USE OF CONDUCTIVITY ELECTRODES AS ON-CHIP SENSORS IN CHROMATOGRAPHIC MICROFLUIDIC DEVICES

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Abstract

We report the use of direct-conductivity sensing as a means for measuring mobile-phase composition and flow rate inside an HPLC chromatography chip. Instantaneous on-chip solvent percentage during gradient elution is determined from the measured mobile-phase conductivity. The method can show changes in mobile phase organic solvent percentage of 1%, even at 90% acetonitrile. In addition, flow rates in the range of 100 to 1000 nL/min are determined by measuring the time-of-flight of a heat pulse as it moves from a heater trace to embedded conductivity sensing electrode.

Keywords: chromatography, conductivity, flow-rate, gradient

1. Introduction

Microfluidic devices employing partition chromatography have been shown to be capable of efficient separations of biomolecules such as peptides and proteins [1]. Most importantly, the narrow bandwidths produced by short, small diameter separation channels in microfluidic "chips" allow analysis of exceptionally low sample volumes with concentration sensitivities similar to those of traditional, larger columns. Chromatographic separation requires a mobile-phase pump. Pumps currently in use were originally designed for standard or narrow bore columns and employ splitters to produce the sub-µL/min flow rates required for microfluidic nanoflow devices. Although these pumps work well for this application, the time required for solvent changes at the pump proportioning valve to reach the microfluidic chromatography channel is larger in comparison to standard bore columns. In addition, improper tubing and fitting connections between the pump and microfluidic device can cause leaks that may go undetected due to the extremely low flow rates used. For these reasons, on-chip sensors that can measure gradient solvent strength as well as actual on-chip flow rate are used to monitor mobile phase conditions in the device, rather than only at the pump.
2. Experimental

The microfabricated devices are made by laminating together polyimide films with patterned gold electrodes and laser-ablated features [2]. All mobile phases were prepared from 0.1% (v/v) formic acid in water (solvent A) and acetonitrile (solvent B). The pump was an Agilent 1100 Series Nanoflow Binary Gradient pump. The separation channel was packed with 3.5 μm silica C-18 reversed phase stationary phase. A Stanford Research Systems SR850 lock-in amplifier supplied a 1 kHz sine wave for conductivity detection. Heat pulses for flow rate monitoring were generated by applying typically 1.5 V for 100 msec to the heater trace.

3. Results and discussion

An example of one of the devices used is shown in Figure 1. The chip is placed directly between the rotor and stator of the injection valve, allowing flow from the pump to enter the separation channel at the left end. At the right end of the separation channel, mobile phase flows past the heater (for flow rate measurement) and conductivity electrodes. In use, effluent from the chip would normally be connected to the inlet of a mass spectrometer. However, the experiments reported here were conducted with the chip alone.

**Organic solvent composition monitoring**

Mobile phase conductivity measurements can be used to monitor the concentration of organic solvent in the mobile phase during a gradient run. Since formic acid is dissociated in water and not in organic solvent, the electrical conductivity of the mobile phase changes from high to low as the percentage of organic solvent increases during the gradient. A plot of mobile phase conductivity during a linear gradient from 0 - 100% acetonitrile is shown in Figure 2. It can be seen from the plot that conductivity of the mobile phase is not a linear function of organic solvent percentage. For practical use, it would be necessary to incorporate an algorithm that calculates organic solvent percentage from measured conductivity into the instrument monitoring software. The main advantage of incorporating an on-chip diagnostic such as mobile phase monitoring is the ability to correct for delay volumes and other discrepancies between instrument settings and real-time conditions at the microfluidic device. Although the gradient shown in Figure 2 is programmed at the pump to start at the beginning of the run, the volumes of tubing and the channels in the chip between the pump gradient select valve and the conductivity electrodes cause a delay of approximately 9 min in the application of the conditions applied by the pump. Real-time mobile phase solvent composition monitoring on the device allows this delay to be measured and taken into account.
Flow rate monitoring

We determine the mean flow velocity of the mobile phase by measuring the time-of-flight of a heat pulse. The pulse is generated using an embedded heater trace and measured via direct-conductivity sensing using planar gold electrodes (Figure 1). The flow rate of the pulse is then computed by taking into account the known cross-sectional dimensions of the channel and heat partitioning between the flowing liquid and the channel walls [3]. As shown in Figure 3, the heat pulse is measured as a change in conductivity at the sensing electrode. The timing of the pulse generation is signalled by a sharp dip in conductivity caused by an artificial baseline pulse. The time of flight of the pulse is measured by the time delay between these two signals.

The implementation of this concept has uncovered two issues. The first issue is that although the conductivity of the mobile phase increases with temperature, we detect the heat pulse as a dip in conductance between the detector electrodes. At this time we do not have a validated model for this effect. Possible causes include the effect of temperature changes on formic acid dissociation and on solvent and ion diffusion rates. The second issue is that the measured mean velocity is lower than the expected velocity by a constant scaling factor. This second effect is explained by heat partitioning between the liquid and the channel walls. As the heat pulse moves in the channel, there is heat exchange with the walls, thus changing the effective velocity of the pulse since there is heat extraction at its leading edge and heat addition at its trailing edge. The decrease in heat pulse velocity by partitioning has been validated by analytical and numerical modeling. In practice, it would be necessary to account for this heat-partitioning effect by calibrating the measured velocity and derived flow rate against a known, calibrated, flow-rate source, (an HPLC pump).

4. Conclusions

On-chip, real-time measurement of organic solvent composition and flow rate during gradient elution can provide precise determination of flow conditions inside microfluidic devices and compliment those measured off-chip at the external pump source.

References

Figure 1. Chromatographic microfluidic device used for linear velocity and solvent composition monitoring. The chip is placed between the stator and rotor of the injector.

Figure 2. Mobile phase conductivity during a linear gradient from 0 - 100% acetonitrile, from 0 - 20 min. The mobile phase composition was returned to 0% acetonitrile at 20 min. Both solvent A (water) and solvent B (acetonitrile) contained 0.1% (v/v) formic acid.

Figure 3. Mobile phase conductivity perturbations caused by application of a heat pulse upstream from the conductivity sensor electrodes. Flow rate can be determined from the time of flight and the known channel dimensions.