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Edinburgh Polysaccharide and Oligosaccharide Substrates

High-voltage paper electrophoresis service

REFERENCE: S.C. Fry (2011) High-voltage paper electrophoresis (HVPE) of cell-wall building blocks and their metabolic precursors. Pp 55–80 *in*: The Plant Cell Wall Methods and Protocols, Edited by Z.A. Popper. Springer New York. ISBN 978-1-61779-007-2.

A seriously under-valued method for the analysis of small (<1000 Da), hydrophilic, ionic compounds [e.g. charged monosaccharides, oligosaccharides and derivatives (including phosphates, nucleotides, uronic acids, aldonic acids, aldaric acids, etc.); neutral sugars that can acquire a charge when binding ions such as molybdate; amino acids; oligopeptides...].

The electrophoretic mobility of compounds is governed by their charge:mass ratio in a highly predictable way [Offord RE (1966) *Nature*, 211, 591–593]. Specifically, the mobility is determined by the ratio of a molecule's charge (Q) to its 'surface area' (represented by the molecular weight raised to the power $2/3$). Thus, high-voltage electrophoresis reports a molecule's $Q : M_r^{2/3}$ ratio, which is a very useful parameter, especially one of these variables can be assumed, for example that the charge is -1 at the pH of the electrophoresis buffer.

Once a standard technique, available in many biochemical laboratories, high-voltage paper electrophoresis is now available in very few, specialised laboratories. The equipment requires sophisticated safety arrangements. The technique can effect high-quality separations of sugars, sugar acids, amino acids, sugar-nucleotides, sugar-phosphates and related hydrophilic compounds. The separated compounds can be detected by staining, or can be eluted from the paper for further analyses, including bioassays. It is semi-preparative if the electrophoresis performed on thick paper. Since the electrophoresis buffers used are volatile, the eluted compounds do not require de-salting prior to further analysis.

We offer a high-voltage paper electrophoresis service in which samples can be run under the following conditions:

pH 2.0 (running buffer = formic + acetic acids; white spirit as coolant) —

- all common amino acids (except cysteic acid) run towards the cathode;
- hydroxyproline oligoarabinosides run at characteristic slow rates towards the cathode;
- amino sugars run towards the cathode;
- neutral sugars immobile;
- sugar acids (e.g. uronic and aldonic acids) almost immobile;
- phosphorylated compounds (e.g. sugar-nucleotides, sugar phosphates, CoA derivatives), and sugar sulphates run towards the anode.
- low- pK_a carboxylic acids (such as oxalic acid, oxalyl esters and 2,3-diketogulonic acid) run towards the anode.



pH 3.5 [*not currently available*] (running buffer = pyridinium acetate buffer; white spirit as coolant) —

- Asp, Glu and cysteic acid run towards the anode;
- Lys, Arg and His move towards the cathode;
- other common amino acids have low mobility;
- amino-sugars run towards the cathode;
- sugar-acids run towards the anode and many are resolved from each other;
- phosphorylated compounds (e.g. sugar-nucleotides, sugar phosphates, CoA derivatives) and organic acids run towards the anode;
- neutral sugars immobile.

pH 6.5 (running buffer = pyridinium acetate buffer; toluene as coolant) —

- Asp, Glu and cysteic acid run towards the anode;
- Lys, Arg and His move towards the cathode; other common amino acids practically immobile;
- amino-sugars run towards the cathode;
- sugar acids, phosphorylated compounds (e.g. sugar-nucleotides, sugar phosphates, CoA derivatives) and organic acids run towards the anode at a rate related to the charge:mass ratio (useful to resolve aldobiouronic acids from monomeric uronic acids);
- neutral sugars immobile.

Molybdate, pH 3.5 (running buffer = Na₂MoO₄/acetic acid; white spirit as coolant) —

- Excellent resolution of reducing sugars from alditols
- Discrimination between differently linked disaccharides, e.g. (1→2), (1→3), (1→4) etc. (after reduction with NaBH₄)

Services offered

Electrophoresis of up to 16 samples (on one sheet of chromatography paper in any one of the buffers listed above).

