flow electrophoretic separation are now available commercially. They are described in more detail in Ivory's excellent review (79).

The Biostream rotationally stabilized free-flow electrophoresis device, based on the Philpot-Harwell design (Figure 4), uses an annular geometry stabilized against radial convection by rotation of the outer cylinder. Carrier buffer and feed are injected at the base of a vertical annulus and move axially upward to fraction collectors at the top.
An electrical field of several tens of V/cm is applied radially between the inner cylinder of the annulus (generally the cathode) and the rotating outer cylinder. The device has a capacity of 1 to 2 L/h of feed, or several g/h of protein. Several analyses of hydrodynamic dispersion and Joule heating have been published, e.g. Beckwith and Ivory (88). Though solute dispersion measured in the separator is several times greater than theoretical predictions, the apparatus can still perform well when buffer composition has been optimized to maximize the difference in solute mobilities (79).

Thin-film free-flow electrophoresis devices have been studied since the late 1950s (Figure 5).

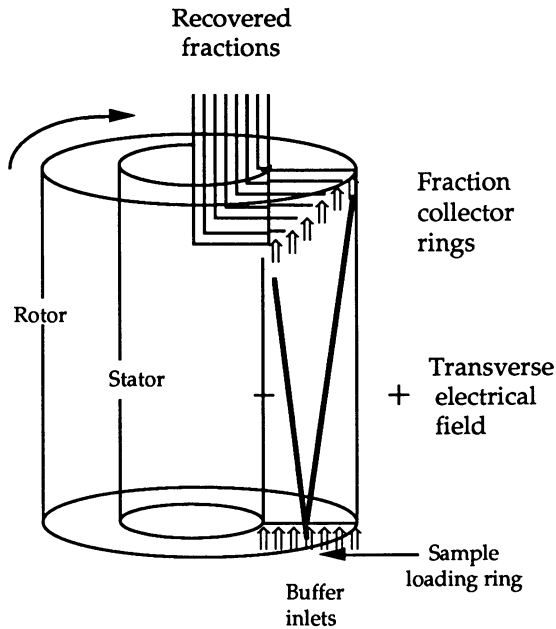

Figure 4. Philpot - Harwell Device

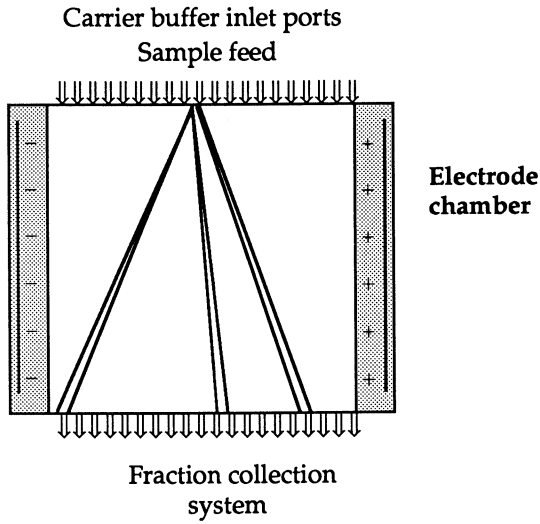

Figure 5. Thin-Film Electrophoresis

These essentially consist of a pair of closely spaced, vertical rectangular plates bounded on the sides by the electrodes. The sample and carrier buffer are fed from the top of the slit and travel down in laminar flow to a battery of fraction collectors at the bottom. Unlike the Philpot-Harwell device, which is essentially adiabatic, the thin-film separator can be cooled at the plates. The commercially available device, the Elphor®, has a throughput of around 0.1 g/h of protein when operated for multi-component separation. It has been used to separate not only proteins, but cells and other particulate materials. Like the Philpot-Harwell apparatus, it uses a relatively large quantity of carrier buffer and the products are substantially diluted during separation.

Much work has been devoted to modeling thin-film separators in the hope of improving their scaling characteristics. Ivory (79) cites three major impediments to expanding their capacity: natural convection due to thermal gradients in the slit; overheating at the column centerline; and the "crescent phenomenon", the hydrodynamic dispersion of solute into a crescent-shaped profile by a combination of horizontal electro-osmotic flow and the vertical parabolic velocity profile of laminar flow in the slit. The first two effects can be overcome by running the system in microgravity. The company McDonnell-Douglas has flown several electrophoresis experiments on the space shuttle, but the work has been impeded by delays in the space program.

