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Heat Index Flow Monitoring in Capillaries with Interferometric Backscatter Detection

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Independent control of fluid is important for small volume fluid applications including separation science techniques and analytical microchip devices. To effectively control fluid, a monitoring system is necessary to ensure proper response to the control input. Proof of principle for a new online technique for flow monitoring is demonstrated here. It offers near real-time measurements of small-volume flows in a noninvasive, simple, and robust design. The measurement is accomplished by the introduction of a plug of heat into the fluid and detection of the time of its appearance a short distance downstream. Using heat indexing and laser induced interferometric backscatter as the detector, flow monitoring is demonstrated in 184-μm diameter capillary tubing over a range of 2−25 cm/s (500 nL/s to 7 mL/s.).

An important factor for optimizing small-volume analytical techniques is the flow rate. Flow impacts resolution, time of analysis, and efficiency for many analytical techniques, including capillary electrophoresis (CE), microbore liquid chromatography, capillary electrochromatography, and flow injection analysis. In addition, flow of materials is essential for operating the fluidic microdevices that are being explored for a wide variety of uses. To control the flow, its rate is used to confirm the appropriate response from the control system. The flow data can be used to maintain a stable or constant flow, to stop flow, or to reverse flow in a dynamic, real-time manner. In addition to standard analytical techniques, this issue of flow control becomes more important on microchip devices, where complex flow patterns in interconnecting channels will be generated. Flow-monitoring techniques that can be easily applied to present systems will be quite difficult to use in smaller settings.

An ideal small-volume monitoring system for analytical techniques and microdevices would have the following properties: it could operate in real time on nanoliter to sub-nanoliter volumes; it would be unaffected by analytes present; it would function under a wide variety of solvent conditions (solvent gradients, etc.), yet be noninvasive, simple, and robust. The importance of determining small-volume flow rates has been recognized and investigated. Several methods have been described wherein each satisfies some, but not all, of these ideal properties.

The chemical marker method is the most common technique to monitor flow.[1−3] This uses an innocuous chemical marker with some easily detectable quality that is introduced into the flow stream and detected a known distance downstream. By monitoring the elapsed time, the flow rate may be calculated. This has been a useful and common technique for a variety of analytical methods, but does not fulfill the requirements for an ideal monitoring technique. It requires the introduction of a foreign material and an injection mechanism, thus resulting in an adulterated sample and engineering complexities.

Several flow-monitoring techniques require an end-column detection apparatus and dilution or alteration of the flow stream.[4,5] These include a conductivity measuring device and a fluorescence detection scheme. The first is based on the ionic strength of the buffer reservoir changing with the delivery of a more concentrated buffer from within the capillary. The second delivers a known amount of fluorescent dye to the flow stream, where the dilution effects are proportional to the flow rate. These techniques require that the sample be significantly altered, and the end-column design cannot be adapted to certain systems (such as interconnecting channels or end-column detection schemes). Another end-column (or precolumn) system involves measuring the mass transferred in a given amount of time; clearly, this system could not be widely used or adapted to complex systems.[6−8] Other techniques have been developed specifically for monitoring electroosmotic flow in CE systems. Some of these determine the zeta potential, which is then used to calculate the rate of flow,[9−13] while others monitor the current across the capillary upon the introduction of a new buffer.[14,15] These techniques not only lack real-time monitoring, but also require highly sensitive measuring equipment.

A variety of laser-based cross-beam techniques have been developed. Laser Doppler velocimetry monitors the Doppler shift of a particle as it passes through the intersecting region of two

