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Development and characterization of "push–pull" sampling device with fast reaction quenching coupled to high-performance liquid chromatography for pharmaceutical process analytical technologies

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ABSTRACT

A push–pull sampling system interfaced on-line to high-performance liquid chromatography (HPLC) was developed for micro-volume real-time monitoring of reaction mixtures. The device consists of concentric tubes wherein sample was continuously withdrawn through the outer tube and reaction quenchant continuously delivered through a recessed inner tube. The device allowed sampling rates of 0.1–6.0 μL/min from a reaction vessel and stopped the reaction by passive mixing with quenchant to preserve the conditions observed in the reaction vessel. A finite element model of the system showed that reaction mixtures could be completely mixed with quenchant within 4.3 s at a flow rate of 1.0 μL/min. The model also showed that an offset distance of 1 mm between the push capillary and sample capillary tips is sufficient to avoid leakage of quenchant/diluent into the bulk sample for push flow rates up to 95% of the pull flow rate. The maximum relative push flow rate was determined to be 90% of the pull flow rate experimentally. Delay between sampling and delivery to the HPLC was from 111 ± 3 s to 317 ± 9 s for pull flow rates from 1.0 to 3.0 μL/min in agreement with expected delays based on tubing volume. Response times were from 27 ± 1 s to 52 ± 6 s over the same flow rate range. The sampler was tested to determine the effects of sample viscosity. The sampler was also used to demonstrate periodic sampling capabilities. As a test of the system, it was used to monitor the base-catalyzed hydrolysis of aspirin for 1.5 h, demonstrating its utility for monitoring an ongoing reaction.

1. Introduction

A trend in the pharmaceutical industry is to combine real-time chemical measurements with statistical analysis to improve pharmaceutical manufacturing. These techniques, collectively known as process analytical technologies (PAT), are designed to ensure timely feedback so the process may be controlled and improved, with the ultimate goal of real-time release of product [1,2]. PAT has been dominated by spectroscopic methods such as near IR [3], Raman [4], UV, fluorescence, and pulsed tetrahertz spectroscopy [5] because they provide rapid feedback and are relatively non-invasive. Despite the power of spectroscopy for PAT, it is desirable to utilize separations methods in the frequent case of complex mixtures or mixtures of compounds with overlapping spectra. Trends in high-performance liquid chromatography (HPLC) [6,7], such as higher pressures [8], higher-temperature [9], and use of monolithic columns [10], have reduced the separation time to acceptable levels for PAT. To utilize HPLC for PAT applications, it is also necessary to incorporate automated sampling and sample preparation procedures. Microscale devices provide advantages in automation and sample preparation, while also minimizing sample volume requirements [11]. In this work, we describe a microscale sampling system suitable for on-line coupling to HPLC that integrates sample dilution and reaction quenching.

Samplers for coupling to HPLC should satisfy several criteria. The developed sampler should be able to be inserted directly into the reaction vessel. The sampler should enable rapid quenching of the reaction to preserve a snapshot of the conditions inside the reaction vessel. The sampler should be versatile enough to work with different solvents, mixtures of different viscosities, and mixtures containing particles.

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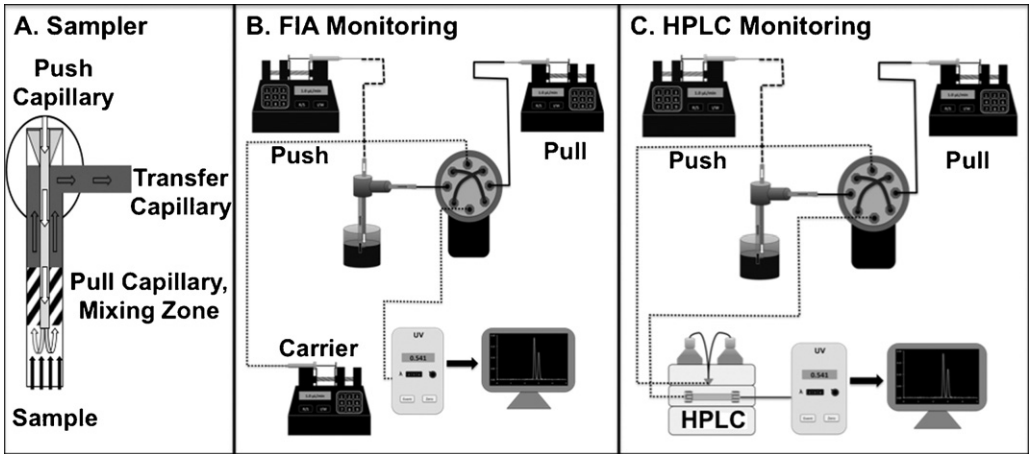

