Using Aspen to Teach Chromatographic Bioprocessing: A CASE STUDY IN WEAK PARTITIONING CHROMATOGRAPHY *for Biotechnology Applications*

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Course projects are an effective way to focus students' attention[1] as students learn best when they become actively involved in solving problems.[2] Properly chosen projects can serve to prepare engineering undergraduates for industrial settings where specialized process simulators (*e.g.*, Aspen Plus, CHEMCAD, HYSIM, and PROSIM) are used extensively.[3-6] Steady-state process simulators in separations and/or design courses are already used in most chemical engineering departments.[7, 8] Recently, programs such as Aspen Chromatography allow students to model and solve liquid-phase ion exchange systems that are often operated as unsteady-state processes. Commercial simulators model these sorption processes through the solution of partial differential equations governing heat and mass transfer and algebraic equations describing equilibrium and pressure drop.[3]

Many chemical engineering students enter the field of biotechnology and bioprocessing where they are confronted with difficult purification challenges. Monoclonal antibodies represent a large percentage of new biopharmaceuticals and those currently in clinical trials. This important class of proteins often requires several downstream processing steps including: clarification, protein A chromatography, anion exchange chromatography followed by hydrophobic interaction chromatography (or cation exchange chromatography), virus filtration, and finally, ultrafiltration or diafiltration.[9] The elimination of even one of these steps can significantly reduce operating costs.

The recent use of weak partitioning chromatography (WPC) in the downstream processing of antibodies by Wyeth BioPharma (Andover, MA) has generated significant attention.[10-12] In that process, WPC is employed to purify monoclonal antibodies in anion exchange systems as part of a two-column separation platform (with Protein A chromatography) as compared to traditional three-column separation platforms. WPC is an isocratic chromatographic protein separation method performed under mobile phase conditions where the product

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protein binds weakly to the resin, in contrast to flowthrough operations[12] where no binding of the product protein occurs. A major advantage of WPC is that it can enable significantly stronger binding of impurities, resulting in improved purification as compared to the flowthrough mode of operation.[12] In addition, product losses are reduced by loading larger amounts of product. Finally, a short wash step can also be employed to attain even higher purity and resin capacity using WPC.

In this paper we describe a project that was used in an advanced chromatographic separations course (15 weeks long, offered every Spring term) taught to senior undergraduate students and graduate students for the past three years. The biotechnology-related separations challenge was derived from the WPC work presented by S. Vunnum[10] at the 2006 national American Chemical Society conference and recently published in several papers.[11-16] Many of the details of using the commercial simulator employed (Aspen Chromatography) and teaching courses with simulators integrated into lectures have been reported previously.[3, 8] The course project described in this paper was designed to instruct the students in basic simulator operation and to enable them to apply course material to an important separation challenge from the biotechnology industry.

2. THE PROJECT

2.1 Learning Objectives: The specific learning objectives[17] for this project were as follows:

At the end of this project students should be able to 1) generate and interpret adsorption isotherm and partition coefficient plots, 2) use a simulation tool for chromatographic modeling, 3) explain the subtleties of a novel mode of chromatography (WPC) through varying calculations and simulations, 4) illustrate the benefit of fractional factorial simulations as a tool for directing experiments, and 5) apply their chromatographic simulation and optimization experience to modeling other separation processes.

2.2 Project Goal: The students were instructed that their goal was to optimize the product yield (recovery) associated with the anion exchange step while satisfying several constraints. The feed mixture for this system consisted of a therapeutic monoclonal antibody (product) and two compounds representing typical impurities in these biological mixtures (*e.g.*, nucleic acids, endotoxins, viruses, or host cell proteins). These impurities are in general more strongly bound than the product of interest under anion exchange chromatographic conditions.[12, 18, 19] The process must result in a product yield greater than 90% while satisfying a 95% purity constraint. Further, the process must maximize the production rate (amount purified per unit time) and be accomplished in less than eight hours (typical work shift).

2.3 Overview: This project is based on a recent publication describing the WPC process.[12] Students were introduced to key WPC concepts, provided with batch adsorption data, instructed to plot adsorption isotherms, and then required to generate partition coefficient plots. Aspen Chromatography was then employed to guide the students through specific column simulations (varying feed loading volume and salt counter-ion concentration). Students were then charged with the open-ended task of optimizing this separation through simulations varying parameters of their choosing (factorial simulation).

2.4 Introducing Students to the Problem: The students were given extensive equilibrium adsorption data for the product and the impurities at three different salt concentrations, some of which are shown in Figure 1 as symbols. They were then asked to fit the isotherm data to an extended Langmuir isotherm with counter-ion dependence [Eq. (1)]. This isotherm is readily available in Aspen Chromatography and was chosen for its ability to represent nonlinear chromatographic behavior at different salt concentrations.

$$Q_i = \frac{\left(IP_{1i}\right)\exp\left[\left(-IP_{2i}\right)\left(c_b\right)\right]\left(IP_{3i}\right)\exp\left[\frac{IP_{4i}}{c_b + IP_{5i}}\right]\left(C_i\right)}{1+\sum_{\substack{k=1\\k\neq b}}^{nc}\left(IP_{3k}\right)\exp\left[\frac{IP_{4k}}{c_b + IP_{5k}}\right]\left(C_k\right)} \quad (1)$$

In this isotherm equation Q_i is the amount of solute i bound to the stationary phase and C_i is the amount of solute i in the mobile phase. The five isotherm parameters are represented by IP_{1i} through IP_{5i} for each solute i, c_b is the salt counter-ion

