

This article describes advancements in process modeling in biopharmaceutical manufacturing focusing on fermentation and chromatographic separation.

Process Modeling Proposition in Biopharmaceutical Manufacturing

by Sei Murakami, PhD, Peter Watler, PhD, Takashi Ishihara, PhD, and Shuichi Yamamoto, PhD

Introduction

Process modeling and simulation are expected to enable the identification and evaluation of product and process variables that may be critical to product quality and performance. They also may identify potential failure modes and mechanisms and quantify their effects on product quality before and during the actual processing.

However, in spite of those benefits, process modeling for biopharmaceutical manufacturing has not been developed extensively due to its reaction and molecular structure complexity. In order to contribute to the process understanding in the biopharmaceutical industry, advancements in process modeling in biopharmaceutical manufacturing focusing on fermentation as an upstream and chromatographic separation as a downstream representative unit operation will be described.

Upstream - Fermentation Limitations in Similar Figures Scale-Up

Large-scale mammalian cell culture for biopharmaceutical production is always intricate due to mammalian cell's extreme fragility and complex nutrient requirement. Avoiding cell damage, while optimizing oxygen supply

and carbon dioxide extraction, is the key to the mammalian cell culture scale-up. In order to characterize such fermentation conditions for predicting scale-up results, various empirical equations have been introduced. Since most of such equations are dependent on the figures, there is similarity between experimented equipment and scaled-up fermenter. Accordingly, fermentation scale-up has been accomplished with similar figures in order to keep reliability of such empirical equations usage. However, all fermentation characteristics cannot be kept constant simultaneously during the similar figures scale-up.¹ For example, when we scale-up a fermenter from 200 to 10,000 liters with constant impeller tip speed, volumetric power input decreases to almost one quarter - *Table A*.

Accordingly, the actual manufacturing record for the cell culture fermenter shows a rapid decline in volumetric power input during scale-up, resulting in a restriction of constant impeller tip speed - *Figure 1*.

Model Development

To facilitate a more flexible scale-up with quantitative culture environment predictions, a fermenter model independent of figures similarity needs to be provided. Modeling of a fermenter requires addressing the following prob-

Table A. Empirical equations for proportional scale-up.

Characteristics	Empirical Equations			200 L		10,000 L		
Power Input	Volumetric Agitation Power	Pg/V	$\propto \frac{n^3 Di^5}{V}$	1.00	1.00	0.20	0.27	13.6
Mass Transfer	Volumetric Oxygen Transfer Coefficient	k_{La}	$\propto \left(\frac{Pg}{V}\right)^{0.4} Us^{0.5}$	1.00	1.92	1.00	1.14	5.45
Hydrodynamic Intensity	Shearing Rate	$(dU/dz)_{max}$	$\propto nDi$	1.00	1.54	0.90	1.00	3.68
Homogeneity	Circulation Rate	Qi/V	$\propto \frac{n Di^3}{V}$	1.00	0.42	0.24	0.27	1.00

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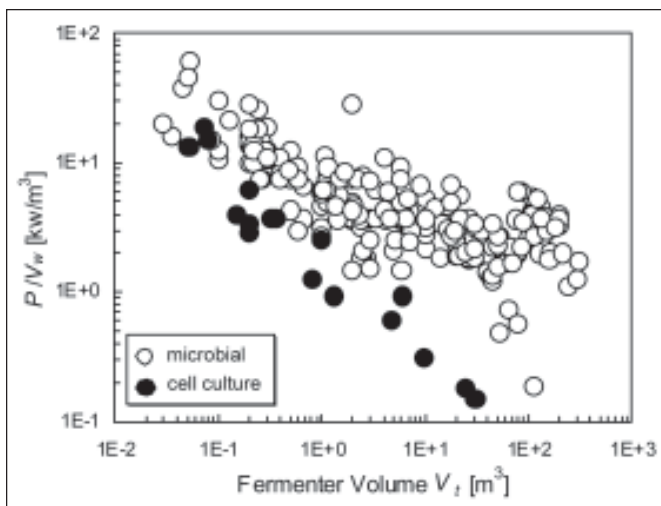


Figure 1. Volumetric power input of various scale fermenters.²

lems: turbulent flow, gas-liquid multi-phase flow, and moving boundary conditions of impellers and baffles.

