Teaching a Bioseparations Laboratory: From training to applied research

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Introduction

One of the problems in the teaching of many laboratories is the lack of direct participation of students in the experiments. This trend has become more acute as the instrumentation used in laboratories has become more and more expensive. Their lack of direct participation in the experiments has not precluded us to expect them to produce meaningful data in spite of their lack of experience. We start evaluating the students during their basic learning process increasing tensions in the classroom and leaving little room for errors.

A widespread practice in the teaching of laboratories is the generation of a laboratory guide that the student follows step by step. The guides are often quite comprehensive and they leave little room for improvisation. After completing their work, the students write a report following a pre-established template that often defies the minimum rules of good writing. At the end of the semester, the student moves happily into the next set of courses. After graduation, the student cannot function in a laboratory setting because she was never trained to function in one but rather to follow a series of commands. We strongly believe that such a deficiency needs to be addressed. Poor communication between the basic sciences and our discipline places that burden on us.

The students at the Chemical Engineering Department of Missouri University of Science and Technology (formerly the University of Missouri-Rolla) take a series of laboratory courses in the Chemistry, Biology and Chemical Engineering Departments. The Missouri S&T Chemical Engineering curriculum has a Biochemical Engineering Emphasis program in which the unit operations laboratories have been replaced by a Biochemical Separations and a Bioreactors Laboratories. In addition, the students take several Biology classes including molecular genetics, general biology and microbiology; two of them, at least, have laboratory sessions. I have been teaching the Bioseparations Laboratory for the last 15 years. It did not take me long to realize that the ability of the average student coming into my class (normally first semester seniors) to function in a laboratory setting is, at least, questionable. Most of them are not able to use a balance properly, do not know how to operate a micro-pipette, but more importantly, do not know how to “move” or function in a laboratory.

The difficulties that the students have functioning in a laboratory may be difficult to quantify but it is very easy to find a few examples here and there. Once, a young student came to see me in distress. There was some fear in her eyes. I sat down ready to listen some disastrous news. “I needed to use the small centrifuge but when I tried it did not work properly. The centrifuge was moving very fast along the bench; I was afraid it was going to fall down so I stopped the experiment”, she said. I sighed in relief. Nothing has been broken and the students were in one piece. The case of the stubborn balance happened a few years after. A

student was (trying) weighing potassium phosphate on a top load balance to prepare a buffer. I was in the vicinity when I heard: “This balance is definitely not working!!!”. Oh Oh, I thought, here it goes my budget for the semester. As I asked him what was the problem he began demonstrating to me how he was adding a lot of material to the weighing dish but the numbers in the digital display did not change. When I noticed that he did not remove the balance cover I couldn’t but laugh. More tragic (and costly) are the story of constant pH pH-meter caused by the smashing of the electrode against the bottom of a beaker and the story of the “broad” range micro-pipette (or how to measure 1.1 ml with a 100 to 1000 µL adjustable micro-pipette). These stories are all from my Bioseparations Lab and they were partly caused by what I like to call “engineer” hands. Rugged and fat hands that, without proper training, are good to disassemble a centrifugal pump but whose dexterity to handle micro-liters of material is questionable. Somehow, I feel sympathetic to my chemistry and biology colleagues who do not allow the students to use anything in their laboratories. Of course, their approach does not teach the students anything but, at least, it is cheap. Obviously, better approaches are necessary.

Our Approach

We decided, a few years back, to attempt to alleviate the poor laboratory training of the students by dividing our Bioseparations Laboratory into two parts: Training and Project. During the first weeks of the semester, the students learn the fundamentals of various separations techniques (mainly membrane filtration and chromatography) as well as ancillary techniques (for example, centrifugation, spectroscopy, use of pH and conductivity meters, gel electrophoresis, etc.). This portion of the semester consists of six to 8 short experiments (one to two weeks each) in which the students follow recipes and write technical reports in scientific journal format. There is not penalty for failure as long as they have been careful in their work. This training section requires close monitoring of the students. We have found that with the exceptions of Ph.D. candidates in the author’s laboratory teaching assistants are not very useful in our laboratory. Therefore, many semesters I have taken the sole responsibility of teaching the class. Although the number of contact hours is considerable during the training portion of the lab that is somehow compensated during the project part in which the students work mostly alone.

A few laboratories that we use for training purposes are included in Table I. This list is not exhaustive as the type of experiments included in the training section is tailored to the type of projects planned for the second part of the semester. The students need to be trained in general laboratory practices but at the same time they must prepare themselves for the operations that they will use in a particular project. The only recurrent themes in the laboratories included in Table I are chromatography and ultrafiltration because of the undisputable importance of these operations in the bioprocessing sector. We have included in the table the main tasks involved in each laboratory as well as the overall goals of each training section.