Fig. 1. Illustration of the sampling system showing (A) a detailed view of sample mixing with quenchant/diluent, (B) an overview for flow injection analysis (FIA) monitoring, and (C) an overview for HPLC monitoring.

Samplers can be batch systems that remove a single sample of pre-set volume or continuous systems that continuously remove samples. Batch systems have been used for process monitoring of monoclonal antibody production in fermentation vats. A system that injects fermentation broth sample (including a small number of cells) directly onto an HPLC column, without filtration or dilution, was reported [12]. A similar system for monitoring ethanol production in a fermentation removes aliquots from a fermentation broth followed by automated filtration, dilution and injection onto the HPLC [13].

Microdialysis (MD) coupled to HPLC is an example of a continuous monitoring system. MD allows for selective sample collection, leaving particulate matter and proteins in the reaction vessel while transporting smaller analytes of interest for analysis via HPLC. MD coupled to HPLC has been used in many applications including monitoring in vivo [14–16], sampling from whole blood [17], and determination of metals in aqueous samples [18]. However, MD is not suitable for all applications of PAT because the membranes are not compatible with organic solvents, and solids or larger analytes of interest are excluded from the sample.

We have developed a microscale sampler that performs automated sampling with rapid (seconds) mixing and on-line HPLC analysis. The developed sampler is inspired by push–pull perfusion systems used for neuroscience [19,20] and is adaptable to a wide variety of analytes. We have demonstrated the ability of the sampler to monitor reactions with a temporal resolution of less than 1 min, and a delay time of several minutes (limited by length of capillary from sample to HPLC) with automated data collection and system control. Finite element modeling of the sampler shows that it can rapidly dilute or quench reactions.

2. Experimental

2.1. Materials

Unless otherwise noted, all chemicals were purchased from Fisher Scientific (Chicago, IL, US). Erioglaucine disodium salt (FD&C Blue 1) in the form of blue food color (water, propylene glycol, erioglaucine, 0.1% propylparaben) was obtained from McCormick (Sparks, MD, US). Acetylsalicylic acid and salicylic acid were obtained from Acros Organics (Morristown, NJ, US).

2.2. Sampler assembly

The sampler was constructed using three fused silica capillaries (Polymicro Technologies Phoenix, AZ, US) and a mixing tee (Valco Instruments, Houston, TX, US) as shown in Fig. 1A. One end of the push capillary (40 μm I.D., 100 μm O.D.) was connected to a Fusion 400 syringe pump (Chemyx, Inc., Stafford, TX, US) and the other end was inserted completely through the mixing tee and sheathed within the pull capillary (100 μm I.D., 250 μm O.D.). One end of the transfer capillary (150 μm I.D., 360 μm O.D.) connected the sidearm of the tee and the other end was connected to an 8-port switching valve (Valco Instruments). The valve was fit with two injection loops, allowing one loop to fill while the other was injected. A second piece of capillary connects the switching valve to a PHD2000 syringe pump (Harvard Instruments, Holliston, MA, US) in withdrawal mode to allow fluid to be pulled through the sampler (Fig. 1B).

Sampling was monitored using direct detection, flow injection analysis, or HPLC. For direct detection experiments, mixed sample was pulled directly through the flow cell of a Spectra 100 variable wavelength detector (Spectra Physics, Mountain View, CA, US). For flow injection analysis (FIA), the switching valve was fitted with 0.5 μL sample loops (Fig. 1B). Sample mixed with quenchant/diluent was pulled through the valve to fill the injection loop and then injected from the loop into the carrier flow stream every 1 min and pumped into the variable wavelength detector unless otherwise noted. Data was recorded using a USB-6008 DAQ card and an in-house LabVIEW program (National Instruments, Austin, TX, US).