In addition to using a direct solving method of the three-dimensional Navier-Stokes equation, turbulent flow has been addressed utilizing the eddy-viscosity model, the Reynolds stress equation model, the large eddy simulation, and the vortex method. Among them, a subset of the eddy-viscosity model, $k-\epsilon$ model,³ has been widely used due to its wide applicability and a moderate computational resource requirement. In spite of limitations of the $k-\epsilon$ model, such as dependence of six empirical constants and an isotropic turbulence assumption, its applicability to mixing vessel simulation has been confirmed and excellent representation of experimentally measured values, especially flow velocity, have been reported.⁴ Modeling of multi-phase flow includes the Eulerian and Lagrangian models.

The Eulerian model assumes each phase to have a separate velocity field and a common pressure field, whereas the Lagrangian model tracks representative bubbles through the domain. A large number of bubbles often observed in fermenter operation renders it unrealistic to apply the Lagrangian model's bubble tracking. A simpler subset of the Eulerian multi-fluid model is drift-flux model,⁵ which assumes the dispersed phase, bubbles, move relative to the continuous phase, liquid, at their terminal velocity. In the

drift-flux model, bubble acceleration and physical property change, usually not significant in fermentation, are neglected. With gas hold up, bubble diameter, and hydrodynamic parameters, a volumetric oxygen transfer coefficient $k_L a$ can be described. Consequently, dissolved oxygen and carbon dioxide distribution can be calculated by solving oxygen and carbon dioxide transport equations.

If a fermenter vessel does not have baffles, boundary conditions can be simpler and do not need to be changed with time by defining it rotating with impellers. However, most fermenters are furnished with baffles or other internal fixed structures making the simulation model's boundary condition multifarious. In order to avoid the complexity of time dependent boundary conditions, a method with an equivalent cylindrical block having experimentally determined boundary conditions of flow velocity and turbulence representing the rotating impellers has been introduced. Although it is simple to calculate, an impeller representative cylinder always requires actual scale experimental measurement and cannot be used for unrealized scale-up study. To avoid the empirical dependence, the dynamical multi-block method⁶ and the sliding mesh method⁷ have been introduced where impellers and baffles are modeled in a separate block rotating relatively to each other.

One of the descriptions of hydrodynamic damage to the cultured cells is Kolmogoroff Eddy Length Scale, which is assumed to be the minimum eddy size before dissipated by viscous force. If the Kolmogoroff Eddy Length Scale is smaller than that of solid particle such as suspended cells, the particle may not move to release the hydrodynamic force and may receive some damage on its surface. On the other hand, if the Eddy is larger, the particle can float on the eddy, and thus, hydrodynamic damage can be avoided.⁸

Based on the above modeling, empirical equations for fermentation characteristics shown in Table A can be substituted by the following elemental and overall parameters independent of figures similarity among different scales.

Because these parameters are independent of reactor figures, they can be used for various fermenter shapes.

Simulation

Using models described above, one now can perform fermentation environments simulations, which provide liquid flow

Characteristics	Elemental Parameters		Overall Evaluation	
	Power Input	Turbulent Energy Dissipation Rate	$\rho\epsilon$	Total Turbulent Energy Dissipation Rate
Mass Transfer	Local $k_L a$	$(\alpha Db) f(Sc, \nu, k, \epsilon)$	Total $k_L a$	$\frac{1}{V} \iiint (k_L a) dx dy dz$
Hydrodynamic Intensity	Kolmogoroff Eddy Length Scale	$(\nu^3/\epsilon)^{1/4}$	Minimum Eddy Length Scale	$\left[\left(\frac{\nu^3}{\epsilon} \right)^{1/4} \right]_{\min}$
Homogeneity	Local Concentration	C	Standard Deviation of Concentration	$\sqrt{\frac{1}{V} \iiint (C - \bar{C})^2 dx dy dz}$

Table B. Elemental parameters and overall evaluation for fermentation model.