A common goal of each training laboratory is to gain general laboratory skills. Table II summarizes the basic skills that the students learn in the training laboratories. Notice the redundancy of many of them. We have found that repetitive direct instructions are necessary for the students to perform properly. The training in the use of each piece is quite intensive.

For example, the students learn the working principles of a pH electrode, how to operate a pH meter and to calibrate it.

Table I. Examples of training laboratories		
Laboratory	Tasks	Goals
Gel Permeation Chromatography	Buffer Preparation Swelling a Gel Packing a Low Pressure Column Running a Low Pressure System Preparing a Calibration Curve	Learn how to pack a column. Learn how a low pressure chromatography system works. General Lab. skills
Ion Exchange Chromatography	Buffer Preparation Swelling a Gel Packing a Low Pressure Column Running a Low Pressure System	Learn how to pack a column. Learn how a low pressure chromatography system works. Learn how to make gradients. General Lab. skills
Partitioning	Preparation of Phase Systems Mixing Centrifugation Sampling the Phases Protein Assay Enzymatic Assay Calculation of Partition Coefficients Statistical analysis of the Results	Learn how to do a Liquid/liquid extraction. Learn enzyme kinetics. Learn protein quantification protocols. Learn statistical analysis of data. General Lab. Skills
Isoelectric Focusing	Preparation of Gels Running the Gels Developing the Gels Documenting the Gels	General Lab Skills. Photo-documentation. Electrophoresis
Cross-Flow Filtration	Preparing buffers and solutions Running a Cross-Flow Filtration Unit Statistical analysis of results Calculation of Mass Transfer Coef.	Learn about concentration polarization. Applied mass transfer principles to filtration. General Lab Skills
Dialysis	Preparation of Dialysis bag Performance of Dialysis Calculation of Diffusion Coefficients	Learn about diffusivity through porous media. General Lab skills.

Table II. Mapping between basic skills and training laboratories.							
Laboratory	pH-meter	Balance	Conductivity meter	Micropipettes	Centrifuge	Spectrophotometer (Absorbance)	Spect. (Kinetics)
Gel Permeation	■	■		■		■	
Ion Exchange	■	■	■	■		■	
Partitioning	■	■		■	■	■	■
Ultrafiltration	■	■		■			
Isoelectric focusing		■		■			
Protein Precipitation	■	■		■	■	■	
Dialysis	■	■				■	

As an illustration, we describe below the experiments the students perform in the ultrafiltration laboratory. Ultrafiltration is a means of concentrating dilute biological solutions that are heat sensitive. The solution to be concentrated is added to a cell that contains a membrane that prevents large molecules from passing through. The membrane pore sizes range from 0.001 to 0.02 μm. Ultrafiltration can also be used to partially separate proteins whose size difference is relatively large. Pressure is applied to force liquid through the membrane until the volume has been reduced to the desired amount. A common problem with ultrafiltration is gel polarization, which is a build-up of the solute at the surface of the membrane which reduces the flux through the membrane.

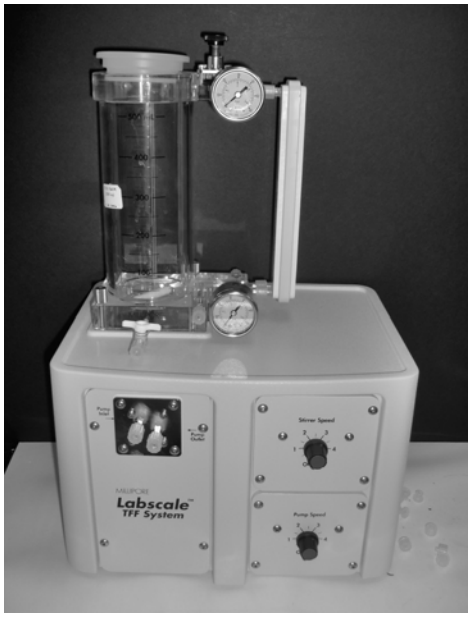

Figure 1. TFF system by Millipore

The learning objectives of this laboratory are: 1) To understand the functioning of a cross-flow ultrafiltration device; 2) To understand the concept of concentration polarization; and 3) To determine the mass transfer coefficient on the retenate side of the membrane (in the pressure independent regime). The students perform all the experiments in duplicate. The students are asked first to collect the necessary materials, which are Dextran (MW 150,000 or higher) and NaCl. Then, they prepare a saline solution (0.050 M NaCl) and dextran solutions at various concentrations in 0.050 M NaCl. The students are then introduced to the particular instrument. For example the Labscale TFF system by Millipore (Figure 1) equipped with a cross filtration membrane modulus with a molecular weight cut off of 100,000. The instructor describes the main components of the instrument, briefly indicates its capabilities and more importantly, he offers the students a copy of the manual and points out at the home page of the manufacturer.