For experiments using HPLC to monitor the sample, 2 μL stainless steel sample loops were installed on the 8-port switching valve used for FIA. Sample was injected every 5 min onto a 150 mm × 4.6 mm I.D. column packed with 5 μm Grace Prosphere HP C4 particles (Grace Davison Discovery Science, Deerfield, IL, US) installed on an Agilent 1100 HPLC system (Agilent Technologies, Inc., Santa Clara, CA, US). The sample was separated isocratically with 35% MeOH, 65% 10 mM KH_2PO_4 (pH 2.3) as the mobile phase. Absorbance was monitored at 254 nm. The arrangement is shown in Fig. 1C.

2.3. COMSOL Multiphysics Modeling

COMSOL Multiphysics 3.5a (COMSOL, Inc., Burlington, MA, US), a finite element modeling program, was used to model fluid flow and mixing within the nested capillary system. The system was modeled using modules for incompressible, isothermal fluid flow and convection and diffusion. Constants for water at 20 °C (density of 998.207 kg m^{-3} and viscosity of 1.002×10^{-3} Pa s) were used for the model. The diffusion coefficient was set to 1.0×10^{-9} m^2/s to approximate a small molecule in solution.

A 3 mm segment of the sampling tip was modeled in three dimensions, with the push capillary recessed 1 mm from the tip of the pull capillary (Fig. 2) and 15 μm from one edge of the pull capillary to match the arrangement used for experiments. Area A is defined as an inlet with laminar volumetric flow and a concentration of 1 mM. Area B is defined as an open boundary for fluid flow with a concentration of 0 mM. Area C is defined as an outlet with laminar volumetric flow and an open boundary for convection and diffusion. Sample and quenchant were defined as fully mixed when the concentration across a plane perpendicular to the capillaries varied ≤5%. Mixing time was calculated using the distance past the push capillary tip at which the sample was mixed, and the flow rate at the center of the widest part of the volume between the push and sample capillaries. Travel time was calculated as the time needed to travel the 1 mm from the tip of the pull capillary to the tip of the recessed push capillary at the linear flow rate determined from the model. The I.D. of the pull capillary was varied from 150 to 400 μm while the centered push capillary had a constant O.D. of 100 μm and I.D. of 40 μm to determine the dependence of mixing time on pull capillary diameter.

To model the maximum push-to-pull flow rate, the pull flow rate was held constant at 1.0 μL/min while the system was modeled with increasing push flow rates. The distance from the push capillary tip where the concentration decreased below 1% of the original concentration was determined at each flow rate.

2.4. On-line dilution

A dilute solution of erioglaucine was sampled at a constant flow rate (1.0 or 2.0 μL/min). The flow rate of water through the push capillary was varied to dilute the sample to different degrees. FIA (see Section 2.2) was used to monitor the degree of sample dilution.

2.5. Delay and response time

Delay and response times were determined using direct monitoring (see Section 2.2). Every 10 min, 100 μL of erioglaucine solution was added to a stirred vial containing a constant volume of water. The pull flow rate was set to 1.0, 2.0 or 3.0 μL/min. Water was pumped through the push capillary at 50% of the pull flow rate. The experiment was repeated with the sampler removed, isolating the contribution to delay and response times due to the length of capillary between the sampler and the variable wavelength detector.

2.6. Viscosity

Samples of varying viscosity were prepared by mixing a constant volume of erioglaucine (in ethanol) with isopropanol and glycerol. Isopropanol was pumped through the push capillary as a model quenchant/diluent. To determine the maximum pull flow rate for a given viscosity, the push flow rate was held constant at (0.1 μL/min). The pull flow rate was initialized at 1.0 μL/min and increased by 1.0 μL/min every 20 min until cavitation was observed in the pull syringe. Peak height reproducibility was monitored using FIA (see Section 2.2), with the push flow rate set to 50% of the pull flow rate.

2.7. Stop-flow sampling

The system was modified by placing a six-port switching valve between the push syringe pump and the push capillary to enable rapid switching between two push flow rates. Syringes were filled with water and connected to the six-port valve adjacent to the push capillary. Two inlet capillaries, of equal dimension to the push capillary, were attached to the six-port valve adjacent to the inlet capillaries, equalizing the pressure drop when the valve was actuated. One syringe was set to 0.9 μL/min (sampling "off") and the other was set to 0.1 μL/min (sampling "on"), while the pull flow rate was set to 1.0 μL/min. Erioglaucine in water was used as the sample. The resulting mixed sample/water solutions were monitored using FIA (see Section 2.2).

