# THE DEVELOPMENT OF A MINIATURIZED WIRELESS MICRODIALYSIS-MICROCHIP ELECTROPHORESIS SYSTEM FOR *IN VIVO* MONITORING OF DRUGS AND NEUROTRANSMITTERS IN AWAKE AND FREELY MOVING SHEEP

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## ABSTRACT

This paper describes a novel miniaturized on-animal separation-based sensor using microdialysis coupled to microchip electrophoresis (MD-MCE) with electrochemical detection. In contrast to previous studies using MD-MCE in which animals need to be anesthetized or tethered to the microdialysis system, this work describes a miniaturized system employing telemetry that can be placed on-animal, making it possible for them to freely roam. The ultimate goal of this project is to be able to monitor biological analytes and correlate *in vivo* concentrations with the behavior of the freely roaming animal.

**KEYWORDS** Separation-based sensors, microdialysis, electrophoresis, *in vivo* monitoring

## INTRODUCTION

To understand the biochemical basis of neurological diseases as well as to develop pharmacological interventions, it is important to be able to simultaneously monitor biological analytes (i.e. neurotransmitters, second messengers) and behavior in near real-time. Microdialysis is a powerful *in vivo* sampling technique that can be employed to continuously monitor the concentrations of compounds in the extracellular space of the brain and other tissues. Microdialysis can be coupled with microchip electrophoresis to produce a separation-based sensor that can monitor several compounds in the extracellular space simultaneously in near real-time. Our lab has previously coupled microdialysis to microchip electrophoresis with on-chip derivatization and fluorescence detection for the continuous monitoring of excitatory amino acids in the brain [1]. These experiments were performed on anesthetized animals. Awake animal systems generally require tethering the animal to the pump and/or analysis system through wires or a liquid switch, which can add milliliters of dead volume resulting in a loss of temporal resolution as well as a significant time delay between the event and the detector response.

An alternative to the awake animal system is an on-animal system. In this case, the analytical system is close to the point of sampling, providing excellent temporal resolution and minimizing the time delay. However, this requires that the entire analytical system be miniaturized, including the pump, power supplies, and detector. The system must also be able to remotely control the power and data acquisition systems.

In this paper, a unique system that will allow researchers to directly correlate neurochemistry with behavior in freely roaming animals is described. Sheep were chosen as the test animal based on their size compatibility with the device as well as their role in behavioral research. The device employs microchip electrophoresis with electrochemical detection, which is an ideal detection scheme as it is sensitive, selective, and amenable to miniaturization through microfabrication.

## EXPERIMENTAL

*Glass Microchip Fabrication*

Soda-lime glass was patterned separately using photolithography for microfluidic channels and electrodes. The channels were wet etched in a solution of 49% hydrofluoric acid, nitric acid, and water in a 20:14:66 ratio for 2.5 min, resulting in channels 50 μm wide and 18–20 μm deep. The electrodes were etched using 10:1 buffered oxide etchant for 1 min, resulting in 400–420 nm troughs to countersink the electrodes. Using a lift-off process, platinum electrodes were created in a DC magnetron sputterer. The chips were pre-assembled using a calcium (II) acetate solution as described by Chiu *et al.* [2], followed by a final assembly under running water. The assembled chips were then aligned under a microscope. Aligned chips were bonded at 650ºC for 1 h in a programmable muffle furnace.

*Glass/PDMS Microchip Fabrication*

Silicon masters were patterned with SU-8 10 photoresist, using standard soft photolithography. All channels were 35 μm high and 40 μm wide except the microdialysis channel, which was 100 μm wide (Figure 1).