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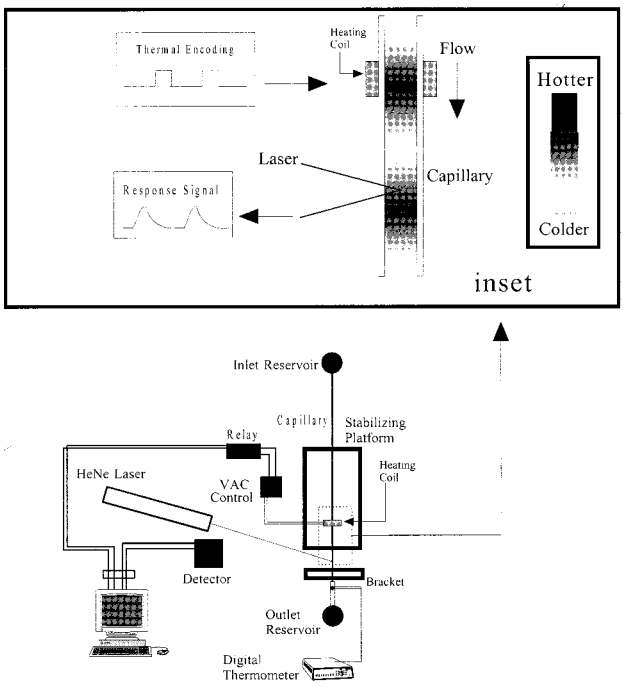

Figure 1. Block diagram of the experimental apparatus. The inset shows thermal encoding being imprinted into the moving fluid stream. The encoding causes an increase in the fluid temperature that alters the RI of the fluid. The zone of differing RI is detected by the LIB detection system as it passes through the detection zone.

lasers.[16,17] However, the introduction of particles into the fluid stream for this technique limits its usefulness. Time-resolved cross-beam thermal lens measurements[18] and pulsed photothermal deflection spectroscopy[19] have also been used. For the thermal lens technique, a position sensitive detector is used to monitor the deflection of a probe beam as it comes into contact with a heated zone in a fluid stream. For the pulsed technique, thermal diffusion makes the velocity measurements difficult. These cross-beam methods require highly accurate alignment and generate nontrivial output signals.

In this study, flow has been accurately monitored using heat indexing and laser-induced interferometric backscatter (LIB) in 184-μm internal diameter capillaries (Figure 1). This is accomplished by heating a small plug of fluid a short distance away from the LIB and letting it travel with the fluid, thus providing a noninvasive measurement of fluid velocity. The LIB technique uses side-illumination on a fluid-filled capillary tube which produces interference fringes (zones of high and low light intensity) radially in the plane of excitation and is typically viewed in a direct backscatter configuration.[20] The position of the interference fringes is sensitive to the refractive index (RI) of the fluid in the capillary. A change in temperature of the fluid alters the RI, resulting in a translational change in the position of the fringes. To generate a heat plug, a resistive heater is wrapped around the capillary and current is passed through it. The plug then is then carried to the detection zone at the flow rate. The distance from the heating zone to the detection zone was 9 mm. Fluid flow rates from 2 to 25 cm/s in fused silica capillary tubes were measured. This monitoring system is not susceptible to vibrations associated with precise optical alignment, nor does it alter the flow stream (other than extremely mild heating). Measurement was reproducible for the many individual capillaries and for several minor design changes during development. This method provides a simple system based on rugged, standard, and inexpensive equipment.

EXPERIMENTAL SECTION

The block diagram of the optical configuration is shown in Figure 1. Fused silica capillary tubing (Polymicro Technologies, Inc., Phoenix, AZ) with an internal diameter (i.d.) of 184 μm. and an outer diameter (o.d.) of 349 μm (70.9 cm in length) was used. A detector window (5 mm in length) was created on the tubing by burning away the polyimide coating. A 632.8-nm, 5 mW He−Ne laser (Melles Griot, Irvine, CA) was positioned such that the beam struck the capillary at the detector window. Flow through the capillary was generated by pressurizing the inlet reservoir with N_2 with a 2−30 psi pressure regulator. The capillary tube was held in place with a stabilizing platform on the upstream side of the detector and an outlet bracket on the downstream side. Embedded into the stabilizing platform was a coiled piece of 130-μm diameter NiChrome wire 8 cm in length. The wire was coiled around the capillary tubing three times and attached to a variable AC controller (Superior Electric Co., Bristol, CT) that provided current. A relay system was used to turn on and off the controller output. The relay was engaged by a signal from Labview (National Instruments, Austin, TX) hardware and software installed on a PC. The signal to the relay system was monitored through an A/D controller card (PC-1200 National Instruments, Austin, TX) and stored in a spreadsheet program. This system allowed for the determination of the time at which the heated plug of fluid was generated. A 3-cm piece of 360-μm-i.d. plastic tubing (Upchurch, Oak Harbor, WA) was attached to the capillary at the outlet bracket, into which a thermocouple (Omega, Stamford, CT) was embedded so that it made contact with the fluid. The plastic tubing was then sealed with epoxy (ITW Devcon, Danvers, MA) to prevent leakage around the thermocouple but to allow flow into the outlet reservoir. The signal from the thermocouple was sent to a digital thermometer (Fluke, Everett, WA) to monitor the temperature of the fluid in the capillary.