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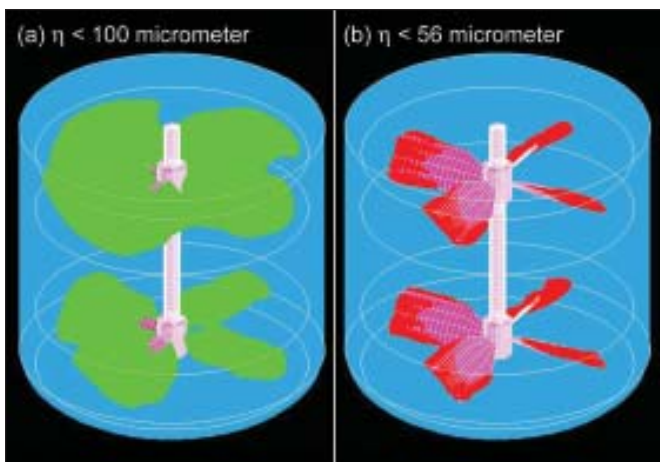


Figure 2. Kolmogoroff Eddy Length Scale distribution.

vector, gas hold up, hydrodynamic damage, volumetric oxygen transfer coefficient, $k_L a$, dissolved oxygen, dissolved carbon dioxide, etc. Due to culture liquid physical characteristics that cannot be predicted precisely prior to the simulation, primary simulation results might differ from experimental results. Consequently, appropriate adjustment for a particular fermentation is essential. This adjustment can be done by performing a couple of simulations for established bench or small-scale fermentations, then comparing the simulation and experimental results.

Process Optimization

Hydrodynamic damage, dissolved oxygen, and dissolved carbon dioxide are the key critical fermentation conditions for mammalian cell culture. The following discussion describes some of the applications of the proposed model and simulation for evaluating and optimizing a fermentation environment.

As a hydrodynamic intensity evaluation example, Kolmogoroff Eddy Length Scale distributions are shown in *Figure 2*. Depending on the size of the particle, such as a suspended cell and a micro carrier, appropriate impeller type and agitation speed, which has larger Eddy Length Scale, can be seen by changing the impeller profile in the simulation.

The dissolved carbon dioxide that is often increased by

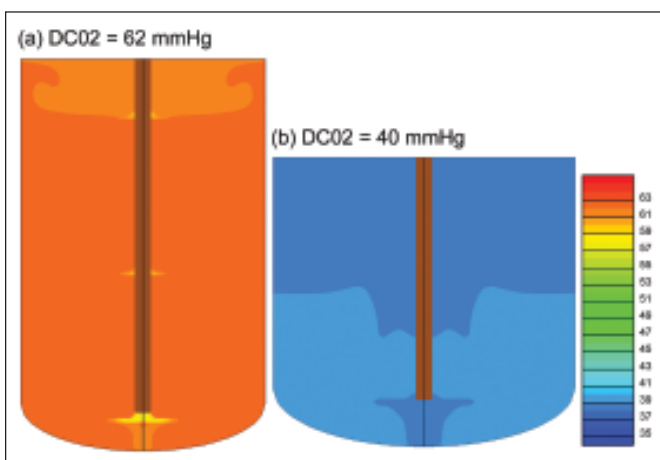


Figure 3. Dissolved carbon dioxide distribution.

scale-up will likely reduce cell growth and productivity. Because liquid surface mass transfer has a significant effect on carbon dioxide extraction, a fermenter aspect ratio needs to be optimized. *Figure 3* shows an effect of bioreactor aspect ratio on dissolved carbon dioxide reduction.

As described above, flexible fermenter configuration and operating conditions evaluations provide minimum hydrodynamic damage and dissolved carbon dioxide which lead to productivity maximization. This simulation for process optimization can be executed not only in a scale-up, but also in a scale-down experiment for an existing fermenter.