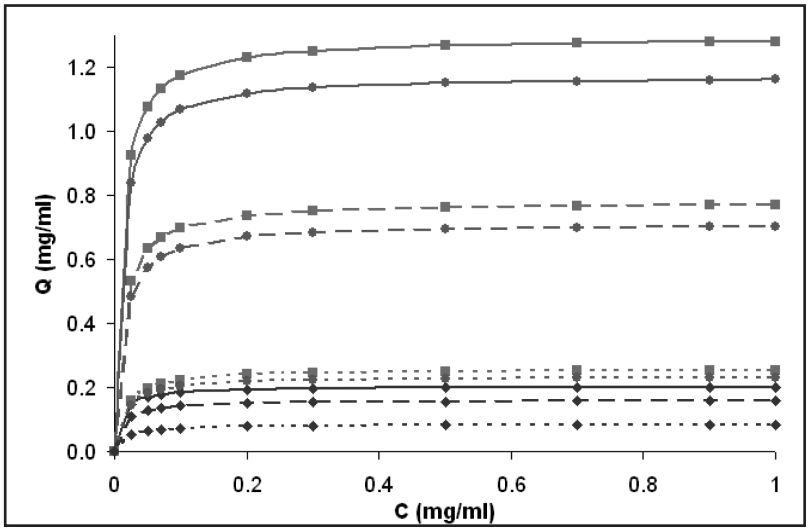


Figure 1. *Adsorption isotherms for the product (◆), impurity 1 (●), and impurity 2 (■). Isotherms are given for varying NaCl concentrations of 190 (dotted lines), 200 (dashed lines) and 225 mM (solid lines).*

concentration in the mobile phase, and C_k in the denominator summation is the mobile phase solute concentration for all solutes (except the salt counter-ion, b).

Once the isotherm plots were generated and the isotherm parameters of the product and the two impurities were obtained, the students were then instructed to determine the effect of varying salt concentration (counter-ion, c_b) upon these isotherms. Adsorption isotherms for counter-ion concentrations of 190 (dotted lines), 200 (dashed lines) and 225 mM (solid lines) are shown in Figure 1. The diamonds represent the product, the circles represent impurity 1, and the squares represent impurity 2. The students were asked to comment upon the relative binding affinity of the solutes, how the affinity of each solute changes with the salt concentration, and what their initial thoughts on possible separation strategies might be. Additionally the students were asked to comment on the salt concentration at which the isotherms of all of the solutes begin to overlap corresponding to conditions that would make it very difficult to separate the product from the impurities. For the data given in this problem a salt concentration of 270 mM was sufficient to compress the isotherms such that any process separation would be difficult. Figure 1 and all of the other figures presented in this text are representative of typical student work.

2.5 Partition Coefficient Plot and Weak Partitioning Chromatography: The generation of partition or distribution coefficient plots served to introduce students to the WPC mode of chromatographic separation. The partition coefficient [Eq. (2)] is defined as the ratio of solute bound to the stationary phase (Q) to that in the mobile phase (C) as the concentration in the mobile phase approaches zero (corresponding to the linear regime of the isotherm). The calculation of partition coefficient values is straightforward using the isotherm [Eq. (1)] with the fitted parameters.

$$K_p = \left(\frac{Q}{C}\right)_{C\to 0} \qquad (2)$$

A partition coefficient plot is a log-log plot of K_p vs. C_{salt}.[12] A representative student-generated plot is shown in Figure 2 for the product and most highly retained impurity (note: in this plot the salt concentration ranged from 150 to 300 mM).

To help the students interpret this plot they were informed that protein ion exchange separations are typically operated as either 1) bind-elute or 2) flowthrough separations. For bind-elute separations the product K_p is high under the column loading condition (often >100) while for flowthrough separations the product K_p is low (usually <0.1). WPC separations define the regime in this plot that lies between bind-elute and flowthrough separations. Typical K_p values for WPC range from 0.1 to 20.

Each of these separation modes (bind-elute, flowthrough, and WPC) has distinct advantages and disadvantages. The choice of chromatographic operation depends upon the solutes in a particular system. Students were instructed to comment on the trends observed in their partition coefficient plots at both high and low counter-ion salt concentrations. They were also asked to rate the potential utility of these three separation modes for this particular feed mixture. This enabled the students to learn about the behavior of WPC as compared to more traditional modes of chromatography and satisfied learning objective 3.

2.6 Preliminary Project Student Reports: Prior to employing Aspen Chromatography the students were required to write preliminary reports that included the following: plotted adsorption isotherms, fitted isotherm parameters, isotherms at varying salt concentrations, partition coefficient plots, and written responses to the various fundamental and applied questions mentioned above. These reports should have a detailed discussion of the isotherm and partition coefficient plots and should include the following observations: i) the product is always the least strongly bound component of the feed mixture under all salt conditions, ii) as the salt concentration was increased, the relative decrease in the amount of product bound was less than that observed for the impurities, iii) superior separations may be possible at higher

