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A simple, effective mixing chamber used in conjunction with a syringe pump for flow analysis

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ABSTRACT

A simple, effective mixing chamber used in conjunction with a syringe pump for flow analysis is described and evaluated. A mixing chamber was constructed using a conventional 5 mL pipette tip and its performance compared with a widely used mixing coil. The results demonstrate that the mixing coil does not rapidly and completely mix solutions. Utilizing a configuration that reversed solution positions in the chamber with each mixing cycle, the proposed mixing chamber achieved complete mixing in a significantly shorter time than the mixing coil. The influence of injected sample volume on absorbance signals was evaluated by calculating an $S_{1/2}$ value for the system. As tested with a minimal rinse, the system has no discernable carryover. Testing this new approach in our previously described silicate measurement system resulted in a more than twofold improvement in sensitivity.

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1. Introduction

The efficiency of fluid mixing is critical in many applications, especially those involving chemical or biological reactions. In flow analyses, fluid is transported by laminar flow inside a narrow-bore open tube where the velocities are too low to induce turbulence mixing [1]. Diffusive and convective mass transports are the main processes governing mixing [2], and this is a function of the distance of mixing liquid transport. It is enhanced by stretching and folding of interfaces which can be augmented in laminar flow regimes. That said, the diffusive mixing associated with laminar flow is often insufficient for mixing liquids [3]. Therefore, a number of techniques have been developed to improve mixing. These include: mixing chambers [4], mixing coils [5,6], knitted reactors [7], packed bed reactors [8], miniature stirrers [9], complex geometries [10], turbulent-like flows [11], special confluence connectors [12,13], floating solid particles or beads [14,15], using supercritical fluids [16], single bead string reactor [17], and time pulsing [18,19]. In all these techniques, mixing is easier to achieve in larger scale devices. Unfortunately many chemical and biological applications require using samples as small as possible. Since sample size is limited, it is desirable to minimize the dimensions of the flow system used which inevitably leads to a highly laminar flow regime and raises concerns about mixing efficiency.

In some flow analysis systems, syringe pumps rather than peristaltic pumps are used to transport liquids through tubing and simultaneously mix the solutions. Approaches include sequential injection analysis (SIA) [20], lab-on-valve (LOV) [21], hybrid flow analyzer (HFA) [22], and multi-syringe flow [23]. In such systems mixing coils can be added to the flow path in order to improve mixing efficiency. In most situations the mixing achieved is not significantly different than in similarly arranged systems that use peristaltic pumps. In some cases, SIA and LOV in particular, it has proven difficult to attain the same levels of mixing efficiency. In an analytical system using a syringe pump the syringe itself can act as a primary mixing chamber but nonetheless an additional mixing coil is needed to enhance mixing [22,24,25]. The advantage of using the syringe as a mixing chamber is its larger cross-section therefore higher Reynolds number.

In this study we investigate the efficiency of using the syringe in the syringe pump as the primary mixing chamber with two different secondary mixing chambers: a conventional mixing coil and a standard 5-mL pipette tip. The characteristics of the system were studied by evaluating performance parameters such as repeatability, $S_{1/2}$, and carryover. We then tested both systems in a practical application, dissolved silicate measurement of fresh water samples.