Downstream - Chromatographic Separation Chromatography Processes for Biopharmaceutical Products

Separation and purification processes of recombinant protein-based pharmaceuticals and other biological products involve a series of chromatography steps. Highly advanced simulation software is available for separation unit operations in chemical and petrochemical processes such as distillation. This type of software is now routinely used for operation and optimization of processes as well as process design. However, chromatography processes are still designed and operated on the basis of the knowledge empirically obtained or on the trial-error approaches. Therefore modeling and simulation of chromatography are important for rational design, optimization, and stable operation of processes.

Several modes of chromatography are available to exploit the charge, hydrophobicity, and size differences of the contaminants and the product. In addition, chromatography columns can be operated in various modes such as flow-through or adsorption mode with isocratic or gradient elution. Current chromatography column technology has enabled the size of preparative columns from a few centimeters to up to two meters capable of holding hundreds of liters of packing materials.

To take advantage of these many advancements, it is critical to have a well-planned development strategy and solid understanding of the separation mechanism to successfully design, scale-up, and implement a cost effective chromatographic process. Indeed, this ability is a key factor in the success of a company's process development effort and subsequent product commercialization.

When a certain chromatography process can be predicted by a model simulation, it is easy to optimize the process by tuning the operating variable and designing a better performance process. Plant constraints such as buffer and tank volumes and process time also can be incorporated into the model. The model allows rapid assessment of buffer usage, retention times, peak volumes, and estimates purity, recovery, and productivity. Also it is easy to change the conditions to observe how the separation changes. The model provides a solid understanding of the process, which parameters are important, and how and why they affect the separation.⁹⁻¹²

The following discussion will describe how to apply models to chromatography processes and what can be done with models.⁹

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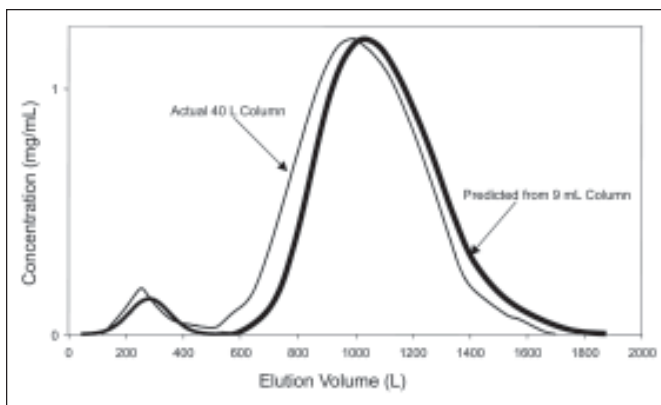


Figure 4. Comparison of isocratic elution curve predicted from the model using a small (9 mL) column linear gradient elution data and actual elution curve of a 40 L column: CM Sepharose FF.⁹

Use of Model for Scale-Up to Industrial Size Column

Chromatography peaks are characterized with the retention volume and the peak width. The retention volume is related to the distribution coefficient K , and the peak width is expressed by $HETP = Z/N$ (Z : column bed height, N : plate number).^{9,12} The model parameters K as a function of salt concentration I , and $HETP$ as a function of velocity are determined from a series of small-scale Linear Gradient Elution (LGE) experiments.^{9,13} These data also can be applied to isocratic elution. As shown in Figure 4, the model can be used to predict isocratic elution behavior of a large-scale industrial column. The predicted chromatogram closely approximated the actual chromatogram from a 40 L column with predicted retention volumes within ~15% of the actual retention volume.

Use of Model for Linear Gradient Elution Optimization

One of the most useful applications of a chromatography model is for screening operating conditions to characterize and optimize the separation. To assess the separation and challenge the model, extremes of column length, gradient length, and initial ionic concentration were evaluated.

A tall bed height with a shallow gradient slope typically maximizes peak separation, but also increases the separation time and the elution volume. Such conditions were screened with the aid of the model by adjusting the operating conditions to a 20 cm bed height and 30 (Column Volume) CV gradient. Under these conditions, the model predicts relatively broad and late eluting peaks with baseline. To verify the model, the actual chromatogram obtained under the same operating conditions is shown in Figure 5. The predicted and actual chromatograms show similar peak shapes and peak widths, and baseline separation with the main peak eluting at ~70% of the gradient. The model provided an accurate prediction of the separation under conditions for high resolution.

