Laboratory Scaledown of Protein Purification Processes Involving Fractional Precipitation and Centrifugal Recovery

M. Boychyn,1  W. Doyle,1  M. Bulmer,2  J. More,2  M. Hoare1

1The Advanced Centre for Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK; telephone: 44-0207-679-7031; fax: 44-0207-209-0703; e-mail: m.hoare@ucl.ac.uk
2Bio Products Laboratory, Hertfordshire, UK

Received 8 August 1999; accepted 9 January 2000

Abstract: The ability to predict the performance of large-scale processes is central to the rapid development of successful operations at the pilot and industrial scale. In this article, we examine the operation, at laboratory scale, of precipitation reactors and centrifuges for protein precipitate recovery and dewatering and how they might best mimic large-scale reactors and centrifuges, in this case, a pilot-scale batch stirred-tank reactor and a multichamber-bowl centrifuge. Novel approaches to bench-top centrifuge operation are provided, in particular with a view to delivery of material for subsequent high-resolution purification, which would be obtained at full pilot scale. Results are presented in terms of properties of the protein precipitates, the fraction of solids recovered, and the extent of dewatering achieved. Good agreement was obtained at bench scale (a 1000-fold scale down factor) for all of these parameters for pilot-scale, batch-feed operation. In addition, the methodology developed allows identification of the extent of break-up that occurs in continuous-feed centrifuges when processing shear-sensitive materials such as the protein precipitates studied here. © 2000 John Wiley & Sons, Inc. Biotechnol Bioeng 69: 1–10, 2000.

Keywords: scaledown; protein precipitation; centrifuge; clarification; dewatering

INTRODUCTION

The scaledown of industrial processes is a powerful tool for minimizing the time required for bioprocess development. It allows the study of more process options at small scale and hence rapid identification of unacceptable ones, and of their operating window, and delayed risk of capital investment. This article examines the translation of scaledown to the laboratory level by developing techniques to mimic the performance of a pilot-scale process using laboratory bench-top equipment. The specific sequence of unit operations, precipitation, feed, centrifugation, and recovery, is central to many industries including blood fractionation, where good separation (clarification and dewatering) is critical for achieving high yields (Bell et al., 1983; Foster, 1994). This process also addresses one of the most challenging aspects of scaledown studies; that is, the key link between the material generated in a reactor and the subsequent high-resolution chromatography columns. Successful scaledown of the latter also depends crucially on the relevant material being available for study—that is, as generated by a good scaledown mimic rather than by an ideal, laboratory-scale process.

The formation and recovery of protein precipitates are important operations widely used in industrial-scale purifications (Englard and Seifter, 1990). Applications range from primary isolation procedures, in which the amounts of contaminating proteins are decreased and/or sizeable reduction of process volume is achieved, to finishing operations (Paul and Rosas, 1990). Precipitation is generally a low-cost, high-yield stage suitable for use with biological streams containing highly concentrated soluble and insoluble contaminants (Stavrinides et al., 1993).

Successful scaledown requires a detailed understanding of the mechanisms involved to identify the key parameters for scaling purposes. Precipitation occurs in four distinct stages (Bell et al., 1983): nucleation; growth of nuclei; aggregation of nuclei; and conditioning of the aggregates. This last stage occurs under low shear rates to promote further growth and increased density and strength of the aggregate by shear-induced rearrangement.

The resultant aggregate characteristics impact on the performance of the subsequent centrifugal separation step. Protein solubility is affected primarily by the type and concentration of precipitant, pH, and ionic strength (Fisher and Glatz, 1986; Foster et al., 1976; Iyer and Przybycien, 1994, 1995). The physical properties of the aggregates depend on several factors: the type of precipitation reactor (Foster et al., 1976); choice, concentration, and rate of addition of precipitating agent (Fisher et al., 1986); initial protein concentration; and nature and extent of mixing and residence

Correspondence to: M. Hoare
Contract grant sponsors: Biotechnology and Biological Sciences Research Council; Bio Products Laboratory; Natural Sciences and Engineering Research Council of Canada; ORS Council, University of London.

