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Capillary electrophoresis for automated on-line monitoring of suspension cultures: Correlating cell density, nutrients and metabolites in near real-time

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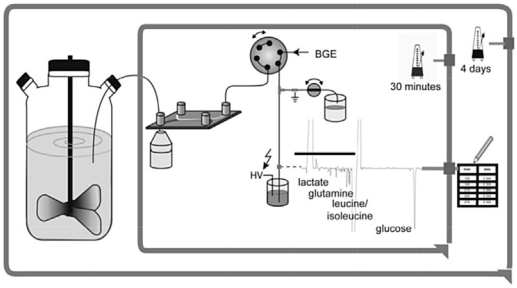
HIGHLIGHTS

- Automated SI-CE system for monitoring suspension cultures every 30 min over a 4 day period.
- CE-C4D separation method using 60 mM TEA/10 mM CAPS, pH 12.4 in HDMB/PSS/HDMB coated PMMA capillary stable for at least 24 h.
- CE-C4D separation using 60 mM TEA/10 mM CAPS, pH 12.4 in HDMB/PSS/HDMB coated PMMA capillary stable for at least 24 h.
- Microfluidic devices enable digital cell density measurement and extraction of a cell-free sample using an H-filter.
- Cell density data provide valuable insight in the metabolic state of the cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasingly stringent demands on the production of biopharmaceuticals demand monitoring of process parameters that impact on their quality. We developed an automated platform for on-line, near real-time monitoring of suspension cultures by integrating microfluidic components for cell counting and filtration with a high-resolution separation technique. This enabled the correlation of the growth of a human lymphocyte cell line with changes in the essential metabolic markers, glucose, glutamine, leucine/isoleucine and lactate, determined by Sequential Injection-Capillary Electrophoresis (SI-CE). Using 8.1 mL of media (41 μL per run), the metabolic status and cell density were recorded every 30 min over 4 days. The presented platform is flexible, simple and automated and allows for fast, robust and sensitive analysis with low sample consumption and high sample throughput. It is compatible with up- and out-scaling, and as such provides a promising new solution to meet the future demands in process monitoring in the biopharmaceutical industry.

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Suspension cell culture
Lactate
Glucose
Glutamine

1. Introduction

Bioprocess monitoring has gained importance over the past few years [1]. The production of chemicals or biopharmaceuticals using biological processes is naturally susceptible to variability because living cells consume substrates and produce metabolites and products in a dynamic way, with variations in metabolic rate across short time intervals. The FDA recommends documentation of nutrient and metabolite time profiles in the process analytical technology (PAT) policy to ensure product quality [2]. Whilst bioreactors are typically equipped with hardware for monitoring a range of physicochemical variables including pH, temperature and dissolved oxygen [3], cell count and the analysis of metabolite levels are usually conducted off-line [1,4,5], making it difficult to control production in a quality-by-design manner. Recent advances in multivariate analysis in combination with spectroscopic techniques can provide more detailed chemical information, for example using UV [6] FTIR [7] or RAMAN spectroscopy [8,9]. Whilst advances in chemometry and the interpretation of spectra has greatly advanced, the resolution of complex analyte sets with similar functional groups remains challenging. Separation science provides many solutions for the analysis of complex samples, for example using high performance liquid chromatography (HPLC) coupled offline [10] or on online [11] to a bioreactor. Though mass spectrometry offers faster analysis, like other spectroscopic techniques the ability to analyze complex samples, or to distinguish isomers with identical mass sequentially without extensive sample clean-up limits its applicability. Additionally, once an automatic sampling routine has been established, the analyte set can easily be expanded by changes to the separation chemistry and/or detector.