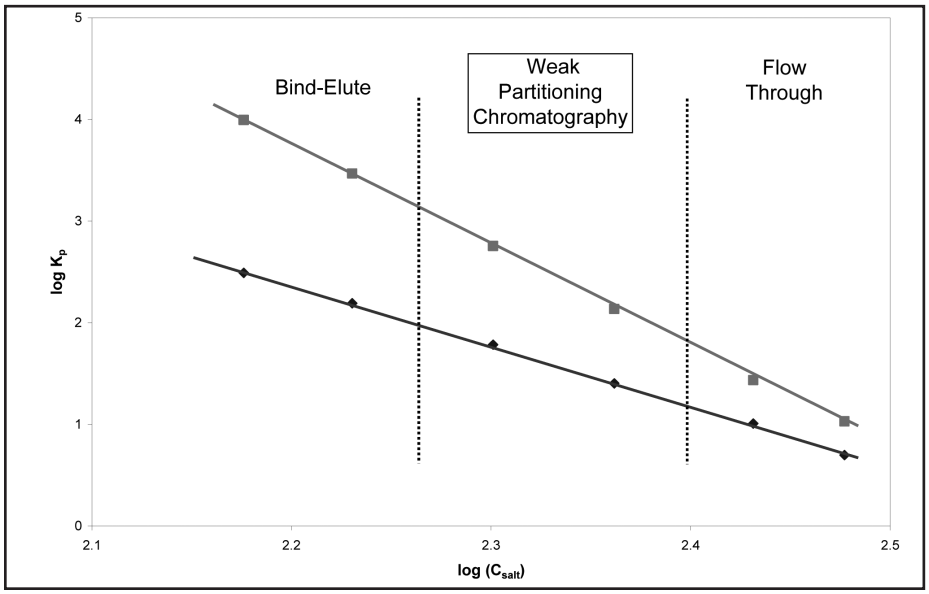


Figure 2. *A representative partition coefficient plot[12] of log K_p versus log C_{salt} for the product (◆) and the most highly retained species, impurity 2 (■) for NaCl concentrations ranging from 150 to 300 mM.*

TABLE 1 Extended Langmuir With Counter-Ion Dependence Isotherm Parameters			
Isotherm Parameters	Product	Impurity 1	Impurity 2
1	33.87	66.09	70.55
2	25.56	3.13	3.02
3	7.58	1.16	1.13
4	0.48	2.39	2.40
5	0	$1.08*10^{-3}$	$9.93*10^{-4}$

salt concentrations, and iv) flowthrough or WPC modes of chromatography may result in improved ion exchange chromatographic processes. These exercises served to meet the first and third learning objectives.

Isotherm parameters (Table 1) were then given to students to compare with their obtained values and for use in Aspen Chromatography.

Data and insight gathered were then used in the Aspen Chromatography simulation platform for optimizing this anion exchange separation. From this point forward, each student was told to use the isotherm parameters provided by the instructor (note: the use of inappropriate isotherm parameters could result in simulator non-convergence since solute profiles can become quite steep for these nonlinear isotherms[3]). Students were then introduced to the commercial simulator through a hands-on computer laboratory lecture. Example problems were reviewed with the students under the guidance of the instructor. In this classroom environment the instructor was able to visit with each student and address the individual questions raised.

3. ASPEN CHROMATOGRAPHY

3.1 Process Flowsheet: The Aspen Chromatography software was used to generate a model using the isotherm parameters and column properties given below along with appropriate Solver Properties (note: representative solver properties are given in the appendix for those unfamiliar with Aspen). While the commercial simulator has been described in detail elsewhere[3, 8] it is instructive to briefly introduce it here.

The Templates and Demonstrations given in the Aspen software package are useful for gaining familiarity with the simulation platform. Clicking on "File" then "Template" or "Demonstration" loads process flowsheets for sample problems already stored within the Aspen chromatography simulator. There are brief descriptions of each example flowsheet. After runnings the simulation, results can be viewed by selecting "Tools," "Report," and then "Chromatography_Report." These Template and Demonstration files help the user get acquainted with the simulation platform and are easily adapted to a range of other separation problems.

For the students to construct their flowsheets with corresponding models (Figure 3) they were also instructed on how to use the Cycle Organizer. By clicking on appropriate