© 2000 John Wiley & Sons, Inc.
time in the reactor (Fisher and Glatz, 1988; Glatz et al., 1986; Rothstein, 1994). The use of a continuous-flow tubular reactor (Bell and Dunnill, 1982) results in large, irregularly shaped particles of low density that are more prone to shear damage than precipitates formed in a batch stirred-tank reactor, with the high flow stresses in the impeller region leading to smaller, denser, and consequently less shear-sensitive particles. This work focuses on the batch tank due to its widespread industrial use (Foster, 1994). These reactors have been scaled successfully for protein precipitate formation by maintaining constant the mean power dissipation per unit volume constant for tanks ranging from 0.27 to 200 L, with the lowest mean velocity gradients giving the largest final aggregates (Hoare et al., 1982). By scaling using constant mean velocity gradient, Maybury (1999) observed good agreement for yeast protein–ammonium sulfate precipitates using vessels ranging from 0.9 to 50 L in size.

The complete design of the precipitation stage requires an understanding of the interaction with the subsequent separation operation, usually centrifugation or filtration. To date, no theoretical model exists that successfully consolidates the integration of precipitation and centrifugation recovery and, consequently, process design is mainly an empirical exercise consisting of laboratory- and pilot-scale experiments through to full-scale operation (Englard and Seifter, 1990).

The multichamber-bowl centrifuge used in this study is representative of the centrifuges used in the biotechnology industry, namely continuous-feed, high-speed machines designed for the recovery of fine, low-density-difference biological particles (Foster, 1994). Characteristics of the multichamber-bowl centrifuge (Fig. 1) include a large solids-holding space with good clarification efficiency and dewatering. Disadvantages of this centrifuge include the need for batch recovery of solids and large temperature increases of process fluid due to the long residence times (Bell et al., 1983). The latter problem is largely overcome by direct cooling of the bowl, centripetal pump, and frame.

One common method for comparing the performance of centrifuges is the Sigma concept, that is the equivalent set, (Ambler, 1959). Maintaining the ratio of flow rate to separation area at a constant level should result in the same clarification for all centrifuges. The settling area is evaluated based on idealized flow patterns, and correction factors must be introduced to account for nonideal flow (i.e., regions of local turbulence), which often occurs in industrial equipment. The extent of centrifugal dewatering of the sediment is also crucial for determining the yield (Bulmer, 1992), particularly when processing a suspension with a high solids content (Ward and Hoare, 1990).

Previous scaledown studies have been done by modification of pilot-scale centrifuges. The throughput of a disk stack centrifuge has been reduced tenfold by decreasing the number of active disks through a series of interlocking inserts (Mannweiler and Hoare, 1992). The volume of process material required for centrifuge characterization has been reduced fourfold using inserts in the solids-holding space (Maybury et al., 1998).

The aim of this study is to examine how an integrated precipitate preparation and recovery sequence can be operated at a laboratory scale (e.g., 10 to 100 mL of process material) to mimic pilot- or industrial-scale operation. The initial target is a scaledown factor of approximately 1000-fold for the precipitation stage and the translation from continuous-flow pilot to laboratory-batch centrifuges with a similar scaledown ratio.

THEORETICAL CONSIDERATIONS

Precipitation

When scaling down a precipitation process, the goal is to be able to mimic a larger reactor operating under feasible conditions and to provide the basis whereby the eventual larger scale process can be operated under identical conditions. Several parameters should be kept constant, including tank geometry (height: diameter ratio), impeller type, diameter of the impeller relative to that of the tank, placement of the impeller, baffle geometry, mean velocity gradient, aging parameter (Camp number), and residence time. However, fluid-flow patterns will change somewhat when increasing the scale of a reactor even if the aforementioned factors are similar for the two scales, thus affecting mixing.

There is a wide range of velocity gradients in the vessel but an average value, $\bar{G}$, can be assumed based on power dissipated, $P$, per unit volume, $V$ (Smoluchowski, 1917):

$$\bar{G} = \left( \frac{P}{V \mu} \right)^{\frac{1}{2}}$$

where $\mu$ is the dynamic viscosity.
The power input, \( P \), is calculated by:

\[
P = P_o \rho N^3 D^5
\]  

where \( P_o \) is the power number (\( P_o = 6 \) for a four-baffled stirred tank with a Rushton impeller and \( Re > 5 \times 10^3 \), where \( Re \) is the Reynolds number, \( Re = \rho N D^2/\mu \), \( \rho \) is the density of the bulk suspension, \( N \) is the impeller speed, and \( D \) is the impeller diameter (Rushhton et al., 1950).