The thin-film separator can also be operated in a binary mode called field-step focusing (89) (Figure 6).

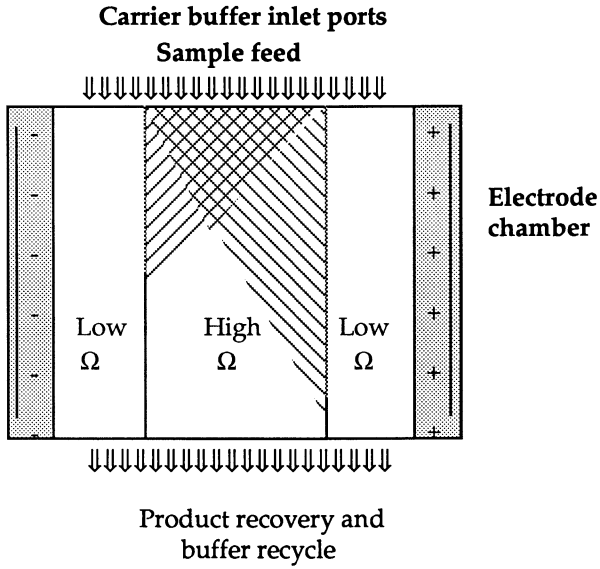

Figure 6. Field-Step Focusing

The sample, dissolved in a low ionic-strength, low-conductivity buffer, is fed to the middle region of the slit, and a high-conductivity buffer is fed to each side, adjacent to the electrodes. The high electrical field in the center causes sample components to migrate rapidly to the left and right, until they are effectively stopped by the low electrical field in the side buffer streams. Two concentrated streams of protein are recovered from the buffer interfaces and can be sent to a second field-step separation after desalting. The authors claim a 10 to 100 fold improvement in throughput over conventional thin-film operation, or 1 to 10 g/h of protein in a binary separation.

• Recycling free-flow methods

The problems of Joule heating and natural convection have been addressed by recycling methods for isoelectric focusing (RIEF) (90), zone electrophoresis (RZE) (91, 92) and most recently, isotachophoresis (RITP) (93). These methods use repeated short electromigrations of solutes to achieve high-resolution batch separations at relatively high throughputs. In both RIEF and RZE, the solution to be fractionated is repeatedly passed through a bank of fractionation channels, bounded by porous membranes to minimize convection, and then through a bank of heat exchangers for cooling. In RITP, no membranes are needed as the thin film configuration limits convection.

Recycling isoelectric focusing operation (90) is started by pre-focusing the ampholyte solution, returning the contents of each compartment back into itself until the entire system achieves a stable pH gradient. Then, sample is added and cycled through the system until each component collects in the channel corresponding to its isoelectric point. Resolution of proteins whose pI's differ by 0.1 pH units is possible in this device; however, the purified fractions must be separated from the ampholytes before further processing. A RIEF device with 60 ml capacity is commercially available (Rotofor®; Bio-rad, Inc.) and is claimed to have a throughput of 0.4 g protein/h over a 4-hour run.

Recycling isoelectric focusing, like its parent method IEF, is an equilibrium process in which each component migrates to a steady-state position and remains there. By contrast, zone electrophoresis is a rate process in which each component moves at a steady-state velocity. In order to convert ZE to a recycle system, it is necessary to provide a counterflow to offset electromigration of the solutes.

The recycle zone electrophoresis (RZE) apparatus of Gobie and Ivory (91, 92) accomplishes this by shifting the reinjection point of each compartment to a port one or more compartments upstream (against the direction of electromigration). The upstream recycle provides an effective counterflow whose magnitude can be adjusted at different positions in the apparatus by changing the distance over which the reinjection point is shifted. The prototype apparatus, with 50 ports, was built with low-, medium- and high-shift regions to produce a binary separation, but n-component purification is theoretically possible in an apparatus with n+1 sections at increasing shift distances. Throughputs of 1.5 g protein/h were reported for the initial apparatus (79).

