Protein Purification using SDS-PAGE

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Practical:

Abstract
Practical 4 of a set of 4 linked practicals for biochemistry modules.

Practical 1 introduces the techniques of measuring enzyme activity; practical 2 uses ion exchange to extract lysozyme from egg white, and measures enzyme activity.

Practical 3 measures protein concentration in the extracts.

This practical uses SDS PAGE to separate the proteins present.

Intended academic level
undergrad2

Duration
This experiment is an all day practical.

Learning Outcomes
Student will have used spectroscopy, ion exchange and gel electrophoresis.

Reinforces practical principles e.g. calibration, dilution of samples, washing of samples etc.

Measurement of enzyme activity.

Ion exchange for purification and effect of pH (and be related to isoelectric point).

Principles of electrophoresis.

Inter-relationship of data from different analytical techniques (activity, concentration, and electrophoresis).

Materials
Various. Gilson pipettes, eppendorf tubes, centrifuge, spectrometers plus cuvettes, test tubes, gel electrophoresis equipment, reagents.

Costs

Further comments
The four practicals are linked with practical 1 introducing the techniques. The four practicals can be related at the end to explain why enzyme activity and protein concentration vary in the samples, and electrophoresis demonstrates this visually.

Reading

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