In addition to particle size it is also important to maximize strength, because precipitate particles are sensitive to shear forces, which may result in breakage in high-flow devices such as pumps and centrifuge feed zones (Bell and Brunner, 1983; Mannweiler, 1989). It has been shown that particle strength is maximized when precipitate suspensions are aged to a Camp number, \( Ca = \bar{G} \), of \( 10^5 \) for a range of shear rates and times (Bell and Dunnill, 1982).

**Centrifugation**

**Clarification**

The Sigma or equivalent “settling area” is commonly used to describe the clarification process in a centrifuge and is an index of its size; that is, the area equivalent to that of a settling tank capable of the sample separation performance while acting under gravity (Ambler, 1959). The concept is based on the Stokes definition of settling velocity of a particle, which assumes a low particle concentration (no hindering settling) and laminar settling of the particle (Ambler, 1959). The concept is considered as the baseline (i.e., \( C_{mc} = 1.0 \)), then the multichamber-bowl has been shown to have a correction factor, \( C_{mc} \), of approximately 0.9 (Svarovsky, 1990).

Different centrifuge designs result in different expressions for the settling area. The equivalent settling area, \( \Sigma \), of a continuous-flow tubular-type centrifuge is:

\[
\Sigma = \frac{\pi L \omega^2}{g} \left( \frac{r_2^2 - r_1^2}{\ln \left( \frac{2r_2}{r_2^2 + r_1^2} \right)} \right)
\]

where \( L \) is the length of a chamber, and \( r_1 \) and \( r_2 \) are the inner and outer radii of the chamber bowl. The settling area of a multichamber-bowl centrifuge (see Fig. 1) is calculated by considering the machine to be a series of tubular bowls:

\[
\Sigma_{mc} = \Sigma_1 + \Sigma_2 + \ldots + \Sigma_n
\]

where \( n \) is the number of chambers.

The Sigma of a batch, laboratory centrifuge with a swing-out rotor is given by:

\[
\Sigma_{lab} = \frac{V_{lab} \omega^2}{2g \ln \left( \frac{2R_2}{R_2 + R_1} \right)}
\]

where \( V_{lab} \) is the volume of material in the tube, and \( R_1 \) and \( R_2 \) are the inner and outer radii, respectively (i.e., the respective distances between the center of rotation and the top of the liquid and the bottom of the tube). For short spin times, acceleration and deceleration phases also contribute significantly to the process (Maybury et al., 1999). Assuming linearity for changes in rotational speed, a modified expression is obtained:

\[
\Sigma_{lab} = \frac{\omega^2 (3 - 2x - 2y)}{6g \ln \left( \frac{2R_2}{R_2 + R_1} \right)}
\]

where \( x \) and \( y \) are the fractional times required for acceleration and deceleration, respectively.

It is possible to compare centrifuges of different designs and sizes as follows:

\[
\frac{Q_{mc}}{C_{mc} \Sigma_{mc}} = \frac{V_{lab} / t_{lab}}{C_{lab} \Sigma_{lab}}
\]

where \( Q_{mc} \) is the flow rate through the multichamber-bowl machine, \( t_{lab} \) is the residence time in the laboratory centrifuge, and \( C_{mc} \) and \( C_{lab} \) are correction factors to account for nonidealities in fluid flow. If the laboratory centrifuge is considered as the baseline (i.e., \( C_{lab} = 1.0 \)), then the multichamber-bowl has been shown to have a correction factor, \( C_{mc} \), of approximately 0.9 (Svarovsky, 1990).

**Dewatering**

A centrifuge is often characterized by the maximum or the mean relative centrifugal force RCF, which is defined for the laboratory centrifuge as:

\[
RCF_{max} = \frac{\omega^2 R_2}{g}, \quad RCF_{mean} = \frac{\omega^2 / \left( \frac{R_2 - R_1}{\ln (R_2/R_1)} \right)}{g}
\]

and similarly for the multichamber-bowl centrifuge.