A new recycle isotachophoretic process (93) uses a thin-film geometry with the electrical field perpendicular to the principal flow direction. Leading buffer, a marker dye, feed and trailing buffer are introduced into one end of the slit. An isotachophoretic stack develops perpendicular to flow as the liquid moves downstream. A fraction collector at the outlet collects the fractions, which are recycled until the stack sharpens. A computerized feedback control system keeps the stack centered in the apparatus. It regulates the withdrawal of trailing buffer and the addition of leading buffer in counterflow to the migration of the stack, based on the position of the marker dye front.

Righetti and coworkers (94) have reported an isoelectric refining method in which a liquid sample is circulated between two gels held at slightly different pH's. The gel segments are prepared with immobilized ampholytes at pH values which bracket the isoelectric point of the target protein. All contaminating species are ionized and eventually migrate into one or the other of the gels, leaving the target species alone in the liquid phase. Although the problem of ampholyte contamination is avoided, isoelectric precipitation of the protein of interest could prove troublesome, as could titration of the gel surfaces by adsorbed or dissolved contaminants. Nevertheless, this technique has potential as a polishing

step for therapeutic proteins because of the extremely high resolution it promises.

• Electrochromatography

Continuous systems using anticonvective packings have also been proposed. The rotating annular electrochromatograph consists of an annular bed of anticonvective medium which may have specific chromatographic interactions with the solutes to be separated. Carrier buffer is pumped axially through the annulus, and the feed is introduced at a fixed point as the bed slowly rotates past it. The electrical field may be either axial, as in the "CRAE" system (95, 96) or radial (97). The CRAE system (Figure 7), with parallel convective and electrophoretic flows, produces a highly tunable one-dimensional separation; the annular electrochromatograph of the Oak Ridge group has the potential to produce a continuous separation in two dimensions.

Both designs for the annular electrochromatograph appear to be limited by heat transfer (79) and to suffer from mechanical and electro-osmotic dispersion of the solute bands. However, electro-osmosis may actually decrease dispersion under some conditions, according to a model developed by **Yoshisato and co-workers** (98). Precise and comprehensive models of annular electrochromatography, now under development, are necessary to guide the design and operation of the equipment.

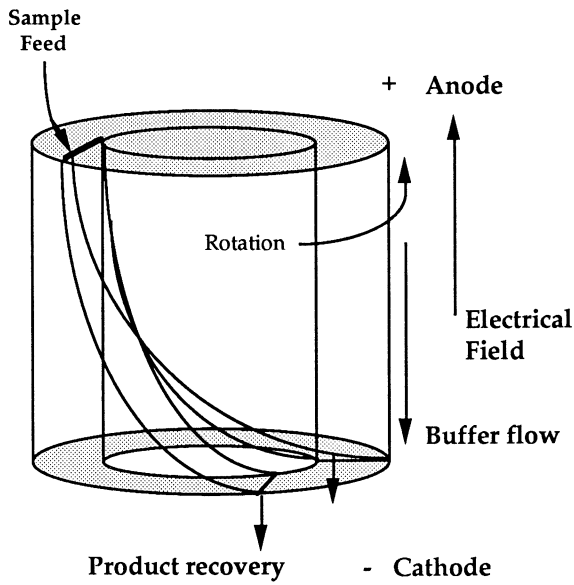

Figure 7. Continuous rotating annular electrophoresis unit (CRAE)

Another electrochromatographic technique, proposed by O'Farrell (99) is counteracting chromatographic electrophoresis (CACE) (Figure 8). In this technique, an axial electrical field is applied antiparallel to convective flow in a cylindrical packed bed of size exclusion gel. The upstream portion of the column is packed with a relatively "excluding" gel and the downstream portion with an "including" gel, so that macromolecules are convected faster on average in the upstream portion than in the downstream portion. By properly tuning the electrical field, a target protein can be made to migrate to the interface and accumulate there while other proteins migrate off the column at either end. Several analyses of CACE have been reported (100, 101, 102). Although this method can be operated continuously, and produces an extremely pure and concentrated product, the throughput is limited by Joule heating and by the pressure-drop limitations of size exclusion gels.