The laser light backscattered from the capillary tubing passed through a 75-μm optical slit (Edmund Scientific, Barrington, NJ) and into the detector assembly. The assembly consisted of a light-dependent resistor (LDR, Radio Shack) in series with a 10 kΩ resistor. An external dc power supply (Jameco, Belmont, CA) was used to apply 5 V dc across the LDR and the resistor. The output signal from the detector assembly was monitored across the 10 kΩ resistor. The He−Ne laser, detector assembly, stabilizing platform, and capillary outlet bracket were rigidly mounted on a 2 ft $\times$ 3 ft optical breadboard (Newport, Irvine, CA) and enclosed in a plastic box painted black to reduce stray light sources. The detector assembly and laser were mounted to translational stages (Edmund Scientific, Barrington, NJ).

To characterize the apparatus, solutions of known RI were injected into the fluid stream and the light intensity monitored by the detector assembly. Monitoring was accomplished by observing a change in light intensity at the LDR due to transla-

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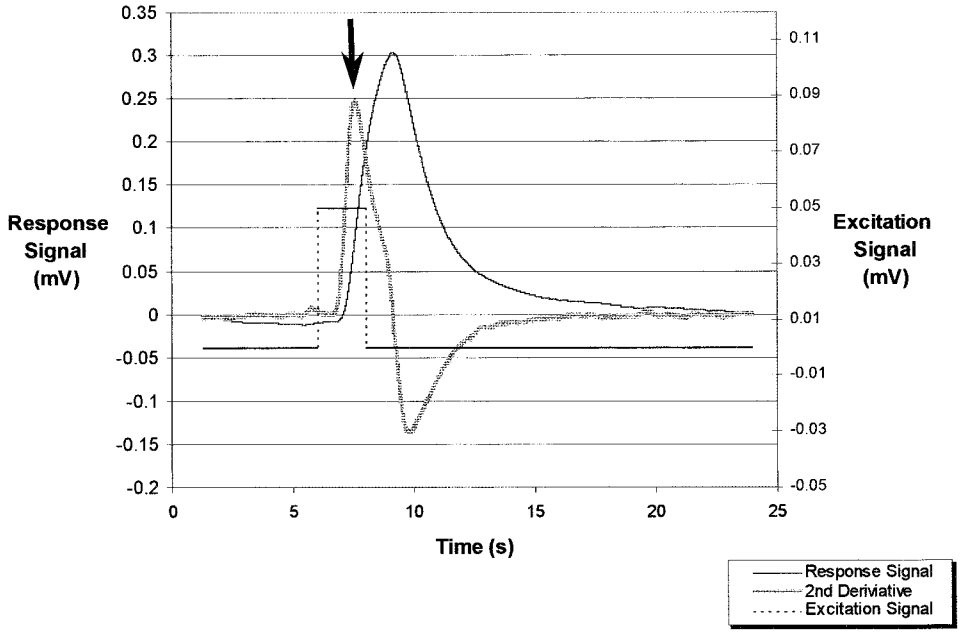

Figure 2. Typical raw data plot as monitored by the LIB detection system. A 2-s square wave excitation signal introduces a plug of heat into the moving fluid. The response is an increase in light intensity incident upon the LDR and is measured as an increase in voltage. To consistently measure flow rate, the maximum of the 2nd derivative of the response signal is calculated (shown by arrow).

tional movement of the interference fringes. One of the intensity zones was positioned at the edge of the optical slit such that an increase in RI of the fluid resulted in an increase in light intensity incident upon the LDR. The output from the LDR assembly was collected at 250 Hz for 12 s and stored in a spreadsheet program.