Capillary electrophoresis (CE) is an alternative high-resolution separation technique to HPLC with the potential to provide detailed chemical information faster and using smaller sample volumes [12]. Tahkoniemi et al. presented a custom-designed pneumatic sampling interface for monitoring accumulation of Cu, Zn, Co and Cd by the Rhodococcus sp. Bacteria incorporating a 0.45 μm filter without reporting any clogging issues [13]. Turkia et al., however, did report issues of growth after 45 h on a 0.22 μm cross flow filter used for obtaining a cell free sample for monitoring carboxylic acids during yeast fermentation [14]. More recently, using off-line derivatisation with a fluorescent label, Turkia at al. used capillary electrophoresis for monitoring the uptake of amino acids in beer brewing [15]. For automated, on-line monitoring, the same group constructed a CE system with flow-through sampling vial and cross filtration unit for online monitoring of the production of carboxylic acids by two different yeasts cultures. In their work, the yeast *kluyveromyces lactis* cultivation was monitored for 173 h and 0.87 L of sample was used for 97 analyses (8.94 mL per analysis) [16].

Here, a robust and reliable electrophoretic separation method was developed for the analysis of the metabolic biomarkers glucose, glutamine, leucine/isoleucine and lactate from media and combined online with sampling and cell density measurements. The platform, schematically depicted in Fig. 1 was used to monitor cell density and these four biomarkers in a culture of human T lymphocytes every 30 min over 4 days, using less than 41 μL of sample per assay. The highly flexible platform is automated, fast and reliable and as such expected to be able to provide new insights in the way chemical changes influence the production of biotechnology products, including biopharmaceuticals, and is anticipated to enable the establishment of boundary conditions ensuring biotechnology product quality.

2. Materials and methods

2.1. Chemicals

All reagents were analytical grade obtained from Sigma–Aldrich (Sydney, AUS) and were used as supplied unless stated otherwise. Milli-Q water (Millipore, Bedford, MA, USA) was used to prepare solutions. Standards containing 0.30 mM sodium fluorescein or 0.42 mM rhodamine-6G (BDH Chemical Ltd, Poole, UK) were prepared in milliQ water and stored at room temperature in a dark place. Fresh standards containing 10 mM lactate, 10 mM L-glutamine, 5 mM L-leucine, 5 mM L-Isoleucine or 40 mM D-glucose standard solution were prepared each week and stored at 8 °C. A 2 M chloride standard was prepared monthly by dissolution of its sodium salt and stored at room temperature. A 90 cm PMMA capillary was coated with a polyelectrolyte coating prepared from (HDMB) and poly(sodium 4-styrene sulfonate homopolymer) (PSS) and (HDMB) to reverse the EOF. The BGE included a combination of 60 mM triethylamine (TEA) and 10 mM N-cyclohexyl-3-aminopropanesulfonic acid (CAPS). The cationic polyelectrolytes poly(ethylenimine) (PEI) (ACROS organics, Geel, Belgium) at 0.075% (w/v) and hexadimethrine bromide (HDMB) at 0.005% (w/v) were added to BGE.

2.2. Fabrication of H-filter

Templates were made using dry film resist with slight modifications [17]. A PMMA slide (80 × 60 × 1 mm), was rinsed with methanol, dried using compressed air, laminated sequentially with three 0.1 mm EP SUEX TDFS sheets (Sudbury, MA, USA) at 60 °C and cooled down with compressed air. The protective film was removed before applying the next layer of resist. After removal of the final protective coversheet of the top film, a transparency mask made by laser printing on an overhead transparency, was positioned on top and the substrate was exposed for 21 min to the output of a UV LED array (OTHL-0480-UV, Opto Technology, Wheeling, IL, USA) [18] and then baked at 110 °C according to the SUEX processing guidelines. Substrates were developed in propylene glycol monomethyl ether acetate (PGMEA), followed by a wash with isopropanol and then dried with compressed air. The microfluidic H-filter devices were created by soft lithography casting polydimethylsiloxane (PDMS) over the template before curing for 1 h in an oven at 70 °C. Access holes were made at the end of each channel using a hole puncher and the device was irreversibly sealed by plasma bonding with a glass microscope slide (75 × 51 × 1 mm). A digital fluorescence microscope (AM4113/AD4113, Dino-lite premier digital microscope, Taiwan) was used for inpsecting the fabricated device.