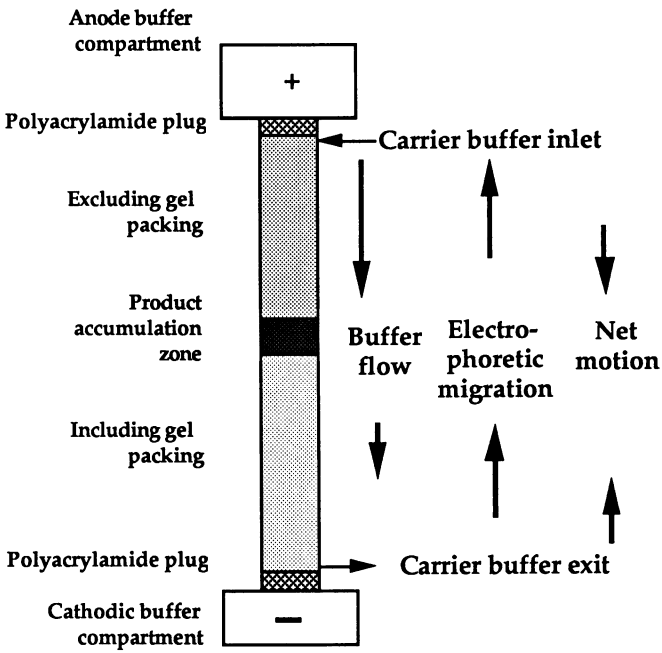

Figure 8. Counteracting Chromatographic Electrophoresis (CACE)

• Electrically assisted separation

Electrophoresis has also been proposed as a way of overcoming diffusion limitations in membrane based processes such as cross-flow microfiltration (103). Lee and Hong (104) used electrophoresis to aid recovery of aspartic acid synthesized by an immobilized enzyme coupled to a membrane. In principle, removal of an electrically charged reaction product by electrophoresis can be used to drive a reaction with an unfavorable equilibrium constant. In their model of facilitated transport of proteins through membranes, **Dall-Bauman and Ivory** (8) found electrical fields to enhance transport of the target protein to the upstream side and away from the downstream side of the membrane. By reducing concentration polarization on both sides of the membrane, small imposed electrical fields allowed the carrier system to function much more efficiently, and produced substantial increases in overall transport across the membrane. Yarmush and Olson (105) have used electrophoresis to elute proteins from affinity membranes. After the protein is dissociated from the ligand by a high pressure environment (~1000 psi), it migrates away from the surface of the adsorbent under an electrical field. Electrical fields may alter the microstructure of a membrane as well as the flux of solute inside it (106). The resulting large changes in permeability and selectivity can be controlled by switching the field on and off. In contrast to other electrophoretic processes in which proteins migrate for distances of millimeters or centimeters, boundary layer disruption requires an electromigration distance of only a few tens of microns. Low-intensity or intermittent fields can be used, avoiding the Joule heating problems which plague conventional large-scale electrophoresis.

Novel separation methods in electrophoresis share a common factor: the complexity of systems where mass transfer, heat transfer, electro-osmosis, dispersion, adsorption, and Donnan effects are all relevant and all interact. Precise and comprehensive modeling efforts are - and will continue to be - of paramount importance in evaluating these new ideas. Important advances in modeling have come from the groups at Oak Ridge National Laboratory, University of Arizona, University of Iowa, University of Washington (Pullman), and North Carolina State University. Likely developments in the near future include a better understanding of the role of electro-osmosis in large-scale separations, the development of unusual geometries to facilitate heat removal from large-scale separation devices, expanded interest in electrophoresis to counteract diffusion limitations, and a steady improvement in experimental apparatus.

Chromatography

Chromatography, the workhorse of protein fractionation, may be defined as the percolation of a fluid through a column of a particulate stationary phase which selectively retards certain components of the fluid. Though very broad, this definition identifies chromatography as a muticomponent separation technique based on differential migration due to adsorption or partitioning of solutes. In the limiting case where the solutes do not move at all, chromatography becomes batch sorption, and additional driving forces must be applied to desorb the solutes.

The past ten years have seen a virtual explosion in every aspect of preparative chromatography - the development of rigid, monodisperse packings for HPLC; the proliferation of stationary phase chemistries, now including systems for chiral and affinity separations; advances in on-line detection systems; and the commercial application of process-scale chromatography in the biotechnology industry, to name only a few. Column chemistries can be counted on to improve steadily, permitting ever finer fractionations. However, many problems remain to be solved. Chromatography is still inherently a batch process; by and large it still uses packed beds of media with their problems of pressure drop, dispersion, and intolerance of particulates in the feed; it still requires large quantities of buffers and yields diluted products.