The students then perform several runs in the instrument varying the concentration of dextran. Their objective is to identify the pressure independent regime. The laboratory is very time demanding because washing between runs takes approximately 90 minutes. After enough data has been collected they used the equation below to estimate the mass transfer coefficient (k_o) and the membrane concentration (C_{AW}),

$$J = k_0 \ln \frac{C_{Aw}}{C_{Ab}} \qquad (1)$$

where J is the mass flow-rate and C_{Ab} is the concentration of the dextran solution.

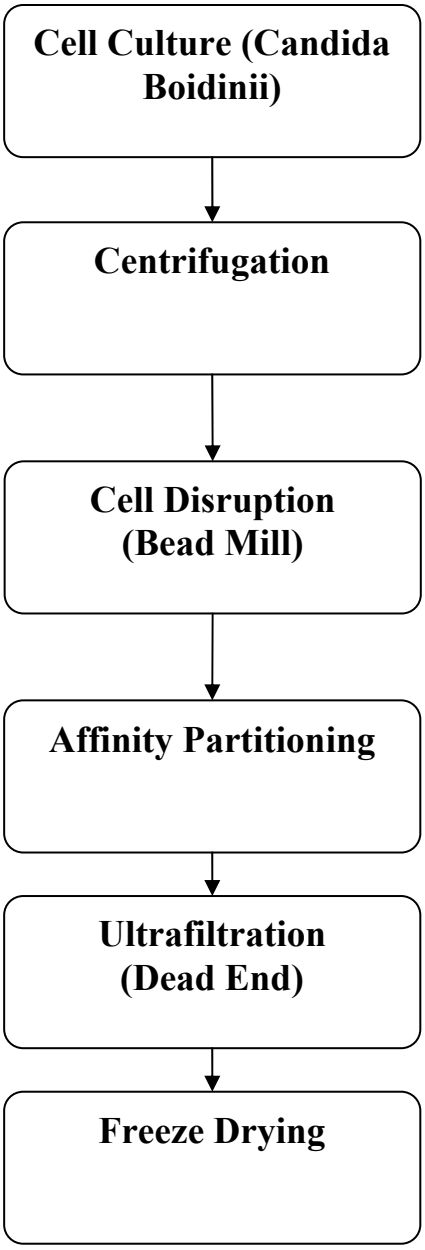

Figure 2. Flow-sheet for the isolation of alcohol dehydrogenase from *Candida boidinii.*

After they have completed the training part, they start a project that consists of a cascade of operations. Examples of projects that we have included in our laboratory are: 1) the isolation of human antibodies from transgenic corn (Lee and Forciniti, 2005); 2) the isolation of alcohol dehydrogenase from yeast (Waldorf et al., 1990); 3) the isolation of coagulation factors from human plasma (Weerasinghe et al., 1985); and 4) the fractionation proteins present in the lenses of mammals (Petitt et al., 1997). During this portion of the semester the participation of the instructor is minimal. The students are responsible to keep the supplies available, they take care of waste disposal, and their working hours are free. Their objective is to finish their project successfully. Because they have been trained before they start the project, it is their responsibility to repeat unsuccessful experiments in their own time. The projects are described to them but it is expected that the students would explore different operating conditions to determine the effect of those changes on yield and purity. The students in the class are divided into groups that pursue different projects. This is necessary because of equipment availability in our laboratory. One student in each group is chosen as the group leader who has the responsibility to distribute work and to report to the instructor. The acceptance of our approach by the students changes from year to year and it depends heavily on their quality.

Figures 2 to 4 show the flow-sheets for the isolation of alcohol dehydrogenase from *Candida boidinii*, the isolation of γ-crystallins from bovine lenses, and the purification of coagulation Factor XII from human plasma respectively.

