Sensors and Actuators B 238 (2017) 633–640

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Multiplexed and fully automated detection of metabolic biomarkers using microdialysis probe

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ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 7 July 2016
Accepted 19 July 2016
Available online 25 July 2016

Keywords:
Microdialysis
Real-time analysis
Metabolic biomarkers
Automated instrumentation
Collection efficiency
Traumatic brain injury

ABSTRACT

We report here, the design and development of an automated near real-time continuous detection system for lactate, glutamate, pyruvate and glucose using microdialysis probe. The system developed can automatically push perfusate through microdialysis probe (20, 100 and 1000 kDa MWCO cutoff probe) at low to medium flow rate of 0.5–2 μL/min with almost 100% fluid recovery. The microdialysate collected from the probe is analyzed automatically for these four metabolite biomarkers. It operates in a continuous mode with measurements of all four biomarkers once every 20 min. The dynamic range for these different markers covers the entire clinical range of traumatic brain injury. The prototype shows a low variation of ~ 7–10% across the entire clinical range for all the biomarkers with fairly good accuracy of ~95%. The instrument canrun continuously for 24 h without user intervention. With a long tubing of 1 m to and from the microdialysis probe and associated dead volume, the total lag time for actual event at the probe site versusreported concentration is roughly 1 h.

1. Introduction

Microdialysis is a sampling technique to monitor continuous changes in biochemical markers from extracellular fluid of tissues. Microdialysis of brain extra cellular fluids is the most widely used application though there are reports of using the technology in other organs like the liver [1], skin [2], blood [3], stomach [4] and ear [5]. This minimally invasive technique is widely used in bedside monitoring of early markers of tissue damage and ischemia for Traumatic brain injury (TBI) [6–9]. Clinical studies have indicated that neurochemical markers like glutamate [10], glucose, lactate [11], pyruvate among others are useful in prediction of development of secondary injury after TBI [12,13]. Increase in Lactate to pyruvate ratio occurs during a metabolic crisis and happens due to mitochondrial dysfunction [14]. It is a well-known marker for monitoring cell damage and ischemia after TBI and Subarachnoid Hemorrhage (SAH). Glucose, Glycerol and Glutamate are additional markers for developing ischemia. During ischemia in brain, the glutamate concentration may increase due to decrease in uptake in glial cells, whereas changes in glucose concentration can happen due to several reasons like ischemia, hyperemia, hyperglycemia or hyper/hypo metabolism [15]. Continuous monitoring of these metabolic markers will therefore be useful at identifying the risk, guide therapy and improve the outcome [6]. Currently, the only clinical bedside monitoring instrument available to analyze minute quantity of microdialysate is ISCUS [16] from MDialysis. While, the instrument is impressive in terms of its performance, it is not truly an inline system. The microdialysis probe and its collection unit are separate from the analyzer. The microdialysate is collected in separate vials which are then individually inserted in the instrument to analyze the content of the vial. It is a tedious, time consuming and error prone process that has to be performed every hour. This paper describes the first automatic inline microdialysis system that can continuously push the perfusate through microdialysis probe, analyze its content for four different biomarkers simultaneously and report the concentrations every 20 min without any intervention. There are few reports of development of inline systems for microdialysis using high performance liquid chromatography (HPLC) for analyzing the microdialysis or using electrochemical probes directly in the flow path of microdialysate. While, the HPLC systems can detect minute concentration of analytes from microdialysate [17–19], it has a large footprint and an expensive system to maintain. Moreover, the interface between microdialysis collection and LC system are not well developed [20]. Similarly electrochemical sensors placed in line with microdialysate suffer from issue of stability of the sensors and difficulty of dynamic calibration. Additionally, lack of availability of EC sensors for many important metabolites makes it difficult to develop a multiplexed detection

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http://dx.doi.org/10.1016/j.snb.2016.07.097
0925-4005/

Table 1
Ratio of different reagents used for detection of four analytes.

No.	Analyte	Sample−μL	Substrate−μL	Enzyme mix-μL
1	Lactate	2	17.2 (27% NAD + 37.85% MTT + 35.12% buffer volume based)	22.83 (95.93% buffer, 2.04% enzyme A, 2.04% enzyme B volume based)
2	Pyruvate	2.5	4 (10% 10 x dye, 90% buffer volume based)	36 (enzyme)
3	Glucose	1.5	14.25 (1.27% dye, 98.73% buffer volume based)	25.75 (0.71% enzyme, 99.29% buffer volume based)
4	Glutamate	4	9 (26.31% NAD, 73.69% MTT volume based)	28.9 (1.63% enzyme mix, 98.37 buffer volume based)

system. Our current system is based on enzymatic reactions and optical detection in 384 well platform, which is robust, well developed and already implemented in many lab tests in different clinical applications. We report here the detailed instrument design and initial results obtained from in-vitro tests.