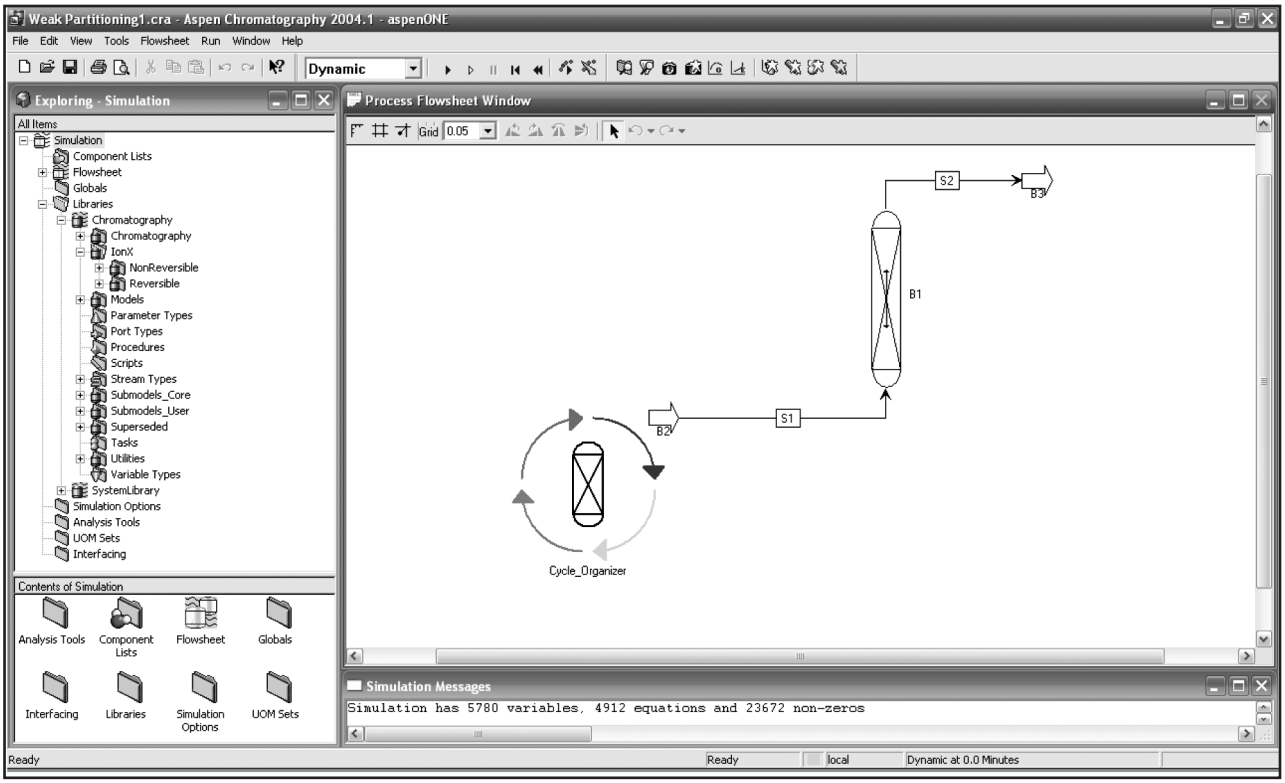
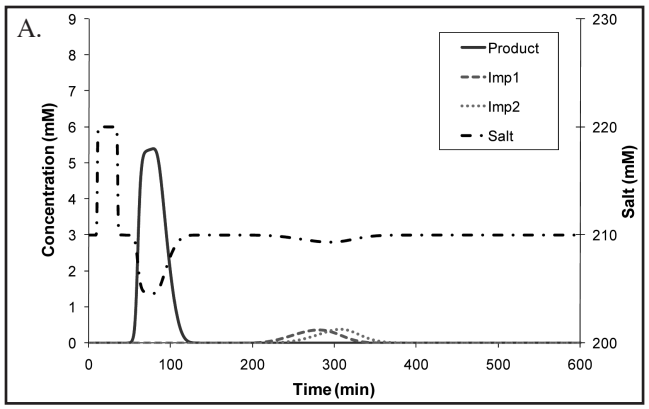
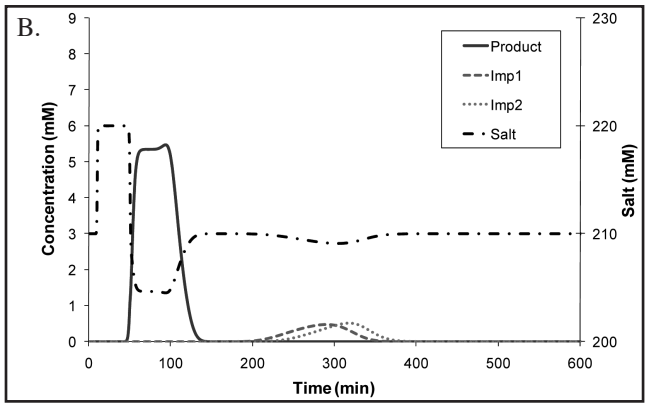
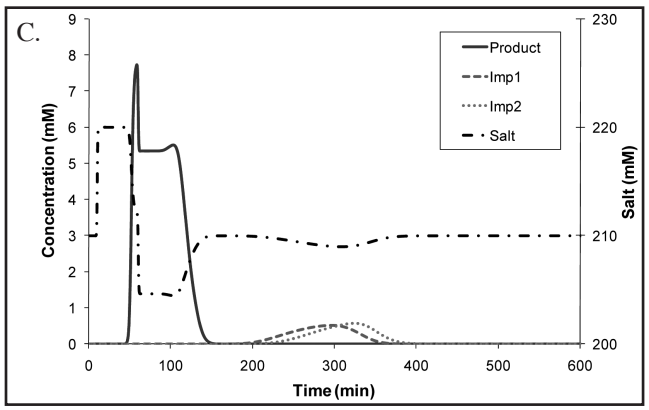
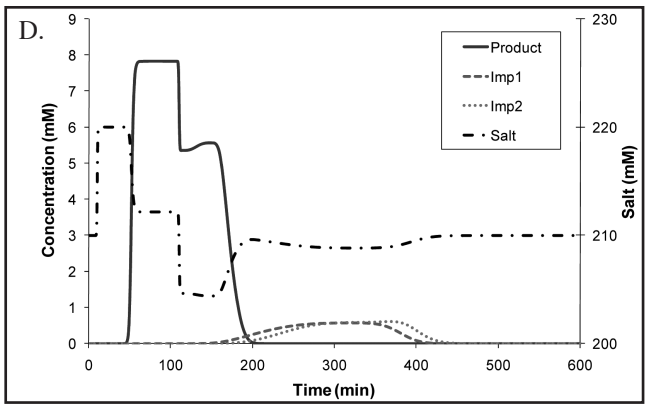


Figure 3. *Aspen Chromatography graphical user interface and representative flowsheet.*









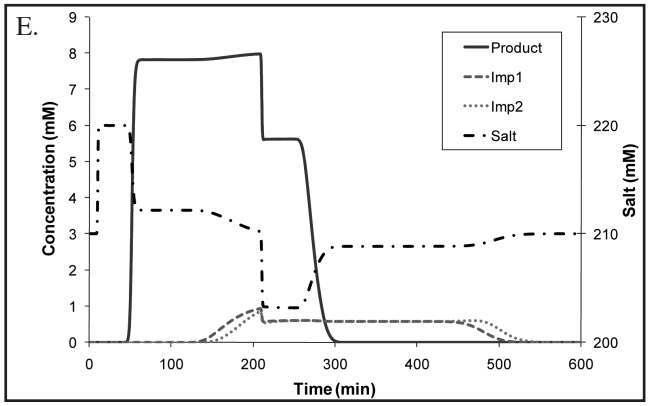


Figure 4, A through E. *Simulated chromatograms for separations with varying feed volumes at constant inlet NaCl concentration of 210 mM (dash-dot line), flow rate (10 ml/min), and feed concentrations [8 mM product (solid line), 1 mM impurity 1 (dashed line), 1 mM impurity 2 (dotted line)]. Feed loading varied from a) 25, b) 40, c) 50, d) 100, and e) 200 minutes.*

directories in the "Exploring-Simulation" window on the left side of Figure 3, they can readily construct the flowsheet and assign appropriate models (*e.g.*, ionx_r_feed, ionx_r_column, and ionx_r_product) to the column.