To study the LIB response to a change in temperature, the interference pattern was monitored at fluid temperatures ranging from ~28 to 30 °C and constant applied pressure (10 psi). This was physically accomplished by applying a constant current to the heating coil and allowing the system to reach a steady state. The data were collected in the same manner as with the RI measurements. The fluid temperature was measured using the thermocouple and digital thermometer. To show that the thermal characterization was not a result of heat conducting along the wall of the capillary, the experiment was repeated without flow.

The flow rate of the system was independently determined by weighing the amount of fluid that passes into the capillary outlet reservoir over a given period of time. At various pressure increments ranging from 2 to 25 psi, the fluid from the capillary was collected in a vial for 5 s. This fluid was then weighed and the flow rate through the capillary calculated. The result was compared with flow rates predicted by the Poiseuille fluid flow model and found to be in agreement within the experimental error of the measurements (~1%).[22]

To measure the fluid flow rate using LIB, the LDR output was monitored for 24 s during a set pressure increment. After 6 s, a heat plug was introduced into the capillary fluid by applying 1 V ac across the heating coil (Figure 2). The response signal data from the LDR was stored in a spreadsheet program. The heat plug travel time (the amount of time required for the heat to travel from the heating coil to the detector window) was defined as $\Delta(t_s + t_f)$, where t_f is the heat plug flow time and t_s the systematic heating time. The systematic heating time includes the amount of time for the heat to conduct through the capillary wall and be absorbed by the fluid as well as any heat losses that occur as the plug moves with the fluid stream. To develop a consistent protocol for the determination of $\Delta(t_s + t_f)$, the second derivative of the response signal was calculated (Figure 2, gray trace). The time at which the maximum of the second derivative occurred was used as the time at which the heat plug reached the detector window. The length of capillary from the heating coil to the detector window was defined as Δd. The heat plug flow rate was defined as $\Delta d/\Delta(t_s + t_f)$. Four trials were performed for each capillary and an average flow rate of the heat plug was determined for each of the selected pressure settings ranging from 2 to 25 psi.

RESULTS AND DISCUSSION

Laser-induced backscatter has been chosen as a promising detection scheme for heat index flow monitoring. Several properties of LIB are important in understanding why it is a well-suited detection method for this system. First, it is a simple technique; the light intensity profile generated by viewing LIB radiation on a flat surface is similar to that of a single-slit diffraction model, with the diffraction pattern changing with the RI of fluids in the illuminated capillary.[21,23] For each of the local maxima, most of the light intensity is concentrated in a broad central diffraction maximum, although there are minor secondary bands on either side of the central maximum. The result is an alternating radial sequence of bright and dark zones (which shift in response to RI) whose intensity can be monitored. This profile is generated almost regardless of the fluid in the capillary tubing, in fact, over

