Sequential Injection Capillary Electrophoresis for Bioprocess monitoring

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From July: Deakin University, Geelong, Australia
When a photograph falls short
Rationale: need for more chemical information

Fast

On-site

Often
• High resolution analytical technique
• Instrumentally simple (unlike HPLC no need for high pressure pumps)
• Fast
• Compatible with miniaturisation
  • Forward compatible with autonomous onsite monitoring
Flow-through vial
9 mL per analysis

Monitoring bioprocesses
Sequential Injection CE

Fill capillary with BGE
Switch 6 port valve and fill interface with sample
Close 2 way valve to inject sample in capillary, change 6 way valve to BGE
Clean interface with BGE and start HV for separation

3000 V
• Requirements
  • CE method
    • Separate lactate from components in medium
    • Stable over >3 days
  • Sampling interface for sampling cell-free medium
    • Sampling volume should not exceed 10% of media volume
      • 20 mL media in standard flask with a surface area of 75 cm²
        • Cannot sample more than 2 mL
Sampling interface for adhesion culture

SI-CE for monitoring adhesion culture

Continuous monitoring lactate human embryonic kidney cell line HEK293

**Conditions:**
85 cm x 50 um i.d. fused silica capillary coated with HDMB/PSS/HDMB; BGE: 25 mM Tris/35 mM CHES, pH 8.65 with 0.02% PEI; +30 kV applied to outlet vial while interface was grounded. Signal was obtained using a TraceDEC conductivity detector positioned 10 cm from the outlet.


*ONLY 1.99 mL of sample for 228 analyses! (8.3 µL/run)*
Mitochondrial activity study

- Lactate level change (normal mice cell line 2)
- Lactate level change (abnormal mice cell line 2)
The next phase: suspension culture

- Jurkat Human T lymphocyte cells
  - Include cell density measurement: digital microscopy
  - Different sampling approach
- Expand analyte set to include other acids, amino acids and sugars

Cell count: digital microscopy vs haemocytometry
Obtain cell-free media: H filter
Si-CE for bioprocess monitoring
60 mM TEA/10 mM CAPS, pH 12.4 with 0.075% PEI and 0.005% HDMB
The BIG data

<8 mL for 192 runs over 4 days (40 µL/run)
Pharmacological studies: drugs affecting lactate production

Alhusban et al, Scientific Reports, 2017, under review
Multiplexing: sampling from 5 parallel cultures

AlHusban Gueven Breadmore and Guijt, Scientific Reports, under review
BGE: 35 mM Tris/35 mM CHES, pH 8.9 with 0.025% PEI
3D pharmacology

Dose response surface over time

Rotenone

A lactate (fmol/cell)

Rotenone (nM)

time (h)

0 2 4 6 8 10

0 20 40 60 80

0 2.5 5 7.5 10

0 2 4 6 8 10

0 2 4 6 8 10

NADH lactate

\(\beta\)-lapachone NAD+ pyruvate

NADH

glycolysis

mitochondria

energy (ATP)

nutrients oxidative stress

rotenone
cloquinol
3D pharmacology

Dose response surface over time
Rotenone

Dose Response surface over time
Clioquinol

Dose response surface over time
β-lapachone
• SI-CE successfully adapted for bioprocess monitoring
  • Adhesion culture
    • Demonstrated to distinguish between normal and mitochondrial knockout cell lines
  • Suspension culture
    • Simultaneous monitoring of cell density, glucose, lactate, leucine and glutamate
      • Correction for cell density essential for correct interpretation of results
    • Study pharmacological effect of drugs known to affect lactate metabolism over time by monitoring 5 parallel cultures
• Hardware is there, needs to be packaged and integrated
My thanks to...

Chippers and CE-ers

• Support
  • ARC: QEII Fellowship, Future Fellowship (Breadmore),
  • Al Zaytoonah University Jordan (Al Husban)
  • Alexander von Humboldt Foundation (Guijt)