In a continuous-feed centrifuge, such as the multichamber bowl, clarification occurs in a short time (the liquid/suspension residence time in the bowl), followed immediately by compaction until the bowl is filled (solids residence time, which is generally tenfold the liquid residence time). Laboratory spin times required to mimic this clarification (conducted at the same RCF mean) are considerably longer than the mean liquid residence time in industrial centrifuges, and approximately four- to fivefold greater. However, the initial spin time accounts only for sedimentation, which is the trajectory of a particle up to its point of impact with a solid surface. To mimic the compaction in a pilot centrifuge, laboratory tubes must be respun at a rotor speed corresponding to the RCF max experienced in the large-scale machine (determined using the outer radius of the outer chamber). The time for which the sediment is to be compacted, \( t_c \), is calculated by determining the average residence time of solids in the holding area of the centrifuge (i.e., the first solids sedimented undergo the full compaction time, but the last ones to settle undergo virtually no com-
errors, and Fig. 2 for an explanation of these terms). We also have liquid, solid, supernatant, and sediment, respectively (see where a, b, c, and d are weighting factors such that

\begin{align}
e_1 &= P_F - (P_L + P_S) \\
e_2 &= P_F - (P_{sup} + P_{sed})
\end{align}

Consistent correction of the mass balance is achieved by using a predetermined weighting for each of the process streams analyzed:

\begin{align}
P_S &= P_S + ai_1 \\
P_L &= P_L + be_1 \\
P_{sup} &= P_{sup} + ce_2 \\
P_{sed} &= P_{sed} + de_2
\end{align}

where a, b, c, and d are weighting factors such that a + b = 1 and c + d = 1. In this study, because the measured errors are distributed approximately equally between the liquid and solid phases, a = b = c = d = 0.5.

From a complete protein mass balance on all process streams displayed in Figure 2, the following augmented matrix can be obtained:

\[ 
\begin{array}{c|cccc}
\text{Inputs} & V_{Sed} & V_{Lsed} & V_{Sup} & V_{Lsup} \\
\hline
P_{sed} & [P_S] & [P_L] & 0 & 0 \\
P_{sup} & 0 & 0 & [P_L] & [P_S] \\
V_{sed} & 1 & 1 & 0 & 0 \\
V_{sup} & 0 & 0 & 1 & 1
\end{array}
\]

where \( V \) is the volume of material in the respective phase and brackets indicate concentration. The solution of the above matrix is the following set of equations:

\begin{align}
V_{Sed} &= \frac{(P_S) V_{sed} - P_{sed}}{[P_L] - [P_S]} \\
V_{Lsed} &= \frac{(P_S) V_{sed} - P_{sed}}{[P_S] - [P_L]} \\
V_{Sup} &= \frac{(P_L) V_{sup} - P_{sup}}{[P_S] - [P_L]} \\
V_{Lsup} &= \frac{(P_S) V_{sup} - P_{sup}}{[P_L] - [P_S]}
\end{align}

From the outputs of the matrix, two key process parameters can be calculated:

\begin{align}
\% \text{ solids sedimented} &= \frac{V_{Sed}}{V_S} \times 100 = \frac{V_{Sed}}{V_{Sed} + V_{Lsed}} \times 100 \\
\% \text{ sediment dewatered} &= \frac{V_{Sed}}{V_{sed}} \times 100 = \frac{V_{Sed}}{V_{Sed} + V_{Lsed}} \times 100
\end{align}

It is these parameters that are used to compare laboratory- and large-scale centrifuges. It is noted that the latter parameter is measured against dewatering in a high-g centrifuge and, as such, is not an absolute value. Absolute values are often not possible to obtain due to an inability to discriminate between free and bound water in precipitate suspensions.

MATERIALS AND METHODS

Chemicals

All chemicals, unless specified otherwise, were obtained from Sigma Chemical Co. (Dorset, UK) and were of analytical grade. A concentrated suspension of polyvinylacetate particles (DM 130) was kindly donated by Hoechst AG (Frankfurt, Germany). The particles were chosen for their small size (0.5 to 3 \( \mu \)m) and low density relative to water (<+2%).
Description of Equipment
The instruments used in the laboratory runs and pilot-plant verification trials, respectively, are listed. Homogenization was carried out at the laboratory scale using an APV Manton Gaulin High-Pressure Homogeniser Lab 40 and at the pilot scale using a K3 machine (APV Baker Ltd., Derby, UK). Debris clarification was done using a Beckman J2-M1 laboratory centrifuge with a JS-13.1 swing-out rotor (Beckman Instruments Ltd., High Wycombe, UK; laboratory centrifuge with a JS-13.1 swing-out rotor (Beckman Instruments Ltd., High Wycombe, UK; laboratory centrifuge with a JS-13.1 swing-out rotor (Beckman Instruments Ltd., High Wycombe, UK; laboratory centrifuge using a suspension of latex particles. Then 0.10 kg of concentrated polyvinylacetate emulsion was diluted in 2 L of distilled water and homogenized at 500 bar for five passes in a Lab 60 high-pressure homogenizer. Exposure to homogenization conditions should ensure that the latex particles are broken down to their minimum size such that they will not be further degraded when feeding to or during passage through a centrifuge. The conditioned particle suspension was diluted into 100 L of reverse osmosis water in a 100-L tank to give a final particle concentration of approximately 0.1% (w/v). The suspension was stirred at 3.33 rps and held for 0.5 h prior to use.