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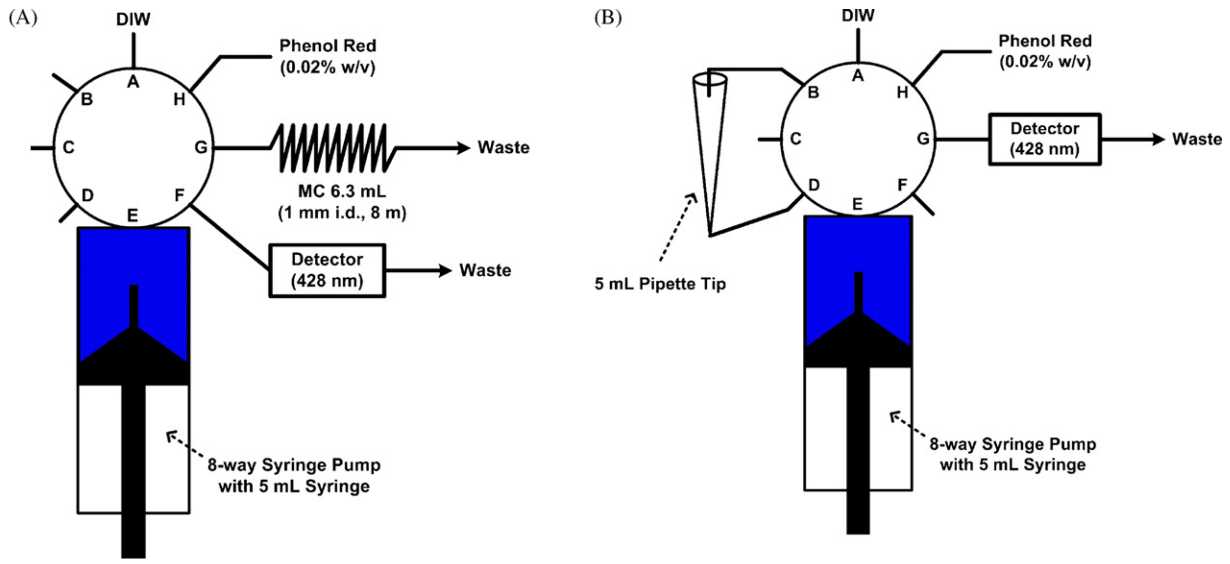

Fig. 1. Configuration of mixing systems with different secondary mixing chambers: (A) mixing coil and (b) pipette tip. DIW, deionized water.

2. Experimental

2.1. Comparison of mixing efficiency of two secondary mixing devices: mixing coil and pipette tip

Fig. 1(A) and (B) shows the configurations of two systems, one using the mixing coil and the other a pipette tip. The efficiencies of the two systems were compared with respect to mixing 0.6 mL of dye solution (0.02% Phenol Red indicator solution, VWR Scientific Products, PA) with 4.4 mL of deionized water DIW.

In both systems a syringe pump (P/N 54022, Kloehn, NV) is used to pull 4.4 mL of DIW into the 5-mL syringe in the syringe pump. 0.6 mL of a Phenol Red solution is then pulled into the syringe. The syringe serves as the primary mixing chamber. To use the mixing coil as a secondary mixing chamber, the mixture in the syringe is pushed into the mixing coil (1 mm i.d., 8 m long Teflon tubing), and then pulled back into the syringe. This constitutes one cycle of mixing. When the 5 mL pipette tip is used as the mixing chamber, one channel of the selection valve of the syringe pump is connected to the top and another to the bottom of the pipette tip (see Figs. 1 and 2). The mixture in the syringe is then pushed to the top of the pipette tip and fills the chamber. It is then pulled back into the syringe via the bottom of the pipette tip. This constitutes one cycle of mixing. The efficiency of the two mixing systems was compared by measuring the number of cycles required to completely mix the solution. In both systems the mixing chambers and detector were cleaned with deionized water 2–3 times after recording each peak signal in order to bring the signal back to baseline levels. A photograph of the mixing chamber with syringe pump system is shown in Fig. 2. It should be noted that the syringe pump used in this work is different from the traditional syringe pumps previously used in sequential injection analysis (SIA). In this syringe pump, a zero dead volume syringe is achieved by a special design piston (see the pointed tips in Figs. 1, 2 and 4). To use it in a SIA technique, a minor modification is necessary (the addition of a holding coil between the distribution valve and the syringe) because, in the syringe pump supplied by the manufacturer, the syringe is directly connected to an 8-way distribution valve.