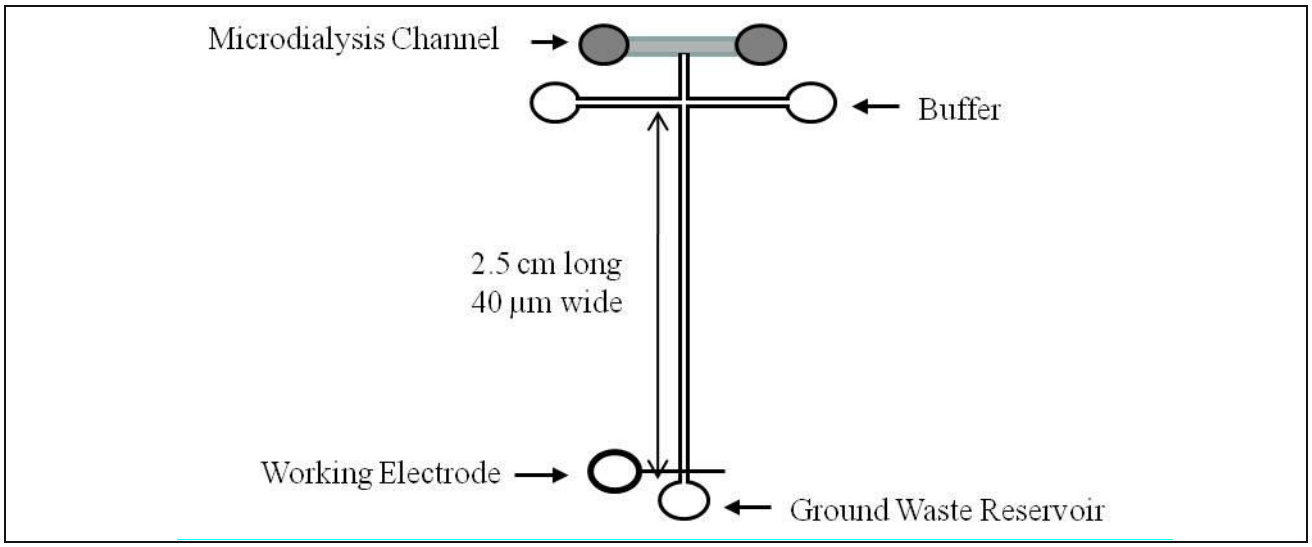


*Figure 1: Schematic of the Microchip Design*

Polydimethylsiloxane (PDMS) was mixed in a 20:1 (monomer:curing agent) ratio, degassed using vacuum, and poured on the patterned silicon wafers. The PDMS was then hardened in an oven at 85ºF for just enough time to retain the shape of the channels without significant deformation. The PDMS was then placed on a fused silica glass plate with a pyrolyzed photoresist film (PPF) [3]. The electrode was aligned in-channel, which means that the electrode is placed in the separation channel and not in the ground waste reservoir. After alignment, the PDMS/glass plate is placed back in the oven overnight.

*On-line MD-MCE System*

All microchips were pretreated using the following procedure before use. All channels were rinsed with filtered 0.1 N NaOH using a syringe. The channels were then rinsed with Nanopure (18Ω) water and the background electrolyte (BGE). All solutions were filtered prior to use. Microchips were then placed in a plastic holder, which was fitted with an interface that contains O-rings to prevent solutions from leaking. The holder contains a dolomite pump connected to a reservoir of BGE. That pump was plumbed to the sidearm of the microchip in order to supply fresh buffer to the reservoir. This has the dual purpose of preventing ion depletion and clearing bubbles that may develop in the microchip during prolonged use. The microdialysis pump, dolomite pump, high voltage, and potentiostat were all controlled by a module housed beneath the holder, which in turn was powered by a portable laptop battery. This system is shown in Figure 2. Samples were injected from the microdialysis channel into the separation channel using gated injection [1].

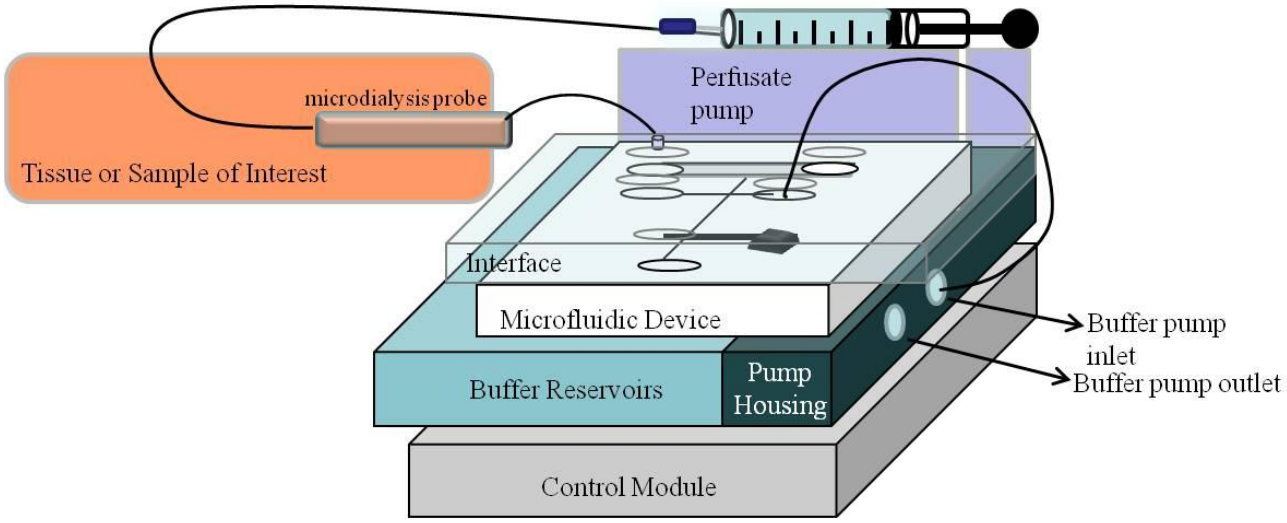


*Figure 2: Schematic of Entire System*

Communication between the on-animal system and the control module was accomplished using Bluetooth, which allows sampling, separation, detection, and microchip maintenance to all be operated remotely. This feature allows the animal's behavior to be minimally influenced by the analysis.