3.2 Simulation Parameters: For the purpose of the simulations a liquid chromatography column (length = 40 cm, Inner Diameter = 2 cm) with a stationary-phase resin (the same used for generating the batch adsorption data and determining the isotherm parameters) having the following properties was used: inter-particle voidage of 0.40, intra-particle voidage of 0.70, bed capacity of 30 M, and 50 micron radius spherical particles.

The following simulation assumptions were used: liquid viscosity of 1 cP, spherical stationary phase resin particles having SFac = 1 (measure of particle uniformity), constant mass transfer coefficients (MTC) of 100,000 min^{-1} for the solutes, material balances assuming convection with dispersion based upon plate numbers (400 plates for the counter-ion and 150 plates for each solute), a solid film model assumption, and a linear lumped resistance kinetic model assumed. The simulations were set to allow varying pressure with constant velocity for the "Pressure Assumption." The "BUDS" partial differential equation discretization method with 100 nodes was sufficient for the calculations.

Initially the students were instructed to use a feed loading time of 25 minutes and a flow rate of 10 ml/min (about 2 column volumes). The concentrations of the product and impurities in the feed mixture were set to 8 mM product, 1 mM impurity 1, and 1 mM impurity 2. While it is a good assumption that the product comprises between 90-95% of the WPC feed, the product and impurities concentration values were chosen for ease of illustration during instruction. The feed stock counter-ion concentration was always set equal to that of the column running buffer for each WPC simulation as these separations are carried out under isocratic conditions. Through this hands-on computer laboratory lecture the students were then able to independently run Aspen, satisfying learning objective 2.

3.3 Factorial Simulations: After appropriately configuring the process flowsheet factorial simulations were employed to optimize the separation. Factorial simulations allow for the study of a given factor's effect upon a response variable as well as interactions between factors. If the number of experiments for a full factorial design is too high, a fractional factorial design may be performed in which some of the possible combinations (usually at least half) are omitted.

As outlined above the process specifications required 90% or greater product recovery (yield) with at least 95% purity. The optimal separation processes should maximize the product purified per unit time while not exceeding a typical eight-hour shift limitation. The students were required to perform factorial simulations using the Aspen Chromatography simulator to vary the feed loading volume and salt concentration in the column running buffer. Further parameters (feed concentration and flow rate) can also readily be examined and were included as an extra-credit option in the present form of this project. Students were initially instructed to approach the problem in an explicitly outlined manner and later asked to optimize the process through more "open-ended" questions.

3.3.1 Varying Column Loading Volume: The following simulations were aimed at instructing the students of WPC separation subtleties and illustrating the benefit of factorial simulations, addressing learning objectives 3 and 4. The first process variable the students were asked to study was the effect of the feed loading volume upon the product production rate. Constant pH, salt (210 mM), flow rate (10 ml/min), and feed concentrations (8 mM product, 1 mM of each of the two impurities) were used. For each simulation the salt concentration in the loading buffer was set equivalent to the running buffer. Simulations using feed loading times of 25, 40, 50, 100, and 200 minutes were required of the students. Results from these simulations are shown in Figure 4 A-E. In these figures the concentration (mM) of the product (solid line), impurity 1 (dashed line), and impurity 2 (dotted line) are given on the left hand y-axis, the salt (dash-dot line) concentration (mM) is given on the right hand y-axis, and the separation time is given on the x-axis (minutes).

Figure 4A shows the WPC separation for a feed loading time of 25 minutes. In this plot an induced salt wave is observed early in the chromatogram due to the desorption of salt during solute binding. The product does not reach its plateau concentration and there is sufficient resolution between the product and each of the two impurities that would allow the process engineer to further increase the feed loading time while still satisfying the constraints on the process.

As the feed loading times increased (Figures 4 B-E) the product peak began to broaden (Figure 4B, 40-minute feed load) and develop a spike (Figure 4C, 50-minute feed load). This spike is due to the competitive binding of the induced salt wave and the product.[20] Further increases in the feed loading time to 100 minutes resulted in broadening of the concentration spike into a two-step concentration breakthrough of the product (Figure 4d). Under these conditions, however, the product began to overlap with the impurities adversely affecting the yield and productivity. A further increase in the feed loading time to 200 minutes (Figure 4e) resulted in greater product losses due to overlap with the impurities.

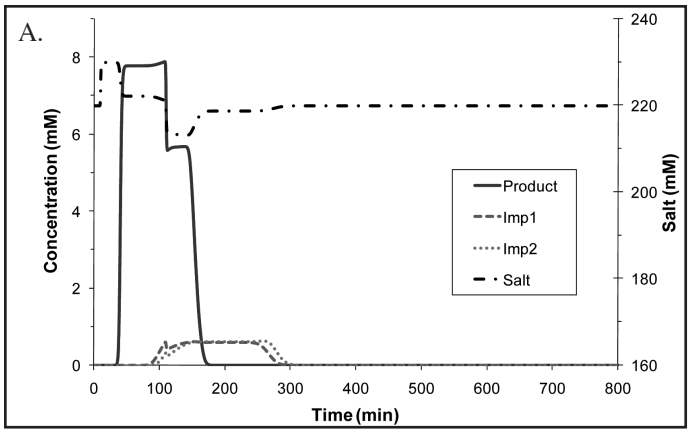
From this set of simulations the students should have noted that there exists an optimum WPC feed loading time for a given salt concentration. Loading times that were too small under-utilize the column capacity available while larger loading times eventually suffer from product losses.