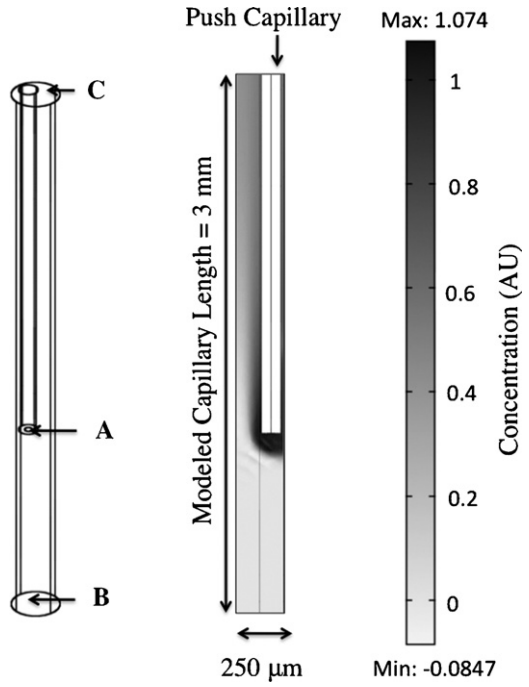

Fig. 2. COMSOL model of the sampler. The model was used for determining the effect of sample capillary diameter on mixing time, the effect of flow rate on mixing time and the correct offset distance between the sampling tip and push capillary to ensure quenchant does not leak into the bulk sample.

2.8. Reaction monitoring with HPLC

Base-catalyzed hydrolysis of acetylsalicylic acid (aspirin) was monitored with the sampler coupled to the HPLC (as described in Section 2.2). A 0.2 M solution of acetylsalicylic acid was prepared in MeOH. A stirred vial containing 14.5 mL of a 25 mM bicarbonate buffer (pH 11.2) was sampled at a pull flow rate of 1.0 μL/min. A constant push flow rate of 0.5 μL/min was applied with 10 mM KH_2PO_4 as the quenchant, which changed the pH of the reaction to approximately 7 and quenched the hydrolysis. After 5 min of sampling, 0.5 mL of the acetylsalicylic acid solution in MeOH was added to the sampling vial, starting the hydrolysis reaction. The reaction was monitored for 110 min using HPLC.

To verify the resulting rate constant was reasonable, the reaction was run again under the same conditions but monitored by a different method. 5 μL aliquots of sample were mixed with 145 μL of 25 mM iron (III) nitrate at a regular interval. Absorbance at 535 nm of the resulting mixture was detected and compared to a calibration curve to determine the concentration of salicylic acid in reaction over time. A rate constant for the hydrolysis of aspirin was calculated and compared to the online method.

3. Results and discussion

3.1. Sampler design

The heart of the push–pull sampler is a pair of concentric fused-silica capillaries. Solution is pulled through the outer (pull) capillary directly to an HPLC valve (or on-line detector) using a syringe pump

Table 1
Effect of pull capillary I.D. on mixing time for a pull flow rate of 1.0 μL/min and a push flow rate of 0.5 μL/min. Decreasing the pull capillary I.D. decreases the time needed to mix quenchant and sample as they travel up the sample capillary.

Pull capillary I.D. (μm)	Mixing time (s)	Travel time (s)	Total time (s)
150	3.5	2.6	6.1
200	3.5	2.8	6.3
250	4.3	3.8	8.1
300	8.6	5.0	13.6
400	17.2	8.3	25.4
400 (centered)	9.4	12.4	21.8

as shown in Fig. 1. At the same time, the diluent/quenchant solution is pushed through the center (push) capillary and mixes with the sample solution upstream in the sampler (Fig. 1A). Solution added through the push capillary can be used to dilute the sample and may include a quenchant to stop the reaction. By varying the flow rate through the push capillary, it is possible to change the dilution. To ensure that the push solution does not contaminate the reaction vessel, the sampler capillaries are arranged so that the push capillary was recessed within the pull capillary. A useful feature of this system is that all materials are largely compatible with many reaction conditions and solvents.