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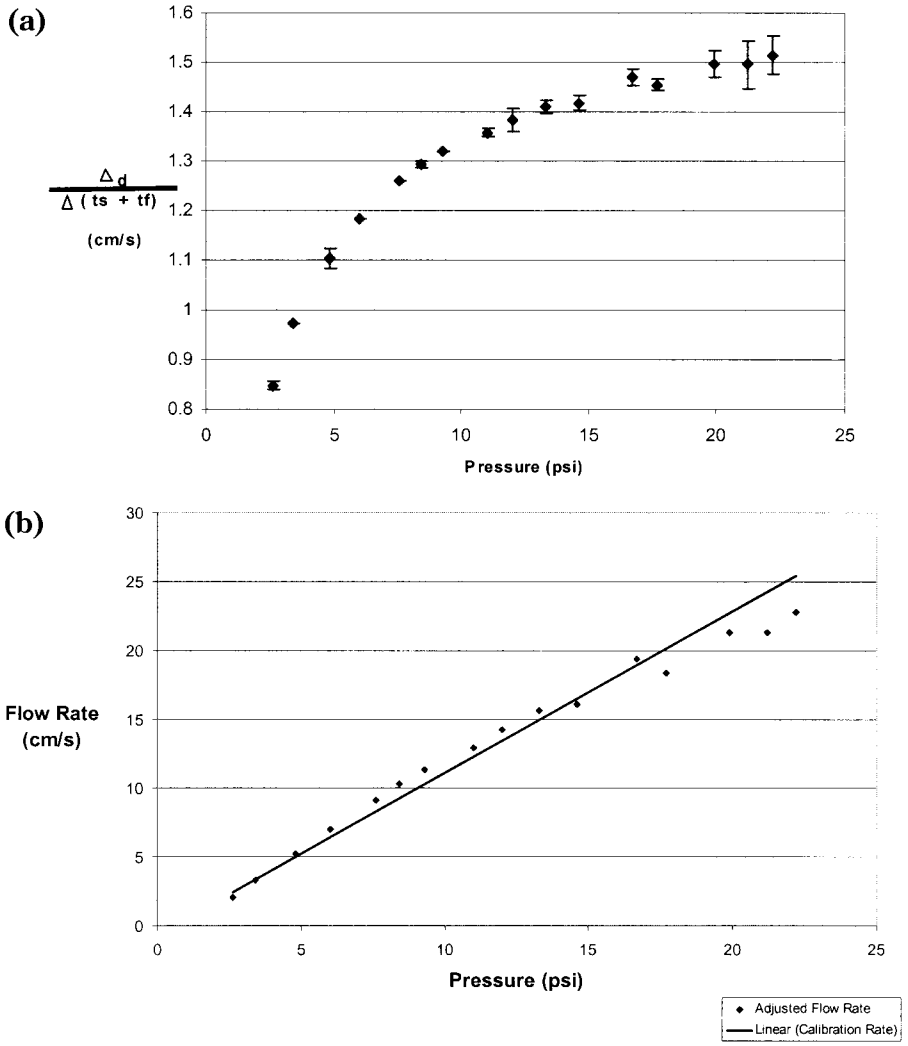

Figure 3. (A) Flow rate as measured by the LIB detection system. Δd is the distance from the heating zone to the detection zone. ($t_s + t_f$) is the amount of time required for the heat plug to traverse Δd plus the lag time due to conductive heating time and heat loss effects. (B) Adjusted fluid flow rate after the conversion algorithm is factored in. The solid line is the calibration flow rate as determined by the weighted effluent method. The data points are the flow rate at various pressures as measured by the LIB detection system after conversion.

a range of RI values from 1.000 to 1.635, a LIB profile is produced.[20] It is also inexpensive; the laser used for most of our preliminary experiments was a common laser pointer (we changed to the He−Ne laser to avoid constantly replacing batteries). The system is stable and robust; mainly because the laser is unfocused. As long as part of the beam encompasses the capillary, the backscatter pattern will form. The He−Ne laser produces a beam width of approximately 2 mm which gives a wide margin for errors in alignment and vibration since the capillary is only 0.35 mm in diameter. In the original LIB work, temperature stabilization was required (on the order of millidegrees centigrade) because of the high thermal coefficient of most fluids, especially water.[21] This characteristic causes thermally induced noise. However, it is exactly this extreme sensitivity to temperature changes that is being exploited for this study. By eliminating temperature-stabilizing devices, a small change in the temperature of the moving fluid can be monitored using a LIB detection system.

To fully characterize the LIB device, both temperature and RI studies were performed. During thermal characterization, the relative position of an intensity zone was monitored and the temperature of the fluid in the capillary was varied. With an increase in fluid temperature, and consequently a decrease in RI, the position of the bright zone relative to the optical slit changes. This causes an increase in the light intensity incident upon the LDR and an increase in detector output voltage. Alignment of the intensity zone at the edge of the optical slit, and empirical knowledge of the direction of movement, allows for sensitive and linear detection ($R^2 = 0.9936$) of temperature changes over a few degrees, as expected.[20] Solutions of varying proportions of methanol and water were placed in the capillary and the signal monitored. There was a linear relationship between signal intensity and solution RI over a range of 1.5×10^{-4} RI units ($R^2 = 0.9795$), consistent with previous results.[20]

Positioning the detector at the steepest portion of the diffraction pattern profile results in a large change in detector output with only a small change in temperature. This allows a small amount of heat to generate a signal at the detector (Figure 2). This reduces the extraneous heating of the capillary walls or fittings that could cause false signals at the detector. In our device, the fluid temperature was typically raised less than 1 °C. As a control experiment, measurements were taken with no flow. No change in signal was detected, thus indicating any heat flow along or

through the walls was not adversely influencing these measurements.