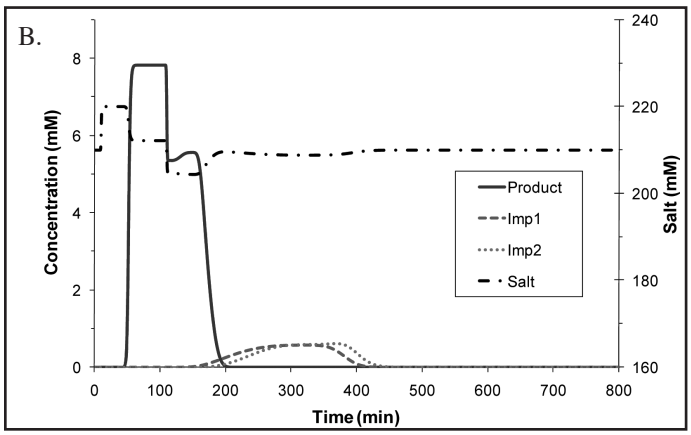
3.3.2 Varying Salt Concentration: The students were instructed to study the effect of varying the salt concentration under which the WPC separations are performed. Simulations at specific salt concentrations (220, 210, 200, and 170 mM NaCl) were required of the students. Figures 5 A-D (next page) show chromatograms for these simulations that were performed using a feed loading time of 100 minutes (note: this feed load corresponded to the conditions where the product began to overlap with the impurities in Figure 4). Students were asked to comment on the changes observed for these parametric WPC simulations (breakthrough times of the product and impurities, time required for the entire separation, shapes of the peaks obtained, etc).

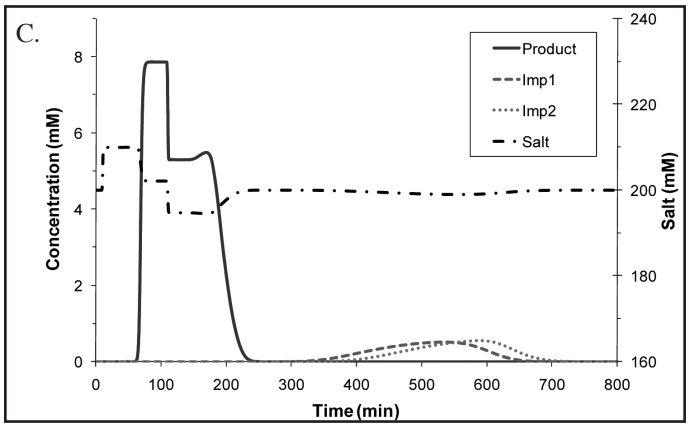
As the salt concentration is increased to 220 mM (Figure 5A) and above, the separation becomes more of a flowthrough operation with product yield diminished due to early eluting impurities. In fact, this illustrates the potential advantage of WPC over more commonly used flowthrough operations. As the salt concentration is decreased (Figures 5 B-C) WPC becomes the dominate mode of separation with increased resolution between the product and impurities. At 200 mM salt complete separation is achieved between the product and the impurities even at this elevated feed load of 100 minutes. Further decrease in salt concentration to 170 mM (Figure 5D) resulted in even greater separation between the product and impurities at the cost of significantly longer separation times. A typical shift time for workers in a manufacturing plant (eight hours) would make such long separation processes impractical. Further decrease in the isocratic operating salt concentration would result in product K_p values characteristic of bind and elute separations. Clearly, as with the feed loading time, there exists an optimum salt concentration for performing WPC separation of a given feed stock.

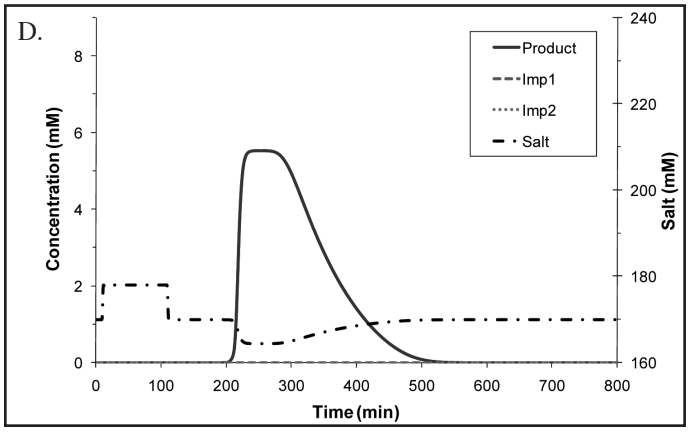
These simulations illustrated the benefits of factorial simulations and enabled students to learn about the behavior of WPC as compared to more traditional modes of chromatography, satisfying learning objectives 3 and 4.

3.3.3 Fractional Factorial Simulations: Data from preliminary project reports along with the parametric simulations carried out (Figures 4 and 5) now positioned the students to solve the open-ended task of optimizing this separation. The









students were asked to use the Aspen Chromatography simulator to determine the optimum separation conditions (maximized product yield per unit time for a given purity constraint) for this system which did not exceed an eight-hour shift limitation typical in industrial settings. Results from a systematic fractional factorial design approach varying the feed loading volume (11 discrete values) and salt concentration (5 discrete values) are outlined below.