After identifying and verifying a high resolution separation, the operating conditions were modified to search for conditions that gave good resolution, but were more scaleable

and offered higher productivity. A shorter bed height will have lower pressure drop upon scale-up, and a more moderate gradient length will lower buffer consumptions. To compensate for the shorter gradient length, gradient slope was reduced by increasing the initial ionic concentration, I_0 . The predicted chromatogram from the more moderate operating conditions of 12 cm bed height, 20 CV gradient, and $I_0 = 28$ mM showed near baseline separation of the peaks with the product peak eluting earlier at ~55% of the gradient and with a narrower peak width⁹ - separation was verified experimentally, showing that the LGE model provided an accurate prediction under moderate operating conditions.⁹

Finally, the separation behavior under extremely low resolution conditions was studied. A very low bed height (5 cm) and a very short gradient (7 CV) required minimal packing materials, buffer and tanks. However, the model predicted that the pre-peaks merged with the product peak, appearing as a small shoulder on the product peak resulting in very poor resolution.⁹ In addition, the model predicted that the product peak eluted toward the end of the gradient (~90%). The actual chromatogram obtained at these operating conditions was very similar to the predicted chromatogram, showing a very sharp, late eluting peak with little resolution.

As discussed above, chromatography models can be used to screen a wide range of conditions in order to optimize and characterize the separation. It is important to empirically verify the proposed conditions prior to specifying them for scale-up. Table C shows that the model predicted how the elution volume and peak width changed at various column and gradient conditions. Predicted retention volume was within 3% of the actual volume for the product peak. The model showed that peak width decreased with sharper gradients and lower bed heights, but under-predicted values by 21-41%.

Use of Model to Improve Productivity

Productivity is defined as the amount of protein of a given purity, produced per unit time per liter of chromatography packed bed volume. Models can be employed to rapidly scout conditions which give the highest productivity for a given purity requirement. For the separation shown in Figure 4, the optimized isocratic operating condition gave a productivity of 0.16 g/L.h - Table D. This is largely because of the long cycle time due to the high distribution coefficient of the product peak under isocratic elution at 40 mM NaCl. One method of addressing a high K value is to use gradient elution. The model showed that a very large gradient volume (60 CV) gave comparable resolution with a somewhat higher productivity of 0.21 g/L.h. From this starting point, operating conditions were varied to search for increased productivity. A moderate bed height of 10 cm and a moderate gradient of 10 CV gave sufficient resolution to achieve 99% purity and very high (99%) recovery. One of the features of the model is that the starting ionic strength can be varied to adjust the gradient slope. Increasing the starting salt concentration to 40 mM, resulted in almost immediate

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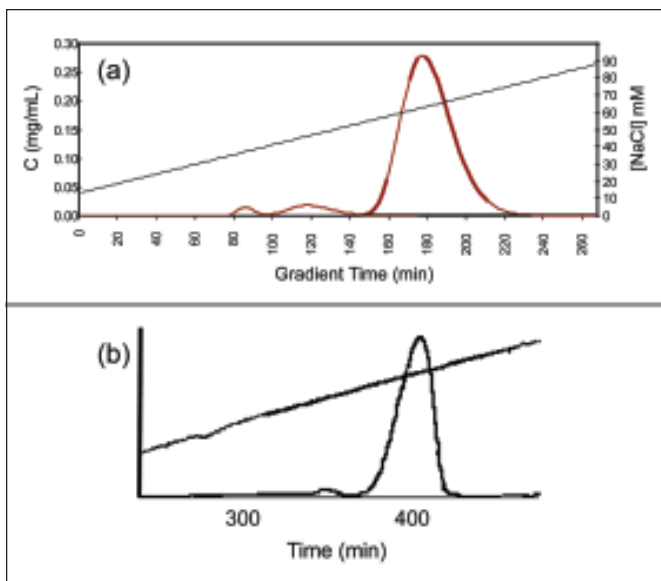