Preparation of Precipitate Suspension
High-activity baker’s yeast (Saccharomyces cerevisiae), provided by DCL (London, UK), was suspended to 28% packed wet weight per volume in phosphate buffer (0.1 M KH₂PO₄, adjusted to pH 6.5 using 4 M NaOH). This was then disrupted by five discrete passes at 500-bar pressure through a high-pressure homogenizer and centrifuged to remove the debris. The clarified homogenate was placed into an agitated precipitation vessel to which a saturated solution of (NH₄)₂SO₄ (519 g L⁻¹, buffered with 0.1 M KH₂PO₄, pH 6.5) was added, resulting in a 40% saturated suspension. At the laboratory scale, the salt solution was rapidly decanted (<2 s) from a beaker in a single dose at the surface and near the impeller. In the pilot plant, addition was carried out in a similar fashion within approximately 5 s. All precipitations were aged at ₋ = 200 s⁻¹ for t = 0.63 h ₋[Camp number] = 10⁴.

Separation of Precipitate Suspension
Scaledown Process
For the scaledown process, the precipitate suspension (30 mL) was placed in a centrifugation tube made of polyvinylchloride graduated in 1-mm (0.2-mL) increments for accurate solids volume measurements (±0.3% as a fraction of total volume). The tubes were centrifuged for purposes of clarification in a Beckman J2-M1 (N = 140 rps, Σlab = 6.1 m², tlab = 0.072 to 0.738 h, mean RCF = 6550), after which the supernatant was removed slowly using a 5-mL pipette down to the sediment–liquid boundary; this boundary became blurred at shorter spin times. The tubes were then respun for compaction (N = 130 rps, t = 0.208 to 1.33 h, maximum RCF = 8830) and the minimal resultant liquid added to that obtained from the clarification spin. The volume of sediment was recorded after the compaction run. The sediment was resuspended to 30 mL with buffer (0.1 M KH₂PO₄, pH 6.5) to redissolve the precipitates prior to analysis.

Pilot-Scale Process
In the pilot plant, suspension (30 L) was fed continuously to the multichamber-bowl centrifuge (N = 167 rps, Σmc =
1100 m², \(Q_{mc} = 20 \text{ L h}^{-1}\) via a peristaltic pump. The supernatant was collected in a vessel for analysis. Excess liquid in the centrifuge bowl was siphoned out to enable recovery of sediment; sediment from all chambers was pooled, weighed \((\rho \approx 1200 \text{ kg m}^{-3}\) (Maybury et al., 1999), and then suspended in buffer for analysis. The multichamber bowl was also run in the batch mode in which the bowl was filled and spun for a set time (\(V_{mc} = 1.4 \text{ L, } \Sigma_{mc} = 187 \text{ m}^2, t_{mc} = 0.38 \text{ h}\)).

Reference for Analysis of Process Samples

For the purposes of this study, 100% separation (100% clarification and dewatering) of the precipitate suspension into its solid and liquid components was achieved by spinning down a 1-mL Eppendorf sample in the microfuge (\(V_{lab}/t_{lab}C_{lab}\Sigma_{lab} = 6.29 \times 10^{-10} \text{ m s}^{-1}, C_{lab} = 1.0, N = 221 \text{ rps, } t_{lab} = 0.5 \text{ h, no cooling}\)).

Analyses

Physical Properties

The density of the protein precipitate suspension was 1115 kg m⁻³, measured with a specific gravity bottle. The viscosity of the suspension was determined to be 0.0035 Pa·s at a shear rate of 200 s⁻¹ using a Contraves Rheomat 115 Viscometer with concentric cylinders (Contraves Industrial Products, Ruislip, UK).