The maximum productivity for the system is given by Eq. (3) where T_{Total} is the total run time for a given column separation, M is the amount of product protein loaded (*i.e.*, V times C, where V is the volume and C is the concentration of protein loaded). Regeneration and re-equilibration times are not included in this since it is expected that these will be the same for all WPC processes for a given feed/column system. Eq. (3) can be rewritten in the form of Eq. (4) where F is the volumetric flow rate and $T_{loading}$ is the feed loading time.

$$f = \frac{M}{T_{Total}} \tag{3}$$

$$\max : f = \left(\frac{\left(T_{loading}\right) \times \left(F\right) \times \left(C\right)}{T_{Total}} \right) \tag{4}$$

The decision variables that can be used to maximize this objective function are the flow rate, feed loading time, concentration of the feed components, and the salt concentration used for the separation. The students were not given explicit separation conditions for these separations but were allowed to vary the parameters as they saw fit as long as the constraints were satisfied.

Simulations performed under these various conditions were then used by the students to produce graphs of the productivity (mass of product per time) as a function of feed loading volume and salt concentration (Figure 6). In this representative productivity bar graph, the data represented by unfilled bars (purity) and half-filled (shift time) did not satisfy the constraints, while the other data (full bars) did.

From this analysis, the students were then asked to select the optimal operating conditions for the purification of this product from the impurities. For example in Figure 6 the optimum result would correspond to a salt concentration

Figure 5, A through D (all, left). *Simulated chromatograms for separations with varying NaCl concentrations (dash-dot line) at constant feed loading time (100 min), flow rate (10 ml/min), and inlet feed concentrations [8 mM product (solid line), 1 mM impurity 1 (dashed line), 1 mM impurity 2 (dotted line)]. Salt counter-ion concentrations were: a) 220, b) 210, c) 200, and d) 170 mM NaCl.*

of 200 mM and a feed load time of 250 minutes. Students were also required to submit representative simulations results illustrating the trends observed in their data and to comment upon how the optimal conditions were obtained. Additionally the students were asked to comment upon the separation conditions for which the constraints were not met. Finally, inspired students were encouraged to take their analysis further by investigating the effect of varying the concentration of the feed components (keeping the relative concentrations constant) and the flow rate (using appropriately modified mass transport coefficients) on the productivity of WPC separations.

4. PROJECT ASSESSMENT

In this section we summarize how learning objectives were satisfied, provide student feedback to various aspects of the project, and provide guidance upon how instructors can assess students through this project. The specific learning objectives[17] for this project were as follows: At the end of this project students should be able to 1) generate and interpret adsorption isotherm and partition coefficient plots, 2) use a simulation tool for chromatographic modeling, 3) explain the subtleties of a novel mode of chromatography (WPC) through varying calculations and simulations, 4) illustrate the benefit of fractional factorial simulations as a tool for directing experiments, and 5) apply their chromatographic simulation and optimization experience to modeling other separation processes. Project description handouts provided to the students contained information on current biotechnology separation challenges and taught them about WPC (objective 3). The tasks they were required to perform taught them how to generate and interpret adsorption isotherm and partition coefficient plots (objective 1) and introduced them to the use of simulation tools for chromatographic modeling (objective 2). Further, as the students performed the required simulations under varying conditions (*e.g.*, feed load and salt concentration) the subtleties of WPC separations became apparent (objective 3). The students were also able to observe trade-offs between maximizing productivity while minimizing the overall process time and satisfying process constraints. The students learned the benefits of fractional factorial simulations (objective 4) during the optimization of their processes. By the end of the project, the students were comfortable and proficient Aspen Chromatography users (objective 5) and they indicated that they felt they had learned the necessary skills to model other separation processes (objective 5).

The student response to the addition of this simulation-based project into the course was quite favorable. While the students had not previously used Aspen Chromatography they had some familiarity with Aspen software through previous chemical engineering courses. The first year that the project was used in the course, the students indicated that more experience with the Aspen simulator in general would have been useful prior to the assignment. This was addressed in the following year by including a hands-on computer laboratory in the lecture sequence. In this studio format, the students were able to get assistance from the instructor and their peers as problems arose, significantly improving the students' experience. Student enthusiasm was apparent, since the entire class stayed well after the instructional period had ended in order to continue learning how to properly use the chromatographic simulator.

Students commented that they enjoyed visualizing the separations that they had been studying earlier in the course and that these exercises significantly strengthened their understanding of chromatographic separations. They also commented that this project enabled them to better understand the implementation of many of the course concepts and theories