Figure 5. Comparison of predicted chromatogram (a) and actual chromatogram (b) for CM Sepharose FF, Gradient = 30 CV, diameter = 1.6 cm, height = 20 cm, l_0 = 16mM NaCl, superficial velocity = 120 cm/h.⁹

desorption of the pre-peaks as was indicated by the $K-I$ curve. At this gradient slope, the product peak elutes earlier resulting in a short cycle time and higher productivity. Another feature of this separation is that the steeper gradient results in sharper peaks and a lower product pool volume. Productivity under these conditions was 0.61 g/L.h, nearly 400% higher than the initial optimized isocratic conditions.

Use of Model for Characterization and Troubleshooting of Separation

To maintain process consistency, validated large-scale processes typically have a constraint to maintain elution volume within $\pm 5\%$. This is often needed as tank volumes are fixed and elution must be completed within planned production times. While column size, flow-rate, and protein loading are fixed by the manufacturing procedure, resin ionic capacity, buffer pH, and ionic strength will vary due to lot-to-lot variations. To assess the consistency of the separation, the model can be used to characterize and troubleshoot the effect of these fluctuations. In this application, the model can be used to elucidate the binding characteristics and to quantify changes in elution volume

with variations of media ion-exchange capacity and buffer ionic strength and the pH.¹⁴ For illustration, a model separation system of b-lactoglobulin near its isoelectric point on a weak cation exchanger, CM Sepharose at pH 5.2, was selected.¹⁴ Although the isoelectric point of this protein is 5.1-5.2, it is retained on both anion and cation exchange chromatography columns at pH ~ 5.2 .¹⁵ According to the manufacturer, the ion-exchange capacity Λ of CM Sepharose FF ranges from 90 to 130 mmol/mL-gel. Such variations can greatly affect the retention time since the distribution coefficient is related to Λ . For the recombinant protein separation shown in Figure 4, a 22% increase in the total ionic capacity from 90 to 110 mM mmol/mL resulted in a 47% increase in the distribution coefficient and the relative retention volume.⁹

In a production environment, it is impractical to consistently obtain resin of a specific ionic capacity. In order to control the retention volume, it is necessary to adjust the salt concentration I_E , of the elution buffer. In the above-mentioned model separation system with b-lactoglobulin, the relationship between the relative elution volume and I_E was examined. It was found that the NaCl concentration must be adjusted by ± 0.015 mol/L to elute the protein within $\pm 5\%$ of the reference elution volume.

This is typically done by trial and error. However, as discussed above, the distribution coefficient as a function of ionic strength can be obtained from gradient elution experimental data. Once this $K-I$ information is obtained for a given Λ , the elution volume can be predicted and the elution buffer ionic strength can be adjusted. Hence, the chromatography model can serve as a convenient tool for tuning and troubleshooting very sensitive isocratic chromatography processes. In addition, there is usually a variation in the salt concentration of elution buffers prepared at production scale. In this example, the salt concentration of the buffer must be within ± 0.002 mol/L in order to meet the $\pm 5\%$ elution volume criteria.

Due to inherent variability during preparation, buffer pH also will vary at production scale. Buffer pH affects the charged state of the of the ion exchanger which affects elution volume. The ion exchange capacity of CM-Sepharose decreases with pH below pH 6.¹⁰ The relative change in elution volume, resulting from changes in the ion-exchange capacity, Λ as a function of operating pH was investigated for the model separation system. Although variations in the relative elution volume are smaller compared with the salt concentration

Operating Conditions			Retention Volume (mL)		Product Peak Width (mL)	
Bed Height (cm)	Gradient (CV)	Initial Salt Concentration (mM)	Predicted	Actual	Predicted	Actual
20	30	16	164	162	71	44
12	20	28	110	110	56	42
12	50	16	230	228
5	7	16	66	68	42	33

Table C. Comparison between predicted and actual experimental retention volume and peak width for the product peak under various gradient elution conditions and column geometries, CM-Sepharose FF superficial velocity = 120 cm/h.⁹

and the ionic-capacity, it is still important that the buffer pH be within ± 0.1 pH unit. It is additionally important to control buffer pH since as discussed above, the interaction between the protein and the ion-exchanger changes with pH especially near the isoelectric point. However, controlling the effect of pH is more complex than controlling salt concentration or the ion-exchange capacity.