The central issue in process-scale chromatography is the problem of increasing the throughput of product per unit amount of packing, subject to constraints of product quality and column life. These constraints, and the scale of the "preparative" process, vary enormously across the field of bioseparations. Perhaps the largest scale chromatographic bioseparation is the refining of ultra-high fructose syrups from an equilibrium mixture of fructose and glucose on calcium-loaded ion exchange resins. World production is on the order of millions of tons per year, and the product is 90 to 95% pure (107). On the opposite end of the spectrum, enzymes and hormones for drug use must meet the most exacting standards of purity, at an output of only kilograms per year.

Historically, the throughput problem has been addressed by heuristics for scale-up of conventional packed beds for multicomponent separations. The most recent scale-up analyses, focusing on intraparticle mass-transfer resistance as a limiting factor, have led away from the traditional long columns to several alternative geometries (108, 109, 110).

Wankat and Koo (110) have shown that the efficient mass transfer achievable with small (~ 10 micron diameter) monodisperse packing can provide excellent resolution on very short columns, even when adsorption isotherms are nonlinear. For high-throughput processes, the most efficient columns resemble squat disks or pancakes (109). The ultimate "column" geometry may well be a membrane or consolidated packing with mobile phase flow through monodisperse pores.

If a pancake column is rolled into a tube, the result is radial-flow chromatography. This geometry has already been commercialized for

ion-exchange separations (Zeta-Prep®, Cuno, Inc.), and the concept is being extended to other chemistries (111). A radial-flow separation module is made by wrapping a sheet of separation medium around a hollow core, then encapsulating the roll in a rigid cartridge. Particulate packings may also be used. Sample and eluant are introduced into the shell side and flow radially to the center outlet. Because of the short bed depth, isocratic resolution is poor, and the column is preferably operated by gradient elution. Throughput is proportional to the cartridge surface area, so scale-up is modular (111, 112).

Chromatography in two different hollow fiber geometries has recently been reported. A hollow fiber can be used as a capillary column analogous to capillary gas chromatography, with the same operating advantages of low pressure drop and rapid mass transfer (108, 113). A bundle of such fibers resembles a consolidated packing or a very thick membrane. Radial hollow fiber chromatography (114) is a miniaturized version of radial flow chromatography and has been demonstrated for affinity purification of fibronectin using gelatin as a ligand. The small volume, low pressure drop and high ligand capacity of the hollow fiber module lead to very short residence times and very efficient use of the ligand. Both of the hollow fiber methods scale up linearly, by using a bigger fiber bundle or multiple modules. Both also suffer from the difficulty of precise flow distribution to a fiber bundle.

• Novel methods in traditional geometries

Binary chromatographic separations are most efficiently run in moving-port and simulated moving-bed processes (115). In these continuous processes, a number of short columns are connected to form a ring. The sample, eluent and withdrawal ports are rotated around the ring to simulate countercurrent movement of the solid phase past a stationary feed port. Weakly bound components move around the column ahead of the feed port and are recovered downstream, while tightly bound components trail behind the feed. Moving-bed and moving-port operation can increase the efficiency of packing use several fold, as there is no waiting for low-mobility samples to clear the column before more feed is injected. The Sorbex process, a simulated moving-bed process, is already standard for process-scale separations of glucose and fructose (107).

Displacement chromatography (Figure 9) is another approach to increased efficiency.