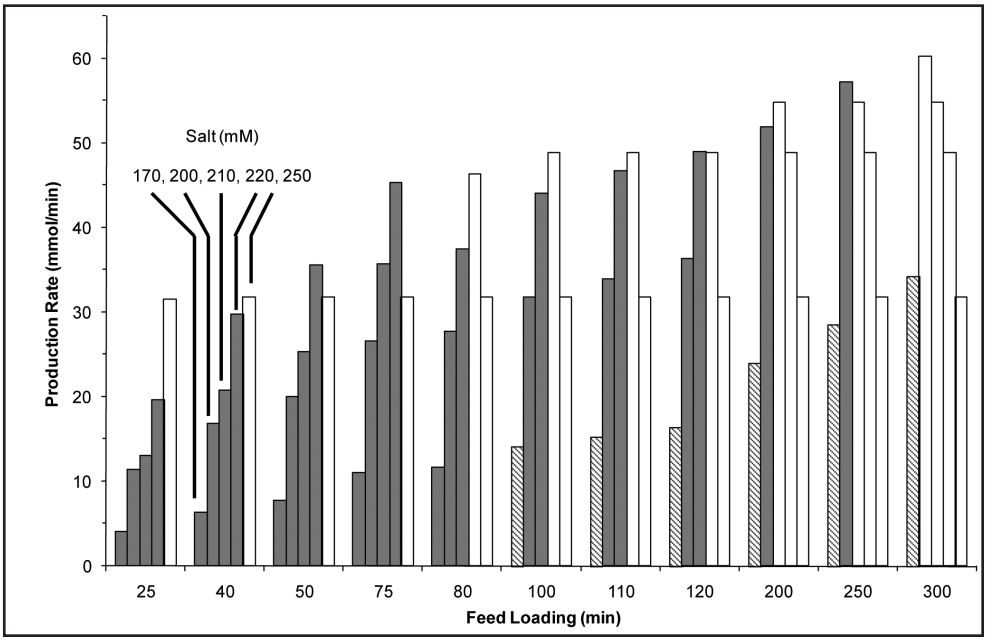


Figure 6. *Representative productivity bar graph for varying feed loading time and NaCl concentrations. For each feed loading time shown on the x-axis results are given at 5 NaCl concentrations of 170, 200, 210, 220, and 250 mM from left to right. The data represented by unfilled bars (purity) and half-filled (shift time) did not satisfy these particular constraints, while the other data (full bars) satisfied all constraints.*

for an actual separation process. Finally, several students commented that this simulation module was their favorite part of the course.

Student assessment was based on two written reports that they were required to submit, one prior to using Aspen and one after. The students were assessed for their ability to generate the required data and figures (*e.g.*, isotherm plots, partition coefficient plots, required simulated outlet chromatograms, and simulations performed for the optimization of the process) as well as for their understanding of these processes as evidenced by the discussions in these reports.

5. CONCLUSIONS

The commercial simulator Aspen Chromatography was employed to study and optimize an important new industrial separation process, weak partitioning chromatography. This case study on antibody purification was implemented in a chromatographic separations course. Students initially were asked to manipulate adsorption data to determine adsorption isotherm parameters and to study the effect of salt on the isotherm behavior. A preliminary report was turned in at this point that also included their responses to a number of questions to probe their knowledge of the subject matter.

The students were then requested to carry out detailed sets of parametric simulations to investigate the effect of operating parameters (*e.g.*, feed load, salt concentration) on the productivity and yield of this separation process. The course project served to teach students basic simulator operation, apply course material to a separation challenge from the biotechnology industry, and encourage open-ended problem exploration for process optimization.

ACKNOWLEDGMENTS

This research was supported by NIH Grant 5R01 GM047372. Helpful discussions with Alexander Freed are also gratefully acknowledged.

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APPENDIX

DIAGNOSTICS (Solver reporting level: low; Properties reporting level: none; Watch group: 0; Watch torn sub-group: 0; Check procedure derives: off; Relative checking tolerance: 0.001; Absolute checking tolerance: 0.001; Check the list variables in equivalences for highest variable steps and residuals).

TOLERANCES (Absolute variable tolerance: 1e-007; Relative variable tolerance: 1e-007; Absolute equation tolerance: 1e-007; Variable change tolerance: 1e-007; Numerical derivative absolute perturbation: 1e-005; Numerical derivative relative perturbation: 1e-005; Explicit event tolerance: 1e-005; Uncheck Solver scaling; Check Eliminate equivalence equations; Check Use Group Decompositions).

TEARING (Procedure tearing: Update; Tear update strat-

egy: Direct; Relative tear tolerance: 1e-005; Absolute tear tolerance: 1e-005; Maximum number of tear iterations: 100).

INTEGRATOR (Integration method: Gear; Maximum order: 5; Absolute integration error tolerance: 0.0005; absolute tear error tolerance: 1e-005; relative integration error tolerance: 0.0005; relative tear error tolerance: 1e-005); Uncheck include sensitivity errors; Uncheck reconverge torn variables; Select that the integration error test includes States only; Variable Initial step size of 0.001; Minimum variable step size: 0.0001; Maximum variable step size: 0.1; Variable step reduction factor: 0.5; Uncheck Always enforce minimum step size; Check Interpolate communication time; Uncheck Locate model discontinuities; Uncheck Re-initialize after variable step-change; Check Use initial step size after variable step-change; Show 0 highest integration errors; Show 0 highest tear integration errors).

LINEAR SOLVER (Name: MA48; Drop tolerance: 0; Re-analyze threshold: 2; Pivot tolerance: 0; Re-analyze FLOPS window size: 0; Re-pivot every 0 factorizations; Solver searches 3 columns for pivots; Uncheck use transpose).

NON LINEAR SOLVER (Mode: Standard; Method: Mixed Newton; Convergence criterion: Residual; Maximum divergent steps: 20; Maximum step reductions: 20; Maximum iterations: 500; Maximum fast Newton steps: 8; Uncheck Dogleg method; Maximum range fraction tolerance: 0; Maximum approach to bound: 1; Absolute perturbation: 1e-005; Singularity perturbation: 0.01; Maximum variable step: 50; Clip factor: 1e-006; Highest variable steps: 0; highest residuals above tolerance: 0; Print linear algebra for groups of size > 0; Uncheck Enabled homotopy).

ESTIMATOR (Estimator: Least Squares; Solver: NL2SOL; Reporting level: High; Solution convergence tolerance: 0.0001; Maximum iterations: 2000; Relative function tolerance: 0.0001; Absolute function tolerance: 0.0001; False convergence tolerance: 0).

OPTIMIZER (Optimizer: FEASOPT; Reporting level: Medium; Maximum iterations: 100; Solution convergence tolerance: 0.0001; Maximum relative step: 10; Maximum absolute step: 10).

HOMOTOPY (Initial homotopy step: 0.1; Maximum homotopy step: 1; minimum homotopy step: 0.01; Step size increment factor: 10; Step size decrement factor: 0.5). ❒