While this system is similar to a push–pull capillary used for in vivo monitoring [19,20], it differs in two ways. First, in vivo push–pull samplers use the same flow rate for the push and pull solutions to avoid changing the volume of extracellular fluid. Using different flow rates, we can adjust dilution or quenching of the sample. Secondly, for in vivo sampling, the inner and outer capillaries are flush at the end to provide a region of solution exchange with the extracellular space. In our case, recessing the push capillary prevents contamination of the sampled solution. In principle, adding solution at a tee downstream of sampling could fill the role of the push solution for quenching or dilution; however, the approach described here allows quenching to occur more rapidly.

3.2. Sampling tip modeling with COMSOL

We modeled the push–pull sampler system using COMSOL with the basic construction shown in Fig. 2. As shown, the diluent/quenchant and sampled solution mix within the pull capillary and reach homogeneity upstream from the sampling tip. Furthermore, the diluent/quenchant solution does not reach the sampling tip and therefore does not contaminate the reaction solution. Using this basic model, we examined how the pull capillary I.D., flow rates, and distance between the outer tip of the push and sample capillary (offset) affect performance parameters such as time required for mixing of quenchant with sample. We also evaluated what conditions would prevent leakage of diluent/quenchant into the reaction solution.

3.2.1. Pull capillary inner diameter

The effect of pull capillary diameter was modeled (structure shown in Fig. 2) to determine suitable dimensions for construction. The effect of the pull capillary I.D. on time required to reach complete mixing is summarized in Table 1. Increasing the diameter dramatically increases the mixing time since the solutions mix primarily by diffusion under the flow rates tested. Increasing the pull capillary I.D. also increases travel time due to a slower linear flow rate. Although short mixing times are desirable, we found that using a sampler with an I.D. < 250 μm was difficult to fabricate. Therefore, all samplers used had a 250 μm I.D. pull capillary. Further decreases in mixing time can be achieved by centering the push capillary within the pull capillary (see Table 1); however, using the materials and methods described here, it was difficult to achieve centered capillaries.

3.2.2. Effect of flow rate on mixing

We next evaluated the effect of flow rates on mixing time. Time required for complete mixing increases with decreased pull flow rate when the push flow rate is modeled at 50% of the pull flow rate (Table 2). Time required for mixing is increased by less than 1 s for a 10-fold decrease in pull flow rate. Because mixing is primarily diffusion-controlled and thus dependent on the width of the channel, the slower mixing time at lower flow rates is due to a higher percentage of time spent in the wider pull channel before being swept into the more narrow space between the push and pull capillary where mixing occurs more rapidly. The difference in travel time from 3.8 to 37.4 s for the same 10-fold decrease in pull flow rate is much more dramatic. To minimize the effect of travel time, the offset between the pull capillary and the recessed push capillary may be decreased to <1 mm at lower flow rates.

3.2.3. Relative push flow rates and capillary offset

Modeling was used to determine the appropriate offset between the pull capillary tip and the recessed push capillary within. The offset needs to be small to minimize time between when the sample is removed from the reaction vessel and when it is mixed with diluent/quenchant. If the offset distance is too short, however, the diluent/quenchant may be able to leak into the bulk solution, affecting the ongoing reaction that is being monitored. It was determined that for a push flow rate of 95% of the pull flow rate, the distance to which the diluent/quenchant spread (local concentration 1% of the original quenchant concentration) was 0.51 mm (Fig. 3 and Supplemental Fig. 1). Based on this result, a 1 mm offset should be sufficient to ensure that the diluent/quenchant does not leak into the bulk sample for a push flow rate up to 95% of the pull flow rate. In practice, when erioglaucine was flowing through the push capillary, leakage into the bulk solution was observed when the push rate was only 90% of the pull rate. This discrepancy may be due to small fluctuations in the flow rate due to pulsing from the syringe pumps. We used a 1 mm offset distance in all subsequent investigations, but a shorter offset distance could be used to facilitate faster mixing if a lower push flow rate is used.