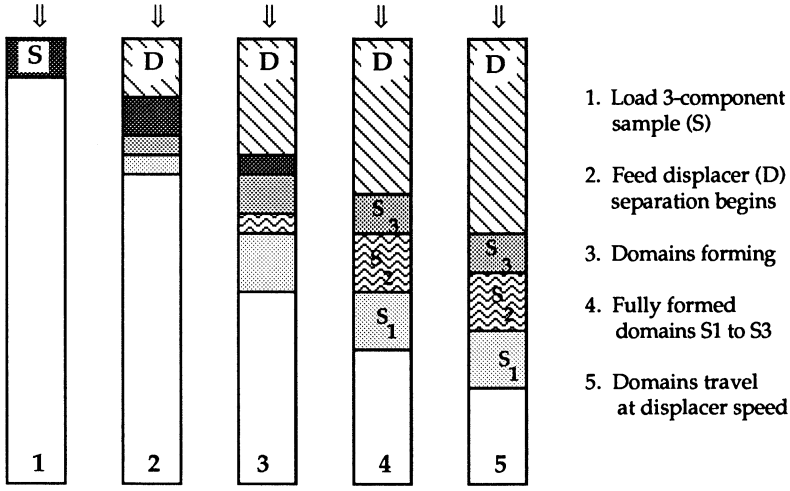

Figure 9. Displacement Chromatography

Ordinary elution chromatography operates in the limit of low solute concentrations, where adsorption isotherms are approximately linear. If the column is overloaded, the usual case in preparative applications, nonlinear isotherms and interactions between solutes cause band broadening and shifts in retention time. At extremely high loading, a new separation mode emerges based on competition between sample components for binding sites on the stationary phase surface. In displacement chromatography, the sample components are displaced from the stationary phase by a front of a highly absorbing solute fed just behind the sample. The displacer drives the sample components downstream, each component displacing the more weakly adsorbed components ahead of it. Ultimately the components form adjacent sharp bands, each traveling at the speed of the displacer front. Displacement chromatography is fast - all sample components are recovered after passage of a single column volume of displacer - and the components are recovered in concentrated form. Separations of antibiotics, amino acids and small peptides have formed the basis for most theoretical work to date (116) and one report of protein separation has appeared (117). Fundamental modeling of nonlinear solute/solute and solute/packing interactions in displacement (118, 119) will provide much needed guidelines for designing recovery strategies and optimizing operating parameters such as feed pulse size and displacer concentration.

Continuous annular chromatography (CAC) has been the subject of several recent experimental studies (120, 121), models (122, **123**) and a brief review (124). The equipment is very similar to the CRAE (Figure 7). Feed, eluent, and regeneration solutions (if necessary) are fed to fixed points or arcs at the top of an annular packed bed which rotates slowly about its axis. As the chromatogram develops, the components separate

into helical bands which are collected at fixed points at the base of the column. Reports to date have centered on ion-exchange and size-exclusion separations, but the apparatus should be able to perform continuously any separation currently done by batch chromatography, e.g. displacement and gradient elution separations with continuous column regeneration (124). Linear models of the CAC have developed rapidly, since the CAC becomes analogous to a conventional chromatographic column if the angular position is transformed to time. The chief difference is a term for angular dispersion of solutes.

Continuous annular chromatography is limited by the elution speed of the fastest and slowest migrating components of the sample. Its throughput per unit volume is the same as conventional column chromatography. Nonlinear gradient and displacement chromatography may prove the best applications for CAC, because of their economy in buffer use. However, these modes are likely to be very sensitive to angular dispersion and to concentration-dependent flow disturbances such as channeling around a viscous feed pulse. Nonlinear models should appear over the next few years.

Countercurrent chromatography, also called centrifugal partition chromatography, is analogous to a multistage countercurrent liquid/liquid extraction system (125). Current technologies using organic and aqueous solvents are suitable for purification of antibiotics or amino acids. A recent report of countercurrent chromatography in an aqueous two-phase system (126) indicates its promise as an initial step for isolation of a macromolecule from a crude fermentation broth, or in classifications of living cells.

Affinity Separation

A significant fraction of biomolecules display natural biological affinity for certain other species, e.g. immuno-ligands, enzyme substrates, hormones. These properties can be exploited in an affinity separation process to recover and purify biomolecules in a more effective way (i.e. with higher yield and higher resolution) than can be achieved with more conventional means of purification (e.g. size exclusion chromatography).

Affinity separations are characterized by the formation of a reversible, specific biochemical interaction between the target molecule (the adsorbate) and an immobilized molecule (the ligand). The two molecules may interact as enzyme and substrate, analogue, cofactor or inhibitor; as antibody and antigen; messenger and receptor; or as complementary nucleic acid sequences (127). The triazine dyes interact with nucleotide-binding sites of a wide range of enzymes. Plant lectins and agglutinins bind to specific sugar moieties, hence are useful for purifying glycoproteins such as mammalian cell surface proteins, and for separating subclasses of mammalian cells based on their surface receptors. Recent advances have focused on "generic" ligands which are useful for

whole classes of proteins. An example is protein G, which binds to any IgG. "Generic biospecificity" may sound like an oxymoron; but it is the most cost-effective approach to ligand chemistry. Optimized conditions for binding and elution on a generic ligand can produce excellent yield and resolution without the need for unique affinity interactions (128).

