

Separating and Quantifying Proteins

by Racheline Rogers

Experiment Overview

This experiment illustrates that molecules can be separated on the basis of their size (or hydrodynamic diameter) using the technique of column chromatography. The sample used, a mixture of bovine serum albumin (Mr 68,000) and a cobalt salt (Mr ~370) is passed through an inert solid matrix (Sephadex G25) through which flows a liquid phase (0.1M NaCl). Fractions of the eluate are collected as they flow out of the column and quantitatively analysed for protein using the Biuret method.

The aims of the experiment are:

- To introduce new technical skills and to engage students in the practice of science
- To emphasise the observational and analytical character of science
- To illustrate the process of gel filtration using Sephadex column chromatography
- To illustrate how to manipulate and present data
- To illustrate the significance and use of a standard curve
- To illustrate the importance of using the same assay conditions for the standard curve and the unknown
- To introduce problem solving skills through the determination of the original concentration of the isolated protein

Learning Experience

This experiment is designed to be an effective learning tool to illustrate all the aims above. However anecdotal evidence from undergraduate students over the years suggests it may be too complicated for some level 2 students.

Aims and Objectives

The aims of the experiment are:

- To reinforce a component of the biochemistry curriculum
- To emphasise the observational character of science and introduce new technical skills
- To introduce skills of problem solving
- To understand the process of gel filtration using Sephadex column chromatography and how the pore size of the Sephadex used is essential in determining the size of the molecules that can be separated
- To understand how to manipulate and present data
- To understand the significance and use of a standard curve
- To understand the importance of using the same assay conditions for the standard curve and the unknown
- To determine the original concentration of the isolated protein

Level of Experiment

Second year undergraduate

Keyword Descriptions of the Experiment

Domain

Analytical Biochemistry

Specific Descriptors

Separating and quantifying proteins

Course Context

This is part of the Level 2 Biochemistry Curriculum on Protein Structure and Function.

Prerequisite Knowledge and Skills

Students are expected to understand how and why proteins can be separated on the basis of their size. It is one of two experiments on the separation of proteins. The second experiment in this series is to separate proteins on the basis of charge using ion exchange chromatography.

Time Required to Complete

Prior to Lab: 20 minutes

In Laboratory: 2 hours

After Laboratory: 20 minutes

Experiment History

This experiment has a long history in the Department of Biochemistry at the University of Adelaide. While the author listed in section (1.9) is responsible for the educational analysis of this experiment, her submission of it to ASELL is done on behalf of all academic staff in the Department (now discipline) of Biochemistry.

Comments

A mixture of three or more proteins could be used in an extension of this experiment and the students could be asked to devise a method by which they could separate them.

References

Elliott, W. H. And Elliott, D.C. (2009) Biochemistry and Molecular Biology. 4th ed. OUP