3.3. On-line dilution

The total volumetric flow rate through the pull capillary is the sum of the volumetric flow rates through the push capillary and the volumetric flow rate from the sample. Therefore, adjusting the ratio of the push to pull volumetric flow rates controls the mixing ratio of the sample with either a quenchant or a diluent. For a constant pull flow rate, increasing the push flow rate should result in predictable dilution of the sample. To confirm this performance,

Table 2
Effect of flow rate on mixing time. When relative push-to-pull flow rate is held constant, an increase in pull flow rate results in a small decrease in mixing time.

Pull flow rate (μL/min)	Push flow rate (μL/min)	Mixing time (s)	Travel time (s)	Total time (s)
0.1	0.05	4.7	37.4	42.1
0.5	0.25	4.5	7.5	12.0
1.0	0.5	4.3	3.8	8.1
2.0	1.0	4.3	1.9	6.2

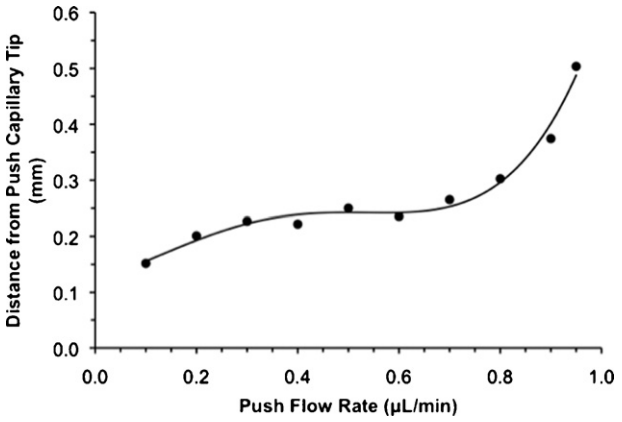

Fig. 3. Distance from push capillary tip (mm) where concentration of quenchant/diluent decreases to <1% of the original concentration. The pull flow rate was kept constant at 1.0 μL/min while the push flow rate was increased. It was determined that even up to a flow rate of 0.95 μL/min an offset of 1 mm was sufficient to prevent leakage.

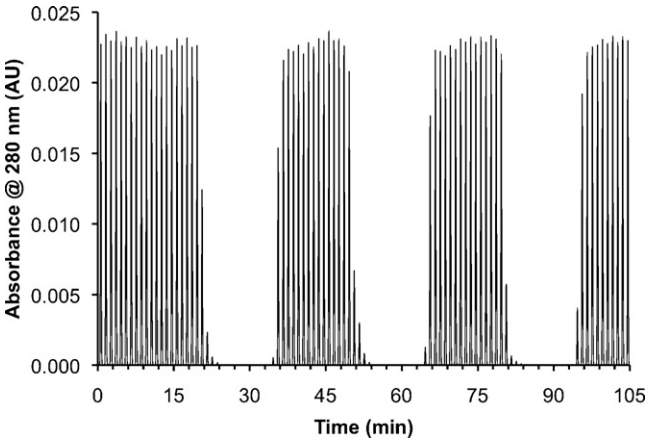

Fig. 4. Plot showing FIA peaks when the push flow rate is switched from 10% of the total pull flow rate ("sampling on") to 90% ("sampling off") and back to 10% every 15 min. The 5 min delay is due to the time required for sample to travel to the detector. It takes approximately 2 min to fully switch from "on" to "off" and back again using this method. This feature may be used to control sample consumption when continuous sampling is not desired.

we varied the push flow rate with a constant pull flow rate while sampling dilute erioglaucine and monitoring the resulting sample by UV absorbance. We found that diluting resulted in the expected decrease in sample absorption (Supplemental Fig. 2). These results also matched modeling data (Supplemental Fig. 1). These results further supported that the push solution does not leak out of the sampler and demonstrated predictable operation of the sampler for on-line dilution.

3.4. Delay and response time

An inherent delay time exists between the time sample is collected and the time that signal is detected downstream of the reactor. Delay time is defined as the difference between the time when sample is added to the stirring sample vial and when 50% of the maximal value of the step change is observed at the detector. Response time is defined as the time required to observe a change from 10% to 90% of the maximal value following a step change in analyte concentration. Delay times ranged from 111.5 ± 3.4 s to 317.0 ± 8.8 s (see Table 3), depending on the flow rate and agreed with expected delays based on tubing volume. Response times varied from 26.6 ± 1.0 s to 52.4 ± 5.8 s over the same flow rate range. Tests performed without the sampler and only the transfer tubing revealed that both parts of the system contributed significantly to the delay and response time. These results suggest the limits of feedback control and rate of reaction that can be monitored by this approach. For most PAT experiments, this performance is sufficient.