2. Material and methods

2.1. Materials

CMA 12 microdialysis probe (20, 100 and 1000 kDa cutoff, 4 mm length except 3 mm for 1000 KDa probe) is purchased from Harvard Apparatus. Ringer's solution and artificial cerebrospinal fluid (ACSF) are purchased from Harvard apparatus or formulated in-house using reagent grade chemicals from Sigma and Fisher Scientific. Ringer's salt solution powder (#M525) is from Himedia Laboratories. Glucose, glutamate and pyruvate used for in-vitro experiments are bought from Sigma. Lactate is bought from Alfa Aeser. Lactate (ECLC-100), glutamate (EGLT-100), glucose (EBGL-100) and pyruvate (EPYR-100) assay kits from BioAssay systems are used. We also have used individual reagents for further optimization of the analyte detection range, stability and sensitivity. Lactate oxidase (#L0638) and pyruvate oxidase (#P4591-100U) are bought from Sigma whereas, glucose oxidase (#0243) is from Amresco and glutamate oxidase (#YMS-80049) is from CosmoBio USA. Clear bottom low volume black 384 well plate is from Corning (#3542). Starting blocking T20 (PBS) blocking buffer from Thermo-Scientific (#37539) is used for plate preparation.

Detailed description of assay for four biomarkers can be found elsewhere [21–24]. These commercial assays are adapted for our application in following way as shown in Table 1. The reagents (substrate and enzyme mix) are mixed with the sample to start a reaction which develops a color. The total volume of reagents and sample adds up to ~40 uL of solution. This volume of fluid is enough for proper detection in a 384 well pate by absorption technique. The intensity of the product color (perceived color against white light: Purple-dark bluish) is measured at 565 nm and proportional to the concentration of the sample. The amount of sample volume and reagents required for proper detection, depends on the concentration of that particular analyte in the sample and can be changed to increase the sensitivity and dynamic range of the detection. In-vitro experiments performed with the instrument used the same reagents as described in Table 1, or a modified version of that depending on the clinical range and sensitivity requirements of some of the experiments. The reagents are drawn from a reservoir which is kept at room temperature for the whole duration of the experiment which can last up to eight hours or more. No significant deterioration of the reagents was observed over eight hours. However, care is taken to store these reagents before being used for experiments. These reagents prepared are kept at −20 °C for storage up to one month. Before the experiment, the reagents are taken out of freezer and thawed at room temperature before being transferred to the instrument.

A push-pull mechanism is implemented for sample collection from the microdialysis probe. This subsystem is composed of an OEM syringe pump module #702225 and two sets of 1 m tubing's (ID 0.12 mm) from Harvard Apparatus, a single channel peristaltic pump from APT (#SP101F.013), a vacuum transducer (PX209-30VAC5 V) purchased from Omega. Flow sensors (LG16-150 and LG-16-480) are from Sensirion and solenoid valve (P/N 038T2S12-32-4) is from Biochem Fluidics. Potentiostat (Emstat3) is from Palmsens or in-house developed current sensor is used. A reagent delivery subsystem is developed in-house. XY slides (ET-150-13) from Newmark systems are used for precise plate movement. These slides have resolution of 7.5 μm, accuracy of 0.0012 mm/mm of travel and repeatability of 30 μm. Multichannel peristaltic pumps (Ismatec-Reglo 4 channel OEM) are from Cole Parmer. Miniature linear actuator (L12-50-100-6-p) is bought from Firgelli along with controller board. CMOS camera (DCC1545 M), Cage cube mounted turning prism mirror (CM1P01) and diffuser (DG-10-600), are bought from Thorlabs. Fujinon Lens (HF9HA-1B) is used with the camera. Multifunction data acquisition board (USB6008) and interface for I^2C communication (USB8451) are bought from National Instruments. LED light source (565 nm), AC/DC converters (1470–2746-ND for 24 V, 285–1984-ND for 12 V and 102–3268-ND for 5 V) are bought from Digikey. The entire prototype is designed using Autodesk Inventor software. The control system for the prototype is written in LabVIEW.

2.2. Development of prototype

There are very few commercially available systems which can perform automatic detection of multiple biomarkers from microdialysate continuously [25]. Development of this type of system requires complex design and software control to make sure both the collection and analysis of microdialysate are performed consistently over many hours of continuous operation. Fig. 1 shows the schematic of overall operation of the prototype system. There are three major subsystems namely a) Dialysate collection unit, b) Reagent delivery subsystem and c) Detection Subsystem. The Dialysate collection unit, uses a push-pull technique to collect microdialysate in a small chamber. The flow rate and pressure inside the chamber are monitored for PID (proportional-integral-derivative) control. Once enough solution is collected, the delivery subsystem will automatically start the assay process for up to four metabolite biomarkers. The assay development will be monitored with the detection subsystem in real time. The final analysis result will be reported before the subsequent assay begins. Current prototype is capable of reporting results of four biomarkers simultaneously at a 20 min interval for up to 24 h. The overall instrument is controlled with LabVIEW running on Windows's based computer.

2.2.1. Dialysate collection unit

Since the dialysate flow rate is usually very low (0.5–2 μL/min), it takes relatively long time to accumulate enough volume of fluid for subsequent analysis. A level detection system within the dialysate collection unit continuously monitors the volume collected and sends a trigger to the detection unit to start its operation as soon as the correct volume of dialysate becomes available. The reagent delivery subsystem will then split the microdialysate in four separate boluses and use them for detection of lactate, pyruvate, glucose and glutamate detection in a 384 well plate. Each of these subsystems is explained in details in next section.