Protein Assay

The protein concentration of all streams was measured using the Bio-Rad assay (Bio-Rad Labs, Herts, UK), which is based on the Bradford assay (Bradford, 1976). Process samples were diluted 50- to 200-fold in buffer (0.1 M, KH₂PO₄, pH 6.5) depending on the protein concentration, and thereafter diluted 30-fold with assay mix (this is Bio-Rad reagent diluted fivefold). The shift in optical density (OD) was monitored by a spectrophotometer at 595 nm. Analysis of solid products was performed by spinning down a 1-mL Eppendorf sample in the microfuge (\(V_s/\Delta T D N\)).

The Enzymatic activity, \(E\), is expressed in terms of units of activity per milliliter of solution and is defined by:

\[
E = \frac{1}{e_{340}} \frac{\Delta A}{\Delta T} \frac{V_c}{V_s} D_N
\]

where \(e_{340} = 6.22 \text{ cm}^2 \text{ µmol}^{-1}\), \(\Delta A/\Delta t\) is the rate of change of OD at 340 nm with respect to time, \(V_c\) is the sample volume added to the cuvette, \(V_s\) is the total volume in the cuvette, and \(D_N\) is the dilution factor. The spectrophotometer was used to monitor the change in absorption of the solution at 340 nm over 60 s at 25°C, from which the reaction rate was calculated by linear regression. Samples were analyzed in triplicate with a reproducibility of ±3%.

Solids Fraction

In independent laboratory experiments, the solids fraction of the precipitate suspension (scaledown and pilot) was measured using the 30-mL tubes (transparent and graduated; spin conditions: \(R_{CFmax} = 24,500, V_{lab}/t_{lab}C_{lab}\Sigma_{lab} = 7.1 \times 10^{-10} \text{ m s}^{-1}, N = 217 \text{ rps, } t = 1.5 \text{ h}\)). A mean solids volume fraction of 0.062 ± 0.003 was obtained.

Moisture Content of Sediment

To determine the absolute moisture content of sediment, the material was weighed before and after drying in an oven at 106°C for 24 h.

RESULTS AND DISCUSSION

Correction Factor of Multichamber Centrifuge

Figure 3 displays the recovery of the polyvinylacetate suspension for both the laboratory and multichamber-bowl centrifuges, from which its correction factor, \(C_{mc}\), was determined to be 0.88 ± 0.05 for \(C_{lab} = 1.0\).

Mass Balance

Table II shows a typical mass balance carried out in triplicate for the characterization of its centrifugal recovery. All process streams are detailed in terms of volumes and normalized amounts of protein and ADH and good reproducibility was achieved with all balances within ±5%. The one notable feature is that the amount of total protein in the supernatant tends to be greater than the soluble amount in the reactor as measured by the microcentrifuge (\(V_{lab}/t_{lab}C_{lab}\Sigma_{lab} = 6.3 \times 10^{-10} \text{ m s}^{-1}\)), indicating the presence of concentrated precipitates due to nonideal separation in the case of the former (\(V_{lab}/t_{lab}C_{lab}\Sigma_{lab} = 7.2 \times 10^{-9} \text{ m s}^{-1}, \text{approximately 11-fold greater than the ideal microfuge}\)).

The mean error of the protein balances was +0.1% and that of ADH was +1.6%; that is, there was no significant activation or denaturation of ADH during laboratory trials. Ninety-seven percent of all protein points fell within a range
of ±6% with ADH data exhibiting a tolerance of ±8%. Furthermore, there was no significant relationship between error in mass balance and time of centrifugation. Approximately the same amount of protein was precipitated in the laboratory and pilot vessels, 30% compared with 32%, respectively, and similar amounts of ADH remained soluble, 93% compared with 90%. The mass balance approach adopted in this study allows for process characterization and comparison independently of these small differences. The greater extent of homogenate clarification at the laboratory scale (97% vs. 75%) leads to differences in debris carryover to the precipitate stage. This results in about a 5% increase in solids concentration after precipitation, again the difference being accounted for within the mass balance.