3.5. Viscosity

As pharmaceutical reactions may take place in solutions over a range of viscosities, we examined the effect of viscosity on the ability to sample by sampling erioglaucine in solutions with different viscosities that were adjusted by the addition of glycerol. Viscosity of the solution was found to affect the maximal pull flow rate that could be used. For any solution, a threshold pull flow rate exists above which cavitation is observed in the pull syringe, resulting in unreliable flow rates. Above a viscosity of 4.5 centipoise (cP), it was not possible to sample without cavitation down to the minimal flow rate tested of 1.0 μL/min. This effect imposes a restriction on the system that lower flow rates must be used for sampling high viscosity solutions. The reproducibility of sampling was evaluated at viscosities ranging from 1.5 to 4.8 cP as a function of flow rate. At a pull flow rate of 2.0 μL/min, and a push flow rate of 1.0 μL/min, the absorbance readings for 1.5 cP, 2.6 cP and 4.8 cP viscosity solutions were $3.1 \pm 0.2 \times 10^{-3}$, $3.3 \pm 0.2 \times 10^{-3}$ and $3.2 \pm 0.1 \times 10^{-3}$, respectively, averaged over 20 injections. The constant absorbance with increasing viscosity shows that the viscosity does not affect quantitative sampling in this range.

3.6. Stop-flow sampling

The sampler was designed for continuous sampling; however, with small samples or slow reactions, it may be advantageous to run the sampler in a non-continuous mode to minimize sample consumption. One approach is to remove the sampler from the solution, but this may not always be feasible depending on the reactor. Therefore, we examined using flow effects within the sampler to stop and start sampling. As discussed above, the total pull flow rate is equal to the sum of the sampling flow rate and the push flow rate. This allows the sampling flow rate to be decreased by increasing the push flow. If the push rate equals the pull flow rate, there would be no sampling; however, the push solution would likely diffuse into the reaction solution. Experimentally, we observed that the push flow rate could be increased to 90% of the pull flow rate without push solution leaking into the reaction vessel. At this fraction, the sampling flow rate was only 10% of the total pull flow rate and nearly stopped, e.g. at 1.0 μL/min, only 100 nL/min of sample

Table 3
Delay and response times, ±standard deviation ($n = 3$). Step changes in erioglaucine concentration were recorded with the sampler connected to the detection capillary as well as with the sampler removed (to show the delay and response time due to only to the detection capillary).

Pull flow rate (μL/min)	Delay time		Response time	
	Sampler (s)	No sampler (s)	Sampler (s)	No sampler (s)
1	317.0 ± 8.8	203.9 ± 3.6	52.4 ± 5.8	19.6 ± 1.3
2	160.0 ± 1.2	103.3 ± 3.9	34.9 ± 2.2	11.9 ± 0.8
3	111.5 ± 3.4	69.1 ± 1.8	26.6 ± 1.0	10.4 ± 0.5

