Journal of Chromatography A, 1323 (2014) 157–162

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

On-line sequential injection-capillary electrophoresis for near-real-time monitoring of extracellular lactate in cell culture flasks

Ala A. Alhusban[a,b], Adam J. Gaudry[b], Michael C. Breadmore[b], Nuri Gueven[c], Rosanne M. Guijt[a,*]

[a] Australian Center of Research on Separation Science, School of Pharmacy, Faculty of Health Sciences, University of Tasmania, Private Bag 26, Hobart, TAS 7001, Australia
[b] Australian Center of Research on Separation Science, School of Chemistry, Faculty of Science, Engineering and Technology, University of Tasmania, Private Bag 75, Hobart, TAS 7001, Australia
[c] School of Pharmacy, Faculty of Health Sciences, University of Tasmania, Private Bag 26, Hobart, TAS 7001, Australia

ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form 31 October 2013
Accepted 1 November 2013
Available online 11 November 2013

Keywords:
Sequential injection capillary electrophoresis
Online monitoring
Bio process monitoring
Capillary electrophoresis
Cell culture

ABSTRACT

Cell culture has replaced many in vivo studies because of ethical and regulatory measures as well as the possibility of increased throughput. Analytical assays to determine (bio)chemical changes are often based on end-point measurements rather than on a series of sequential determinations. The purpose of this work is to develop an analytical system for monitoring cell culture based on sequential injection-capillary electrophoresis (SI-CE) with capacitively coupled contactless conductivity detection (C^4D). The system was applied for monitoring lactate production, an important metabolic indicator, during mammalian cell culture. Using a background electrolyte consisting of 25 mM tris(hydroxymethyl)aminomethane, 35 mM cyclohexyl-2-aminoethanesulfonic acid with 0.02% poly(ethyleneimine) (PEI) at pH 8.65 and a multilayer polymer coated capillary, lactate could be resolved from other compounds present in media with relative standard deviations 0.07% for intraday electrophoretic mobility and an analysis time of less than 10 min. Using the human embryonic kidney cell line HEK293, lactate concentrations in the cell culture medium were measured every 20 min over 3 days, requiring only 8.73 μL of sample per run. Combining simplicity, portability, automation, high sample throughput, low limits of detection, low sample consumption and the ability to up- and outscale, this new methodology represents a promising technique for near real-time monitoring of chemical changes in diverse cell culture applications.

1. Introduction

Cell culture of eukaryotic cells is widely applied throughout life sciences. At the small scale, cell based assays have replaced many in vivo assays because of ethical and regulatory restrictions on working with laboratory animals. Additionally, the production of biopharmaceuticals synthesized by living cells during fermentation or cell culture processes is a rapidly growing field. According to the FDA, approximately 30–40 percent of the authorized medical products in 2012 were biopharmaceuticals. Bioprocessing offers many advantages. For example, the production of vaccines by cell culture technology instead of conventional methods provides the capability for rapid manufacturing start-up in case of a pandemic because characterized cell lines can be stored and are thus readily available. Moreover, the risk of impurities can be reduced because vaccine production takes place in a highly controlled, closed and sterile environment [1]. Cell culture based technologies are intensively employed in drug discovery [2], and are significant tools for drug screening and new potential drug targets studies [3]. In 2012 alone, 35 novel biologics were developed by the biopharmaceutical industry and approved by the FDA [4]. In this context it is important to note that bioprocess monitoring in the production process of biopharmaceuticals is essential to ensure the safety of the product as well as to satisfy economic and regulatory demands.

At present, the majority of cell culture based monitoring is restricted to a few end point based assays that do not reflect the dynamic metabolic processes in cells that influence the final product. Therefore, a detailed and continuous monitoring of the bioprocesses in each production batch would significantly help manufacturers to control product quality, increase production yields and reduce production costs [5]. At the same time, online monitoring of bioprocesses will also significantly enhance our understanding of fundamental dynamic cellular metabolic reactions that cannot be easily ascertained by end point measurements.

* Corresponding author. Tel.: +61 3 6226 2196; fax: +61 3 62262870.
E-mail addresses: Ala.Alhusban@utas.edu.au (A.A. Alhusban), Adam.Gaudry@utas.edu.au (A.J. Gaudry), mcb@utas.edu.au (M.C. Breadmore), Nuri.Guven@utas.edu.au (N. Gueven), Rosanne.Guijt@utas.edu.au (R.M. Guijt).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.11.006

Lactate is one of the major products of eukaryotic and prokaryotic cells and is one of the most important organic acids in extracellular media that can be used to monitor cellular metabolism and energy status. It is produced from glucose and glutamine in mammalian cells [6]. It is important to monitor because it affects the physicochemical stability of the bioprocess medium by reducing pH levels and is outright toxic to some cells. In addition, under certain conditions, cultured cells can use lactate as an alternative source for carbon, even in the presence of glucose [7]. At the same time, lactate can be used as an indicator of biological activity. For example, metabolic shifting from lactate production to lactate consumption was reported to result in improvements of process performance regarding productivity, scalability, process robustness and cell growth [8].