### Centrifugation Performance

Figure 4 displays the purification factor of ADH in the supernatant for a range of $Q/S$ values that decreases with an increase in flow rate. Good agreement was obtained for lab scaledown and pilot-prepared precipitates put through the laboratory scaledown centrifuge, with the lower purification of the pilot-prepared suspension attributable to an increase in the amount of ADH precipitated at pilot scale. The large decrease in purification factor when using the multichamber-bowl centrifuge may be due to several factors. The mass balance for ADH indicated an overall 16% loss of activity, which may be attributable to denaturation (the supernatant temperature was 20°C and that of the bowl was 35°C; the feed temperature was 4°C). In addition, there is the possibility that the clarification and dewatering performance of the multichamber-bowl centrifuge is different from that of the laboratory centrifuge. This will be assessed later.

Figure 5 shows the centrifugation yield of soluble ADH in the supernatant for various equivalent flow rates. The yield calculation only considers the recovery of soluble ADH. The yield curve, initially at a high value, decreased markedly with increasing $Q/S$ as a result of poorer dewatering of the sediment. The yield exhibited a possible minimum at approximately $Q/S = 4.2 \times 10^{-8}$ m s$^{-1}$. Yield increase at lower $Q/S$ could be due to fewer solids being settled, thus decreasing the volume of sediment.

Figure 6 is a plot of the solids sedimented against equivalent flow rate for various processes. First, the relationship between these two parameters was obtained for the laboratory centrifuge. A linear and relatively sensitive dependence

### Table II. Example of mass balance of a scaledown, laboratory process (scaledown precipitator and centrifuge) at an equivalent flow rate of 20 L h$^{-1}$ ($V_{lab}/C_{lab} = 7.2 \times 10^{-10}$ m s$^{-1}$).

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th>Protein</th>
<th>ADH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>42.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Precipitant</td>
<td>28.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Soluble</td>
<td>66.0</td>
<td>92.7</td>
</tr>
<tr>
<td>Insoluble</td>
<td>4.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Sum</td>
<td>70.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Error (%)</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Supernatant</td>
<td>64.1</td>
<td>95.6</td>
</tr>
<tr>
<td>Sediment</td>
<td>5.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Sum</td>
<td>70.0</td>
<td>102.8</td>
</tr>
<tr>
<td>Error (%)</td>
<td>0.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Amounts of feed protein (1270 ± 50 mg mL$^{-1}$) and ADH (17,300 ± 1000 U mL$^{-1}$) normalized to 100.
Figure 4. The dependence of purification factor of ADH in the supernatant on the logarithm of equivalent flow rate per centrifuge separation area for a precipitate suspension. The curve is a trend first-order exponential decay fit. Clarification was performed at $V_{\text{lab}} / t_{\text{lab}} C_{\text{lab}} S_{\text{lab}} = 3.6$ to $43 \times 10^{-9}$ m s$^{-1}$, $N = 140$ rps, $t_{\text{lab}} = 0.072$ to 0.738 h, RCF$_{\text{min}} = 6550$; compaction was carried out at $N = 130$ rps, $t = 0.208$ to 1.33 h, RCF$_{\text{max}} = 8830$. Solid points indicate precipitate (ppt) prepared in the laboratory and open points pertain to the pilot vessel; squares and circle represent the laboratory and pilot-continuous centrifuge. Data points indicate mean values and error bars show the 95% confidence interval for (at least) duplicate runs. Data are not presented for the pilot-batch centrifuge ($V_{\text{mc}} / t_{\text{mc}} C_{\text{mc}} S_{\text{mc}}$) due to the considerable error associated with the calculation.

Figure 5. The linear-log relation of yield of soluble ADH in the supernatant to the equivalent flow rate per centrifuge separation area for the precipitate suspension. The maximum yield was 100% measured at $V_{\text{lab}} / t_{\text{lab}} C_{\text{lab}} S_{\text{lab}} = 6.3 \times 10^{-10}$ m s$^{-1}$ ($N = 221$ rps, $t = 0.5$ h, $C_{\text{lab}} = 1.0$). Centrifugation conditions and symbols are as in Figure 4. Triangle represents pilot-batch centrifuge.

Figure 6. The probability-log dependence of percent solids sedimented on equivalent flow rate per centrifuge separation area for the precipitate suspension. The line is a trend linear fit. Centrifugation conditions and symbols are as per Figure 4. The maximum recovery was >99% measured at $V_{\text{ref}} / t_{\text{ref}} C_{\text{ref}} S_{\text{ref}} = 6.3 \times 10^{-10}$ m s$^{-1}$.