## RESULTS AND DISCUSSION

Initial studies employed a PDMS/glass microchip device fabricated as described above for the determination of nitrite *in vitro*. The separation buffer consisted of 40 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) and 2 mM tetradecyl trimethyl ammonium bromide (TTAB), pH 6.44, BGE with reversed polarity. The system was tested using a 100 μM nitrite solution in BGE, which was introduced to the microchip using the microdialysis syringe pump. Sequential injections of nitrite were detected following gated injection from the microdialysis channel at the PPF electrode at an applied voltage of 700 mV vs. Ag/AgCl (Figure 3). The sample flow rate was 0.5 μL/min and the separation voltage was –1200V.

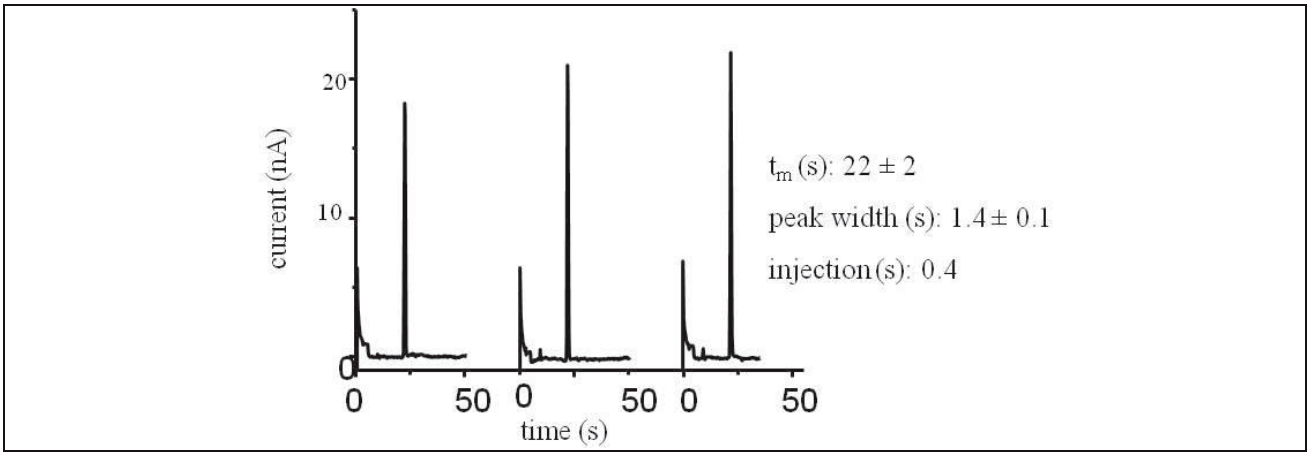


*Figure 3: Electropherogram of the detection of nitrite using PDMS/glass microchip with a PPF electrode*

The high separation efficiencies for the nitrite peaks shown in Figure 3 can be attributed in part to the in-channel electrode alignment. The in-channel electrode placement also influences the apparent potential that is felt by the working electrode due to the effect of the separation voltage. When using reverse polarity for the electrophoresis voltage, a negative shift in the apparent oxidation potential occurs, making the substance appear to be more easily oxidized. It is important to evaluate the electrode potential with each new electrode alignment. The integrated system shown in Figure 2 included a remotely controlled pumping system that could successfully remove bubbles from the channels in the chip and reestablish the electroosmotic pumping for the gated injection. Current work is focused on employing the integrated system for on-animal monitoring of the transdermal delivery of nitroglycerin in sheep. Nitroglycerin is converted to nitrite which will be sampled using a linear probe placed beneath the skin.

## CONCLUSION

A prototype device for on-animal analysis using microdialysis coupled with microchip electrophoresis with electrochemical detection has been developed. Both PDMS and glass chips have been evaluated. An integrated system including the microdialysis pump, high voltage power supplies, miniaturized potentiostat, and telemetry has been developed. Current research is focused on evaluating this device to monitor transdermal drug delivery in sheep. The ultimate goal is to monitor neurotransmitter release and behavior simultaneously in awake, freely moving sheep.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from the National Institutes of Health (5R43NS064644-02) and the Adams Institute for Bioanalytical Chemistry at the University of Kansas for their financial support of this project. We would also like to thank Pinnacle Technology, Inc. for their development of the control module and holder.

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