Protein Purification

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Practical:

Abstract
A set of 4 linked practicals for biochemistry modules.

Practical 1 introduces the techniques of measuring enzyme activity and protein concentration using spectroscopic analysis.

Practical 2 uses ion exchange to extract lysozyme from egg white, and measures enzyme activity spectroscopically.

Practical 3 measures protein concentration in the extracts.

Practical 4 uses SDS PAGE to separate the proteins present.

If required enzyme activity could be omitted and just practicals 2 and 4 used to demonstrate ion exchange extraction from natural material, and gel electrophoresis

Intended academic level
undergrad2

Duration
3 hours each for practicals 1 - 3 (2 may overrun slightly if students are inefficient, 3 may take 2 hours). Practical 4 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 three 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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