Cost-Effective Teacher

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A Computer-Interfaced Drop Counter as an Inexpensive Fraction Collector for Column Chromatography

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Column chromatography is a staple of the undergraduate biochemistry laboratory curriculum. Lab experiments featuring gel filtration *(1–9),* ion exchange *(3, 5, 7, 9),* and affinity *(7, 9)* chromatography have been used to familiarize students with these separation techniques and to teach them skills that they will need for a career in the laboratory. However, many undergraduate laboratories do not have the budget to purchase the fraction collectors or columns that would typically be used in a professional setting. Generally, the protocols that are commonly used in college courses involve compromises and do not provide an opportunity for students to learn some desirable skills. For example, buffer is commonly applied to the top of the column by either pipetting it onto an open column *(1, 3, 4, 8)* or by the use of a reservoir, directly attached to the column *(5, 7, 9).* This does not enable students to learn how to set up a continuous flow of buffer through the column with tubing connections or to control operating pressure. Fractions are often collected into graduated cylinders or tubes *(1, 3)* or by comparing the height of buffer to a calibrated tube *(4).* Protocols that call for fraction collectors *(5, 8, 9)* require modification in classrooms that do not have this equipment.

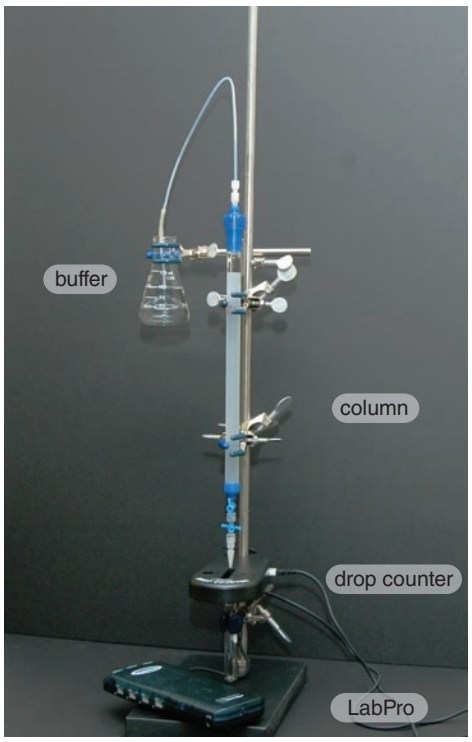

Figure 1. Photograph of the apparatus.

This article describes the use of a computer-interfaced drop counter to monitor fraction collection. Although the sensor and software used by the author is manufactured by Vernier Software and Technology (Figure 1), similar systems are available from MicroLab, Pasco, and MeasureNet. There are significant differences among the various systems, particularly in the design of the software, but the protocol described can be adapted to work with any of the systems. All of these drop counters can function as the sensor in a fraction collector, but test tubes must be moved manually. However, for a lab that is already outfitted with computers running one of these systems, the drop counter costs much less (under $100 per lab station) than the Bio-Rad model 2110 fraction collector ($799 with an educator discount) and requires much less storage space. The protocol described enables students to learn the same skills that they would learn when using a computer-interfaced fraction collector: alignment of the column with the sensor, calibration of drops, and the use of computer software for this application. In addition, Kontes Flex-Column economy columns with Luer fittings and Teflon tubing are used to set up an automatic buffer feeding system. Students have the opportunity to learn how to fill the inlet tubing, control operating pressure by adjusting the height of the reservoir, and set up a drip system that will prevent the column from running dry. Students can also learn techniques for troubleshooting common problems associated with these methods.

The experiment used to illustrate this system was adapted from Dryer *(1).* The experiment was reduced in scale by using semi-micro cuvettes and collecting smaller fractions. This made it possible to improve separation by reducing the column diameter while maintaining the column height. These modifications made it possible to reduce both the time required for the column to pack and the quantity of reagents required. The demonstration of techniques, the experiment, and a lesson on data analysis can be completed in a four-hour lab period.

Procedure

Setting Up the Column

Attach the double-stopcock to the column outlet. Position the drop counter beneath the column. Add buffer to the column to a height of about 8 cm and allow some of it to drain to fill the column outlet. Pour the Sephadex slurry into the column and allow it to pack under gravity to the desired height.[1] An automatic buffer feeding system can be set up as follows: (i) Fill the inlet tubing connected to the column cap and attach a pinch clamp to the tubing. (ii) Screw the cap onto the column. (iii) Place the end of the tubing in the buffer reservoir. Additional details are available in the online material.

Calibrating the Drop Counter and Setting Up the Computer Desktop

Position the drop counter so that the sensor is centered directly under the pathway of the effluent. Connect the drop counter to the computer interface and open the drop counter file. Open the lower stopcock fully and adjust the upper stopcock on the column to obtain the appropriate flow rate. After waiting a few seconds to create a partial vacuum in the column, release the pinch clamp to begin buffer flow. Calibrate the drop counter by collecting buffer into a 10 mL graduated cylinder. Modify the drop counter window to display the output as volume. Then set the alarm (if available) to collect the desired fraction size. Create a graph of absorbance (*y* axis) versus fraction number (*x* axis) that will be used to enter data.