Conclusion

Advancements in process modeling for fermentation and chromatographic separation were described.

For a fermentation process, hydrodynamic and the mass transfer model have been incorporated into CFD, which enables us to overcome contradictions in similar figures scale-up. While all parameters expressing fermentation conditions cannot be kept constant simultaneously during the scale-up, the proposed model facilitates the scale-up environment prediction along with flexible fermenter configuration and operating conditions for productivity maximization. This simulation method also can be used for analyzing and understanding an existing fermentation process for further quality and productivity enhancement. Appropriate adjustment for a particular fermentation by performing a couple of simulations for established bench or small scale fermentations is necessary for more accurate performance prediction.

For a downstream process, the model simulation is a useful tool for process design, diagnosis and operations. It also is helpful to understand the mechanism of very difficult and unstable separations. We have applied the model analysis to the separation of protein variants near the isoelectric points,¹⁵ the separation of monoclonal antibodies,¹⁶ and the separation with monolithic columns.¹⁷ Further study is needed to establish a fast and simple method for determining data needed for the model simulations, and a method for obtaining important information with the aid of rapidly developing "bioinformatics."¹²

Both models for upstream and downstream biopharmaceutical manufacturing described here will provide insight and understanding of the critical process attributes, which enable superior performance prediction, proper process monitoring interpretation, in-process adjustment, and versatile troubleshooting.

FDA's regulatory framework (Process Analytical Technology or PAT) is intended to facilitate progress to the desired state of pharmaceutical manufacturing.¹⁸ In one of the PAT Tools "Multivariable tools for design, data acquisition, and analysis," mathematical relationships and models are expected to provide scientific understanding of the relevant multi-factorial relationships. In conjunction with recent biopharmaceutical manufacturing technology development concerning other PAT Tools, i.e. "Process Analyzers," "Process Control Tools," and "Continuous Improvement and Knowledge Management," these models possibly will contribute to the progress of the PAT framework.

Operating Conditions			Recovery (%)	Productivity (g/L.h)
Bed Height (cm)	Gradient (CV)	I_0 (mM)		
12	Isocratic	40	100	0.16
12	60	0	100	0.21
20	30	16	100	0.24
5	7	16	28	0.53
10	10	40	99	0.61

Table D. Optimization of operating conditions using chromatography model simulations. Comparison of recovery and productivity for various operating conditions, CM-FF, $I_f = 100$ mM NaCl.⁹

Nomenclature

C	concentration for fermenter homogeneity evaluation [-]
CV	column volume [L]
Db	bubble diameter [m]
D	diffusivity [m ² /s]
Di	impeller diameter [m]
$HETP$	height equivalent to a theoretical plate [cm]
I	ionic strength of buffer [M]
I_0	initial ionic strength of buffer [M]
I_E	ionic strength of elution buffer [M]
K	distribution coefficient [-]
k	turbulent energy [m/s]
$k_L a$	volumetric mass transfer coefficient [s ⁻¹]
n	rotation speed [s ⁻¹]
P_g	sparged mixing power [w]
Qi	impeller pumping flow rate [m ³ /s]
Sc	Schmidt number [-]
Sct	turbulent Schmidt number [-]
u	velocity vector [m/s]
Ui	impeller tip speed [m/s]
Us	reactor superficial gas velocity [m/s]
V	reactor volume [m ³]
Z	column bed height [cm]
α	gas hold up [-]
ϵ	turbulent energy dissipation rate [m ² /s ³]
η	Kolmogoroff Eddy Length Scale [m]
Λ	ion-exchange capacity [μ mol/mL-gel]
ν	kinetic viscosity [m ² /s]
ν_e	turbulent kinetic viscosity [m ² /s]
ρ	density [kg/m ³]

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