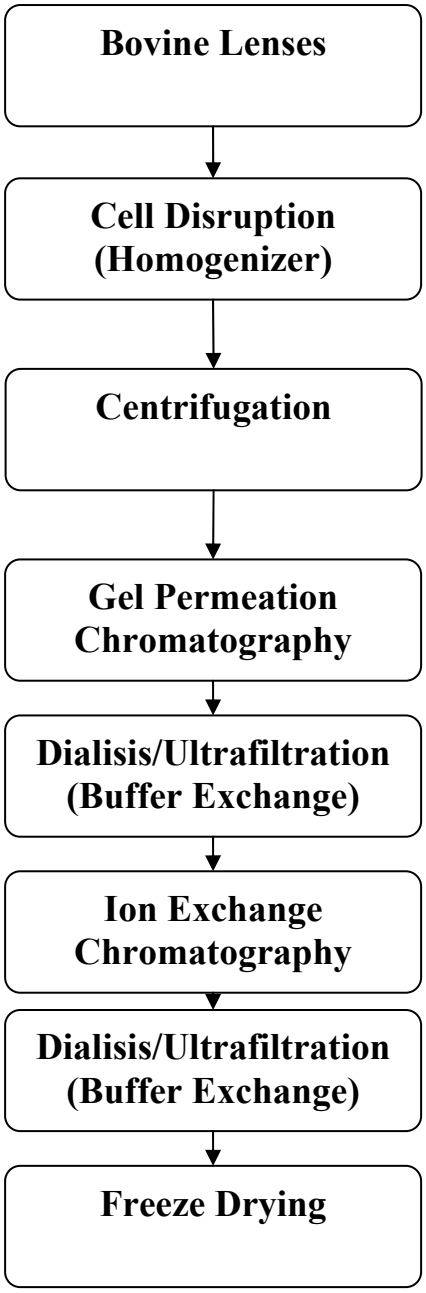

Figure 3. Flow-sheet for the isolation of γ-crystallins from calf lenses.

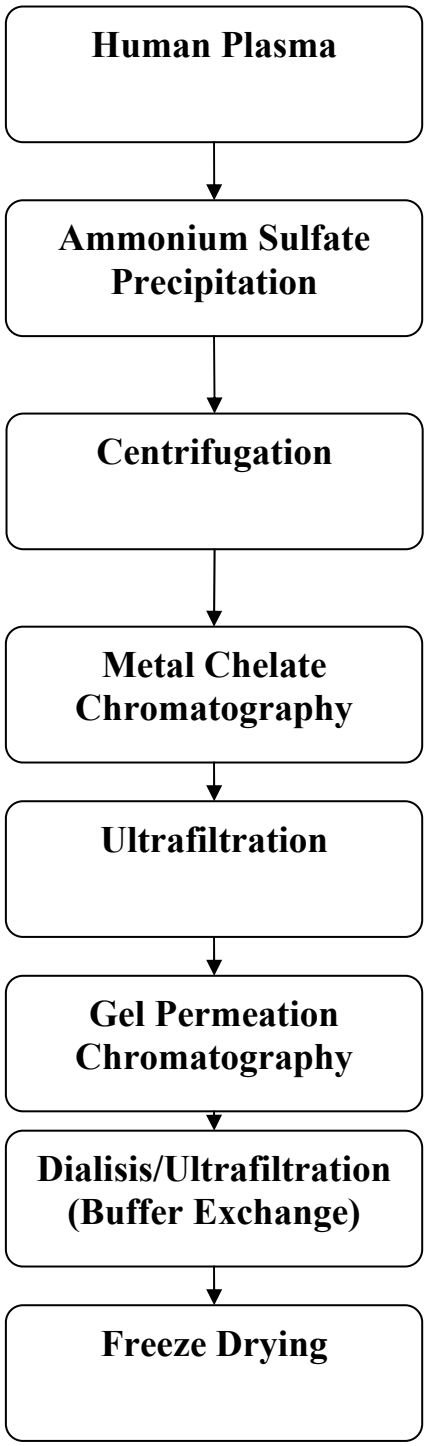

Figure 4. Flow-sheet for the isolation of Coagulation Factor XII from human

The genesis of each laboratory project is to be found in research projects that have been pursued in the author's research group. The projects are only vaguely described to the students (just a little bit more than the flow-sheets included in this manuscript). After the students have become familiar with the project (by reading a couple of key articles in the open literature), they discuss with the instructor the kind of experiments they want to conduct and what kind of conditions they would like to explore. For example, in the isolation of gamma crystallins from bovine lenses, the selection of the type of buffer gradient in the ion exchange chromatography step is key to obtain a good separation.

Laboratory Organization

The number of students in each group varies from year to year depending on the number of students in my course. During the training sessions groups are small, no larger than 3 students per group. During the project portion of the lab group sizes range from 2 (in years with few students) to 5 in years with a large student population. The expectations in each case also change. For example, students belonging to small groups of two students are expected to actively participate in each task of the project but they are not asked to explore a variety of operating conditions. Students belonging to large groups are normally divided into two or three subgroups with very specific tasks. For example, in the isolation of alcohol dehydrogenase project a large group of five students will be divided into three subgroups. A group of two will be in charge of cell grow and cell disruption. They explore how different metabolic stages of the yeast affect the production of the enzyme. They may also explore different milling conditions. A group of two students is in charge of the process separation development whereas the remaining student is in charge of all analytical work (determination of protein concentration and enzymatic activity in each step of the process). The group organizes its own tasks and the lab is run on "open door" policies (including nights and weekends). Graduate students working in the author's laboratory help in keeping the lab open at unusual hours and they provide the "adult" supervision that an undergraduate student should have.

References

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