Applying the Sample

The sample is applied with a disposable pipet by layering it under the buffer. Students should be cautioned to apply the sample slowly and to distribute it evenly over the column bed to avoid disturbing the matrix.

Running the Column

Select the first test tube and clamp it in position under the column outlet. Open the stopcock and begin collecting fractions. Each time the desired fluid volume has been collected (signaled by an alarm in some systems), close the lower stopcock, transfer the tube to a test tube rack, and replace it with an empty tube. Read the absorbance of the fraction on a spectrophotometer at the appropriate wavelength and record this value in the data table. Continue collecting fractions until the absorbance value for the last peak reaches baseline. Print the table and graph.

Hazards

Blue dextran, myoglobin, and glycerol may cause skin and eye irritation and may be harmful if inhaled or swallowed. The separation mixture should be handled with caution. Safety glasses should be worn at all times during this lab.

Results and Discussion

The application of this system, used by biochemistry students at this college, is the determination of the molecular weight of myoglobin by gel filtration chromatography. In this experiment, students plot absorbance versus fraction number by entering data manually into Logger Pro (Vernier), while the drop counter program is running. A sample of their results is illustrated in Figure 2, which is used to determine the elution volumes of blue dextran, DNP-glutamic acid,[2] and myoglobin.

A standard curve is plotted using data reported by Dryer *(1)*. Semi-log paper or graphing software such as Excel or Logger Pro can be used to produce this graph. The K_d value, the partition coefficient, of myoglobin is calculated from elution volumes and is interpolated on the curve to determine the molecular weight of myoglobin. For example, a standard curve constructed in Logger Pro 3.3 was used to determine the molecular weight of myoglobin from the K_d value derived from the sample data shown (Figure 3). Students obtain the true molecular weight of horse skeletal muscle myoglobin from the ExPASy Protein knowledgebase *(10)* and calculate a percent error.

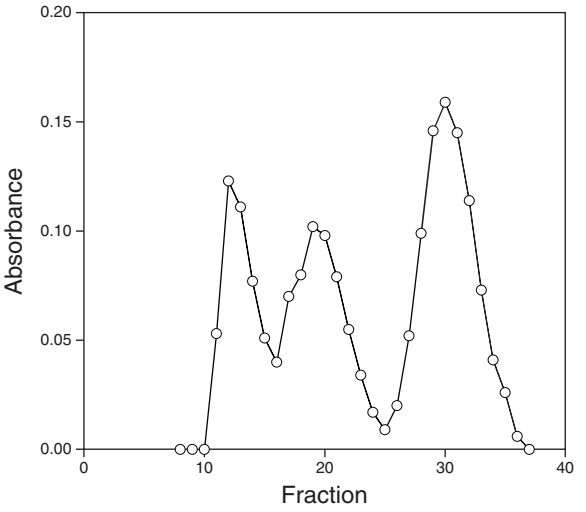

Figure 2. Gel filtration chromatography elution profile. Absorbance values were determined at the following wavelengths: fractions 8–16, 650 nm (blue dextran); fractions 17–25, 500 nm (myoglobin); fractions 26–37, 440 nm (DNP-glutamic acid). Column dimensions: 1.5 × 25 cm. Fraction size: 1.4 mL.

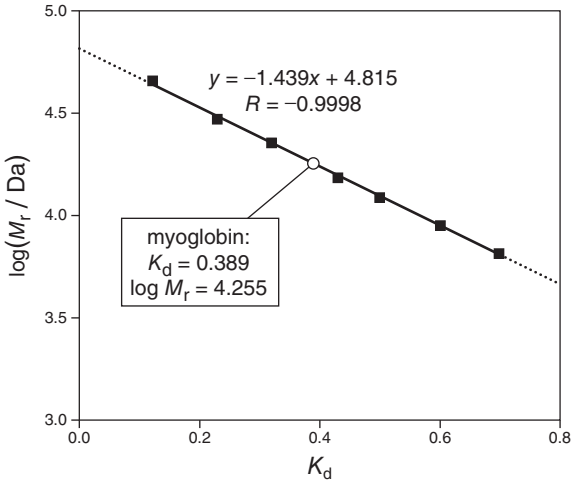

Figure 3. The standard curve for a Sephadex G-75 column. The interpolation function of Logger Pro 3.3 was used to determine the log M_r of myoglobin from its K_d. The molecular weight determined in this experiment was 18,000 (2.5% error). The data for the standard curve are from ref *1* and reproduced in Table 2 in the online material.

Acknowledgments

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Notes

1. While the column is being packed, rotate the drop counter 180 degrees from the position seen in Figure 1 to prevent buffer and Sephadex being dropped on the counter.

2. DNP-glutamic acid, a component of the separation mixture, has been discontinued. DNP-serine is one of several acceptable substitutes and is used in the student handouts.

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Figure 1 in color

Supplement

Protocol for the experiment used to generate the data

Student handout

Instructor notes including answers to a post-lab problem set

Results of a student survey

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