Due to the tight connection between extracellular and intracellular metabolic pathways, cellular bioprocesses are primarily controlled by manipulating the external environment in form of the composition of the cell culture medium [9]. Therefore, timely information about even small changes in the concentration of extracellular lactic acid during cell culture will directly help to control and improve the efficiency of the bioprocess. Realizing this depends on the availability of suitable and functional tools that can be used for monitoring [10]. Currently, a number of different methods are used for lactate detection, the most important being enzymatic assays [11,12,13], and analytical separations such as liquid chromatography (LC) [14,15] and capillary electrophoresis (CE) [16,17]. Enzymatic assays are highly specific, instrumentally simple, but time consuming – typically requiring hours per measurement [18], and are restricted to a single analyte. Chromatographic techniques are sensitive, versatile, and have excellent reproducibility. However, separations are slow and typically sample pre-treatment is required. Capillary Electrophoresis (CE) is a powerful alternative characterized by faster separation compared to LC and has been widely used to separate a diverse range of analytes, from small ions through to macromolecules in many fields [19,20], in particular for monitoring bioprocesses. As discussed in a recent review, CE has been readily employed for the analysis of discrete samples, utilizing commercially available single or multiple capillary instruments [21]. It has also been employed for on line monitoring of a variety of analytes in different matrices and environments, but only a few reports present dedicated, online sampling interfaces for CE analysis [21]. One of those, a CE method with conductivity detection was applied for automated continuous on-line analysis of 23 ions in tap water over two days [22]. CE was also used for on-line determination of perchlorate in biological samples such as breast milk, human urine, serum, red wine and cow's milk using a supported liquid membrane [23]. A filter probe was integrated with a computerized pneumatic sampling system to monitor the bioaccumulation of Cu^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} in the bacteria species *Rhodococcus* sp. [24].

Another CE method using LIF detection was successfully employed for microbial analysis of water collected from two local streams through continuous electrokinetic injection under field-amplified conditions [25].

The low sample volume makes CE ideally suited for monitoring cell culture conditions and cell-based assays. In this study, an automated, robust and portable SI-CE setup was developed by modifying the experimental set up previously reported by Blanco et al. [26]. A flow-through interface was designed to sample cell-free media for monitoring lactate production by the human embryonic kidney cell line HEK293. The unique combination of the sampling interface and SI-CE system minimized sample consumption and analysis time. Using this system, 72 samples were analyzed per day using less than 700 μL of sample per day, enabling monitoring of lactate production by HEK293 cells in vitro over three days, using less than 10% of the total media volume.

2. Materials and methods

2.1. Chemicals

All reagents were analytical grade reagent obtained from Sigma–Aldrich (Sydney, AUS) and were used as supplied unless otherwise stated. Solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA). Lactate standard solution (10 mM) was prepared weekly and stored at 8 °C by dissolution of its sodium salt. Chloride standard solution (2 M) was prepared monthly from sodium chloride and stored at room temperature.

The cationic polyelectrolyte poly(ethylenimine) (PEI) (ACROS organics, Geel, Belgium) was added to BGE. A polyelectrolyte multilayer coated fused-silica capillary prepared from hexadimethrine bromide (HDMB) (Sigma–Aldrich, St. Louis, MO, USA) and poly(sodium 4-styrene sulfonate homopolymer) (PSS) and (HDMB) again in order to reverse the EOF. Cetyltrimethylammonium bromide (CTAB) was added to BGE in case of dynamic coating. Two studied BGEs including a combination of (tris(hydroxymethyl)aminomethane (Tris))/*N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), pH 8.85 and 2-(*N*-morpholino)ethanesulfonic acid (MES)/20 mM L-histidine (His), pH 6.15 were evaluated for optimum separation.

2.2. Instrument design and operation

A scheme of the instrumental set up used for this work is shown in Fig. 1. The SI-CE instrumentation is composed of two peristaltic pumps (PeriWaves, CorSolutions, Ithaca, NY, USA) for sample and BGE delivery to the system. A two-position injector valve (MXP-7980, Rheodyne, Oak Harbor, WA, USA) was used to direct sample or BGE to the analytical system. A PEEK T-piece-connector (P-727, Upchurch Scientific, OakHarbor, WA, USA) was used for interfacing the flow system and the CE capillary. This interface allows inserting the capillary with a small internal volume (0.57 μL). All connecting tubing was selected based on a narrow diameter (381 μm) to reduce dead volume and to minimize turbulent flow. The capillary inlet was fixed near the interface center at a position to eliminate consecutive sample carry over, and the outlet end was immersed in a 25 mL glass vial filled with BGE. A stainless steel syringe needle was cut to yield a 2 cm long, 0.51 mm internal diameter tube and was employed as electrode and connected to the interface through the waste tubing. A solenoid (isolation) valve (HP225K021, NResearch, West Caldwell, NJ, USA) was linked on the waste tubing at the T piece outlet to control solutions direction either to capillary or to waste. A commercial capacitively coupled contactless conductivity detector, C^4D (TraceDec), was supplied by Innovative Sensor Technologies (Strassahof, Austria). Detection parameters were optimized according to capillary internal diameter. The detector operational parameters were selected as following: frequency, 2× high; voltage, 18 db; gain, 200%; off set, 008. The detector sensing head was fixed 10 cm from the outlet end of the CE capillary. A high-voltage power supply was used for CE under reversed polarity, with the anode (+) electrode immersed in the outlet glass vial. The injector valve, pumps and high-voltage power supply were connected with an NI USB-6212 data acquisition interface board (National instruments, Austin, TX, USA) and controlled using LabView v8.1 (National Instruments).

It is important to note that all the described components were sufficiently compact to be placed inside an incubator (BBD6220, Heraeus, Thermo-Fisher, Kendo Laboratory Products, Zurich, Switzerland) that was operated at fixed humidity (90%) and constant temperature (37 °C). A CO_2 Laser Engraver (40 W CO_2 Deluxe Hobby Laser, Full Spectrum Laser LLC, Las Vegas, NV, USA) was used to fabricate a hole in both the sampling tubing interface and cell culture flask. Sequential injection of samples was