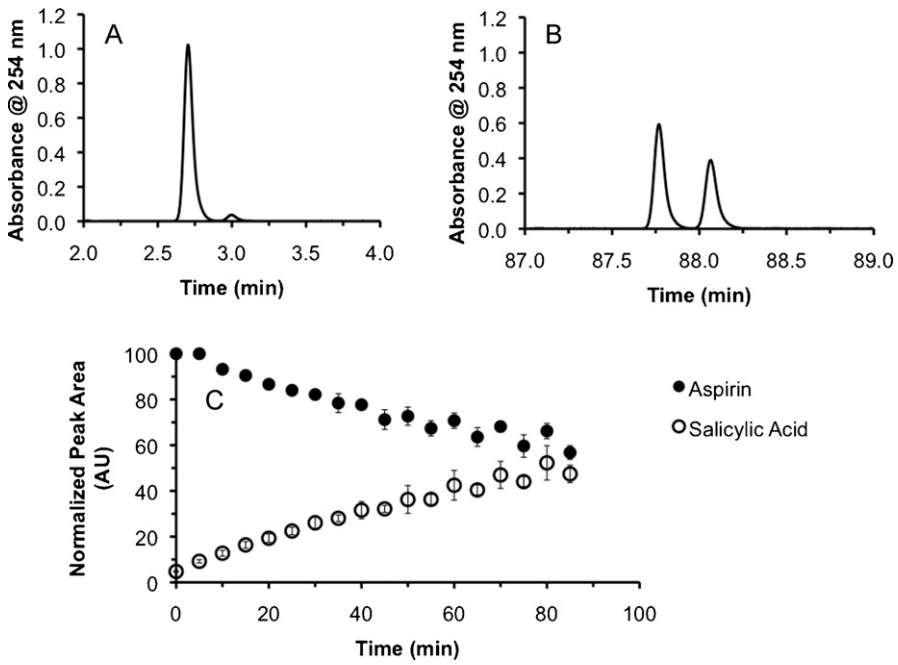

Fig. 5. Chromatogram of aspirin and salicylic acid injected at (A) 0 min following the start of the hydrolysis reaction and (B) 85 min following the start of the hydrolysis reaction. (C) Plot of aspirin and salicylic acid peak areas monitored with HPLC. Peak areas were calculated from chromatograms using in-house software.

was withdrawn. By modulating the push flow rate between 10% and 90% of the pull flow rate, sampling could be effectively switched from "on" to off" (Fig. 4).

To demonstrate this possibility, we sampled dilute erioglaucine at a pull flow rate of 1.0 μL/min and analyzed the mixed solution by FIA. Switching the push flow rates from 0.1 μL/min ("on") to 0.9 μL/min ("off") showed a change in the FIA peak heights in about 3 min (Fig. 5).

3.7. Organic reaction monitored by HPLC

We coupled the system to an HPLC system to show on-line monitoring of a reaction with automated separation and detection. Base-catalyzed hydrolysis of acetylsalicylic acid (aspirin) results in the production of salicylic acid and acetic acid. As acetylsalicylic acid was hydrolyzed, sample was collected, quenched by pumping KH_2PO_4 solution through the push capillary to increase the pH of the sampled solution and quench the reaction, and automatically injected onto the HPLC at fixed intervals over 1.5 h (Fig. 5). For each injection, acetylsalicylic acid and salicylic acid were separated within 3 min (Fig. 5A and B). Peak areas, normalized to the initial acetylsalicylic peak, are shown for three replicate experiments in Fig. 5C. It is believed that the increased standard deviation at the end of the experiment was due to air becoming trapped in one of the sample loops for one of the replicates. A rate constant for this reaction was calculated to be $1.1 \pm 0.1 \times 10^{-3}$ mol^{-1} L min^{-1}. The rate constant for offline monitoring of the reaction with iron (III) nitrate under the same reaction conditions was determined to also be $1.1 \pm 0.1 \times 10^{-3}$ mol^{-1} L min^{-1}, demonstrating that the sampler provides comparable rate data to conventional monitoring methods.

This experiment demonstrated the utility of the sampler for near real-time monitoring of an ongoing reaction. For each sample, the reaction was halted upon the removal by a shift in pH, and was automatically analyzed with HPLC. The reaction progress from acetylsalicylic acid to salicylic acid was observed and individual chromatograms could be obtained for any given time point, showing a snapshot of the reaction in progress. Beyond initial loading of reagents, the entire monitoring process was automated, removing the time and potential errors from manual interventions.

4. Conclusions

A push–pull sampler device was demonstrated for monitoring chemical reactions. The system allowed sampling from solutions with a range of viscosities and automated the infusion of quenchant or diluent to sample. The system was readily coupled to an on-line detector, flow injection analysis, or HPLC. The device is capable of quenching in 5 s at the sampling site. Flow properties prevented quenchant or diluents from leaking into the reaction vessel and allowed the possibility of stopping sampling periodically. The latter possibility is useful for reducing sample consumption. The ability to monitor an ongoing reaction has been demonstrated under completely automated conditions. Computer control of pumps, valve and detector through LabVIEW software allow for sampling to be automated. Future work with the sampler would adapt the materials used for particular reactions that are being monitored and could involve connecting the sampler to other separation/detection instruments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.09.066.

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