Note: for running 16 samples, each sample to be analysed should not contain more than 1 µmol of total ions (equivalent to ~200 µg of galacturonic acid). Non-ionic compounds, e.g. glucose, can be present in higher amounts.

Alternatively, for preparative work, a single sample of up to 25 µmol (equivalent to ~5 mg of galacturonic acid) can be loaded per sheet.

...Or anything in between.

Additionally, we can stain the electrophoretograms (and/or markers), and return them or a scan of them to you with interpretation of the compounds detected:

- Staining with ninhydrin [detects α-amino acids; recommended minimum loading = 0.2 µg per compound of interest; also detects other amino compounds e.g. polyamines, γ-aminobutyric acid, with lower sensitivity].
- Staining with isatin-ninhydrin [detects imino acids (hydroxyproline and proline, including hydroxyproline oligoarabinosides); recommended minimum loading = 0.2 µg per compound of interest. Also detects amino acids, at lower sensitivity.].
- Staining with aniline hydrogen-phthalate [detects reducing sugars (neutral, uronic acids, amino-sugars); recommended minimum loading = 25 nmol per compound of interest, e.g. ~5 µg per monosaccharide, 10 µg per disaccharide, ~30 µg per heptasaccharide].
- Staining with AgNO₃ [detects reducing and non-reducing sugars, aldonic acids, aldaric acids, alditols, many ascorbate metabolites; recommended minimum loading = 1 µg per monomer of interest].



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- Staining with molybdate [detects organic and inorganic phosphates; recommended minimum loading = 20 nmol per compound of interest, e.g. ~5 μg Glc-6-P].

Prices:

The samples will be loaded on the appropriate type of paper and subjected to electrophoresis at the most appropriate pH; the paper will then be dried and returned to you.

First sheet of a related batch: **£600** [includes a preliminary run of relevant markers (supplied by you, if you wish) and their visualisation by staining].

Each subsequent sheet run under the same conditions: **£250**.

Each sheet stained: additional **£125**.



Sample preparation

Acid hydrolysis of polysaccharides or oligosaccharides.

This is the standard technique used for conversion of non-cellulosic polysaccharides, oligosaccharides and glycoproteins to their component neutral monosaccharides. Uronic acids are also released, but in lower yield.

Service comprises: Hydrolysis of sample (1 µg to 10 mg dry wt; for other quantities, please enquire) in 2 M TFA at 120°C for 1 hour; removal of TFA and any acid-resistant polymers.

Cost: £250 for first sample of a batch. Plus £10 per additional sample.

Driselase hydrolysis of polysaccharides or oligosaccharides.

A highly informative technique for hydrolysis of plant cell wall polysaccharides or oligosaccharides, including cellulose and acidic polysaccharides. The sample is digested with purified Driselase, after prior mild acid hydrolysis (to remove any complex arabinose-terminated side-chains from the backbone of xylans) and/or alkaline hydrolysis (if protecting ester groups are likely to be present). Products are monosaccharides and specific disaccharides. In particular, the β-xylose residues of xylans are recovered as xylose + xylobiose, whereas the α-xylose residues of xyloglucan are recovered as isoprimeverose. Any remaining Driselase-indigestible material can then be converted to monosaccharides by complete acid hydrolysis and analysed separately.

Service comprises: Digestion of sample (1 µg to 5 mg dry wt; for other quantities, please enquire). Isolation of ethanol-soluble products (monosaccharides and oligosaccharides) essentially free of Driselase protein and any undigested polysaccharides.

Cost: £400 for first sample of a batch. Plus £50 per additional sample.

Alkaline hydrolysis of glycoproteins to release hydroxyproline oligoarabinosides.

Uses barium hydroxide to hydrolyse the protein backbone, while leaving any Hyp-linked oligosaccharides still glycosidically linked to hydroxyproline. Products are simple amino acids plus Hyp-Ara's from glycoproteins such as extensins.

Service comprises: Hydrolysis of sample (1 µg to 10 mg dry wt; for other quantities, please enquire). Removal of Ba(OH)₂.

Cost: £400 for first sample of a batch. Plus £10 per additional sample.