The Poiseuille fluid flow model indicates that fluid velocity is directly proportional to pressure over a broad range of conditions. By holding the capillary radius, fluid viscosity, and capillary tube length constant, an increase in pressure will result in a linearly faster flow rate. Using the weighted effluent method, the fluid flow rate was determined and compared with the values predicted from theory. The results show that the apparatus used for this study follows the linear dependence of flow rate upon pressure as described by the model.

Systematic heat losses and heat-conduction lag time are inherent in this system, requiring that the measurement of the exact heat plug travel time be carefully considered. To consistently determine a value for $\Delta(t_s + t_f)$, a convenient point along the response signal intensity curve was selected; the point chosen was the maximum of the second derivative of the response curve (Figure 2). Defining the time increment at which this maximum appeared as t_2 and the time increment at which the current was applied to the coil as t_1, $\Delta(t_s + t_f) = (t_2 - t_1)$. Determining t_1 and t_2 and knowing the distance from the heating coil to the detector window, the fluid flow rate was calculated.

Figure 3A shows the relationship between pressure and flow rate as measured by the heat-indexing method. The nonlinear relationship is a result of the heat lag time and heat losses from the fluid in the capillary tubing.[24] Heating the capillary walls and the few nanoliters of water takes a finite amount of time after the current has been applied. In addition, the loss of heat to the walls, which is an advantage for CE, robs the first few fluid elements of their heat, and therefore, they are not detected. The heating lag time is most pronounced at high flow rates since the total time is largely due to the heating time, as opposed to the transport time. The heat-loss issues are most pronounced at low flow rates since the solution has a longer contact time with the capillary wall. These processes add to the measured time, but both can be addressed with a simple correction factor. Specifically, the measured heat plug velocity, ν_H (cm/s), is converted to the actual velocity, ν (cm/s, as determined by the weighted effluent experiments), by $\nu = 0.1 \exp[3.6\ \nu_H]$. The calculated flow is linear over a range of 2−25 cm/s (Figure 3B). This new flow-monitoring system is a promising method for assessing small-volume movements. This technique provides the basis for a simple, noninvasive, and robust method for monitoring flow in capillaries and channels.

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However, several developments must be made beyond this proof-of-principle demonstration for application in target systems such as conventional capillary electrophoresis and microfluidic devices. The technology, both the heating process and the detection functions, must be adapted to smaller, more relevant diameters, internal diameters (less than 75 μm), and significantly lower flow rates (less than mm/s). Ongoing studies, where several issues are currently being addressed, indicate these objectives can be met. The obvious limitations when going to smaller, slower systems are loss of heat (signal) through the wall and the diffusion of heat—imitating slow flow. Systematic heat losses can be reduced by decreasing the distance between the heating and detection zones. This can be accomplished by improvements to the resistive heating mechanism or altering the heating source to microwave or infrared radiation so that the detection zone can be placed within a few micrometers. The heating signal also will have to be of a shorter duration and must be confined to a specific small volume within the fluid.

The low flow limit will be defined by the time it takes for diffusion of heat to reach the detection zone, and this will be exacerbated by the close proximity of the heater/detector. Fortunately, there is information available in the signal to extract the flow information, even when a significant portion of the transport is from the heat diffusion. By recording and analyzing the centroid, the forward-, and back-slope (1st and 2nd derivative) of the heat plug, the velocity for the entire heat plug can be calculated. Finally, another major concern which may significantly limit the universal applicability of this method is gradients and analyte interference. The detector is sensitive to any changes in the RI, which may result in false readings. However, by shifting the underlying frequency of the pulse to significantly higher levels (by reducing pulse duration and spacing facilitated by close proximity) than either the gradients or the analyte peak width, the cross-talk between system properties and flow monitoring will be reduced.

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