Figure 7. The linear-log relationship of the degree of dewatering on equivalent flow rate per centrifuge separation area for the precipitate suspension. The line is a trend first-order exponential decay fit. Dewatering in the reference centrifuge considered to be 100%. Data is not presented for the pilot-batch centrifuge ($V_{\text{mc}} / t_{\text{mc}} C_{\text{mc}} S_{\text{mc}}$) due to considerable uncertainty associated with the calculation.
was observed; for instance, tenfold decrease in $V\Sigma$ resulted in the solids remaining in the supernatant decreasing from about 30% to 1%. A midrange clarification performance at lab scale was used to test the prediction at pilot scale in a continuous-flow centrifuge. However, a large difference was observed between the laboratory prediction (79% clarification) and the observed pilot value (30%). Possible reasons for this discrepancy were then investigated. Similar recoveries obtained for both scaledown and pilot-prepared precipitate suspensions (79% and 74%, respectively) ruled out differences in the settling properties of the precipitates. Operation of the multichamber-bowl centrifuge in batch mode (i.e., prefilled with precipitate suspension) gave comparable but slightly lower performance (67% as compared with 74% clarification), the lower value at pilot scale being explained by some re-entrainment of solids during siphoning of supernatant from the bowl. Hence, the most probable explanation for the much lower recovery in the pilot-flow centrifuge than in the scaledown one is the break-up of precipitate aggregates in the feed region of the continuous machine. High flow stresses generated by the extremely rapid acceleration of material upon entrance into the pilot machine leads to aggregate breakage and hence lower recoveries. Use of a peristaltic pump excluded the possibility of precipitate damage due to pumping, as Hoare et al. (1982) demonstrated that this device showed no disruptive effect on precipitates.

As observed in Figure 7, good agreement in terms of dewatering exists for laboratory- and pilot-scale prepared precipitates processed in the laboratory-scaledown centrifuge (55% vs. 58%), with a marginally drier sediment being obtained in the multichamber-bowl (63% vs. 58%). Dewatering results in Figure 7 are presented not in terms of dry weights but values relative to the maximum compaction possible in the microfuge (RCF$_{\text{max}} = 20,000$).

To validate the analysis, the moisture content of the centrifuge sediments was measured using material prepared at the pilot scale (Table III). The sediment in the reference centrifuge was the driest (37%), followed by that obtained in the pilot (32.4%) and scaledown centrifuges (30%). This confirms that longer spin times are needed for the scaledown centrifuge to mimic the dewatering of the sediment of the multichamber bowl.

## Table III. Moisture content of sediment for samples in the reference (microfuge), scaledown (Beckman J2-MI), and pilot (multichamber-bowl) centrifuges (pilot-prepared precipitate used)

<table>
<thead>
<tr>
<th>Centrifuge</th>
<th>$t_\text{c}$ (h)</th>
<th>RCF</th>
<th>% Dewatered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ($V_{\text{ref}}/\sum C_{\text{ref}}$)</td>
<td>0.5</td>
<td>12,000</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>Scaledown ($V_{\text{lab}}/\sum C_{\text{lab}}$)</td>
<td>0.38</td>
<td>10,000</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Pilot ($Q_{\text{mc}}/\sum C_{\text{mc}}$)</td>
<td>0.75</td>
<td>Chamber 1</td>
<td>32.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chamber 2</td>
<td>31.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chamber 3</td>
<td>32.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chamber 4</td>
<td>33.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>32.4 ± 0.3</td>
</tr>
</tbody>
</table>

## CONCLUSIONS

Scaling precipitation based on a constant mean velocity gradient and aging resulted in particles with similar characteristics, as evidenced by similar clarification in the laboratory-scaledown centrifuge for material prepared at the laboratory and pilot scales. Good agreement was achieved between the scaledown and multichamber-bowl centrifuges in terms of solids dewatering and also for precipitate recovery in the batch-operated, pilot machine. The techniques developed in this study allow identification of the extent of precipitate break-up occurring in the continuous-flow centrifuge, in this case leading to approximately 50% reduction in clarification.

The expert advice of Dr. J. Maybury is recognized.

## References


Paul EL, Rosas CB. 1990. Challenges for chemical engineers in the pharmaceutical industry. Chem Eng Prog 86:22.