The classical affinity separation (enrichment) of a single target product comprises four steps: adsorption, washing to remove non-specifically bound components, elution of the target component, and regeneration of the ligand. The process context is usually column chromatography. A brief review of current practice is given by McCormick (129). Biospecific interactions can also be used to strip specific contaminants such as endotoxins, DNA or T-lymphocytes from a product stream. As expected, the operating requirements for enrichment and depletion modes are quite different (130). Applications of potential importance based on this principle include, i) the purification of viruses for the production of antiviral vaccines (131), and ii) the removal of viruses from blood products and therapeutic recombinant proteins (132). **Tsao and Wang** (132) investigated batch adsorption of viruses from protein solutions onto immobilized quaternary ammonium chlorides (QAC's), a class of antimicrobials which can disrupt cell membranes. The treatment appeared effective against "enveloped" viruses having surface lipids. Adsorption of these viruses via hydrophobic interactions was followed by their inactivation at the solid surface. Results for a non-enveloped virus were less conclusive, and were complicated by competitive binding of soluble proteins. In general, viruses adsorb readily to many different types of solids (133) and future work in this area will require careful analysis of non-specific binding.

Affinity ligands can be covalently immobilized to an immense variety of supports. For chromatographic processes, agarose beads have been a popular support since agar is porous, dimensionally stable over a wide range of pH and ionic strength, and is easily activated for covalent coupling of ligands. However, affinity chromatography by HPLC has grown in popularity and may become the method of choice for large-scale "polishing" affinity purifications (134). Bergold and coworkers (135) have reviewed this technology in detail.

Affinity separations have historically been used in later stages of a purification train, in order to protect the expensive ligands from reactive components of a crude system, and to minimize the extent of nonspecific binding. However, the specificity and the high binding constants of affinity interactions make them especially attractive for isolation of biomolecules from crude medium. Affinity-based product recovery can provide a high yield, some purification and substantial concentration of the product, reducing the processing volume and leaving the initial crude mixture nearly unaffected. Much recent work has appeared on affinity methods for initial product isolations, such as affinity partitioning, adsorption in a fluidized bed reactor (136) and affinity separations using magnetic particles (137, 138). Affinity partitioning is an

attractive technique which combine properties of affinity and aqueous two-phase systems. Since the demonstration of affinity partitioning in 1975 (139), several model systems have been described (140, 141). The triazine-dye has been the ligand of choice for the affinity extraction of glycolytic and other enzymes, e.g. phosphofructokinase from baker's yeast (142). Compared to other affinity purification techniques, affinity partitioning has decisive advantages, e.g. higher capacity, which make it attractive for large-scale, continuous operations using complex systems such as crude homogenates (142).

Both affinity ligands and their adsorbates are typically high-priced, labile biomolecules, hence affinity separations may be costly. The ideal affinity isolation would make efficient use of ligand through careful immobilization, retaining its bioactive conformation at an appropriate surface density of binding sites. The support would have a high surface area to promote rapid binding of adsorbate. The loaded affinity support would separate readily from its surroundings, and washing, elution and regeneration procedures would be chemically mild. Continuous processes involving rapid recycle of the ligand/support system could achieve high throughput per unit quantity of ligand.

Recent advances in affinity separation fall into two main categories: affinity isolation and process concepts for desorption strategies.

• Isolation by Affinity Interaction

In an isolation step, where yield and concentration are more important than purity, the adsorption mechanism can be considered an on/off process, and several alternative contacting schemes can be used. Ligands have been bound to magnetized particles (137, 138) for continuous countercurrent adsorption in magnetically stabilized fluidized beds. Ligands attached to liquid perfluorocarbons (143), to dextran and related polymers (144), or incorporated into liposomes (145), or reversed micelles (146) may be used for biospecific liquid-liquid extraction or "affinity partitioning". Ligands have also been attached to surfactants and biopolymers for selective precipitation of dilute protein species (147, 148).

Affinity escort systems consist of a ligand attached to a high molecular-weight polymer (149) or to a small particle (52). The so-called macroligand will bind an adsorbate and increase its size so that it may be separated by ultrafiltration or cross-flow microfiltration. Even larger particle sizes have been used. Nigam and coworkers (150, 151) immobilized ligands to finely divided solid supports which they encapsulated in hydrogel beads of up to 3 mm diameter. The hydrogel prevented fouling of the ligand by high molecular-weight and particulate materials. Higher ligand loadings and a reduction in internal mass transfer resistances were achieved by encapsulating a liquid phase containing a ligand immobilized to a soluble polymer.

Pungor and coworkers (152) described a continuous affinity-recycle extraction system which allows continuous separation of an adsorbate from crude cell lysate without preliminary clarification. In this scheme, a feed stream is added to a slurry of particulate affinity adsorbent (agarose beads) in a continuous well-mixed contactor. A wash buffer dilutes contaminants as it carries the loaded adsorbent to a desorption stage, where the adsorbate is recovered in concentrated form by a slow flow of desorbing buffer. The regenerated adsorbent particles are then recycled to the adsorption stage. The system survived 24 hours of operation with no observable ligand leakage, and recovered 70% of the product (beta-galactosidase) from a slurry of homogenized bacterial cells. In both of these methods, the ligand is protected from fouling, and the rapid recycle of adsorbent makes very efficient use of the ligand. Multiple contacting stages or a wash stage prior to desorption can be used to increase yield or purity.

• Recovery Strategies

Historically, the removal of the adsorbate from its complex with the ligand has been a critical step in affinity separation. Typical methods have included reversible denaturation of the adsorbate and/or ligand with urea, guanidine salts, chaotropic salts, iodide or thiocyanate. Even after prompt removal or dilution of the denaturant, full renaturation is not always achieved. Conducting elutions at extreme pH's, high ionic strength, or by addition of organic solvents results in the disruption of the ligand/adsorbate interaction, and may denature the proteins, especially after repeated exposure to the hostile conditions. An added drawback to chemical elution methods at process scale is the cost of recycle or disposal of eluents. Recently, "switch" monoclonal antibodies have been prepared for which small changes in environment, such as a pH change of 1 to 2 points, produce major changes in binding constant (153). This and future advances in ligand chemistry could ease the elution problems in large-scale affinity separations.

Several mild and effective elution methods have recently been proposed. Olson and coworkers (154) have reported that brief exposure to pressures of 1000 to 2000 atmospheres can dissociate antibody/antigen complexes and other non-covalently bound protein complexes without affecting the activity of monomeric proteins. Repeated cycling to high pressures did not affect the binding capacity of the ligand, nor did it denature the adsorbate. Electrophoretic elution, described above, is a useful adjunct to this method or can be used alone (105). The use of temperature-programmed elution to dissociate tightly-bound complexes in affinity HPLC has been reported by Bergold and coworkers (135).

Electrophoretic elution and "switch" monoclonal antibodies are combined in a new rapid recycle method: an affinity-mediated membrane transport process reported by **Dall-Bauman and Ivory** (8). In this modeling paper, a "switch" monoclonal antibody incorporated into a supported liquid membrane is used to facilitate the transport of human growth hormone from a high-pH to a low-pH environment. Electrochemical effects, including Donnan equilibria between the membrane and external environments, and imposition of external electrical fields, significantly affected the flux of protein across the membrane. Experimental confirmation of the simulation results could introduce affinity-mediated transport as a powerful new biospecific separation method.

CONCLUSION

The contributions in this book illustrate the important role of downstream processing and purification processes in the application of biotechnology. We expect that this trend will continue, especially with the proliferation of recombinant proteins derived from the recombinant DNA technology. All of the techniques presented in this book can play a critical role in the downstream processing and in the effective production of biological products. Most of the early fears related to the safety aspects of recombinant DNA products have been assuaged since studies showed that quantities of DNA (obtained from chinese hamster ovary cells) at the hundred of μg level did not result in the formation of tumors in newborn rats (155).

The science behind the concepts and techniques of bioseparations is exciting: each fundamental mechanism which is uncovered sets the direction of future development work and motivates further advances.

On the development side, it is important to recognize that a successful bioprocess leading to a safe product results from the integration of techniques ingeniously connected with one another. The fermentation engineer will confer with downstream processing colleagues to design the fermentation process, since the mode of operation (e.g. fed-batch or continuous) can have major effects on product stability and response to handling, as well as on the nature of the impurities which may remain with the product. Looking at a process with an integrated vision not only minimizes the likelihood that serious mistakes will occur, but it also favors optimization of each unit operation in the context of the entire process. This plays a significant role in making a process viable and cost-effective.

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