2.2. Comparing the mixing devices in a silicate measurement system

We adapted our recently described a liquid waveguide-based silicate measurement system [24] to use a 5-mL pipette tip instead of a mixing coil as a secondary mixing chamber (see Fig. 3). All reagents were prepared as previously described. The fluidic system consisted of one syringe pump SP equipped with an 8-way distribution valve SV (P/N 17620) and an injection valve IV (6-port medium pressure, Upchurch Scientific, WA). The syringe pump was equipped with a 5-mL capacity zero dead volume syringe (P/N 24691). The injection valve was equipped with a Teflon injection coil IC (0.78 mL, 1 mm i.d., 100 cm). As Fig. 3 indicates channel H of SV was connected to the top of the pipette tip, and channel F of SV was connected to the bottom of the pipette tip. The arrangement of the system with the mixing coil was as previously described [24].

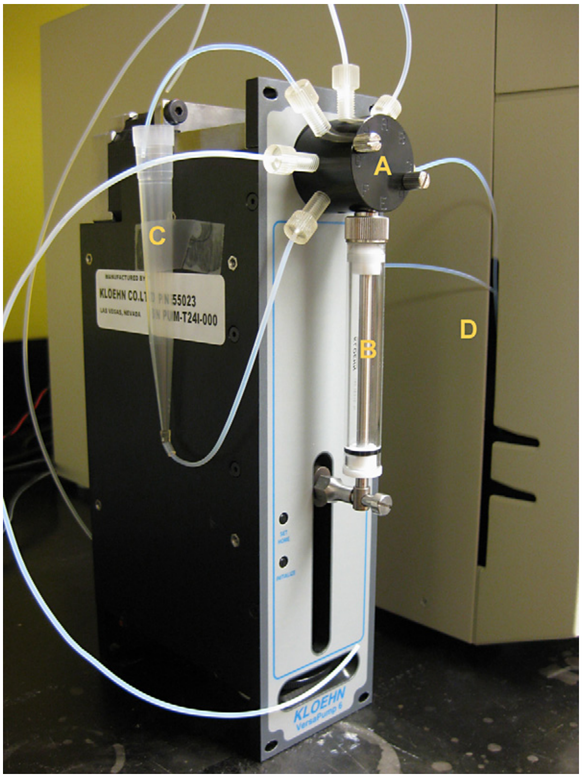

Fig. 2. Photograph of the experimental system: (A) 8-way distribution valve, (B) 5-mL glass syringe, (C) 5-mL pipette tip, and (D) spectrophotometer.

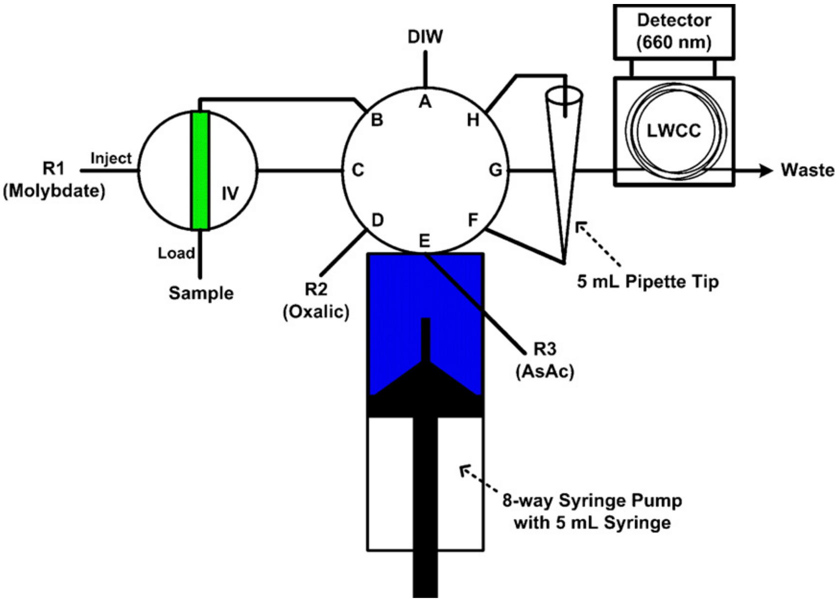

Fig. 3. Liquid-waveguide analyzer for silicate determination adapted for using a 5-mL pipette tip as the secondary mixing chamber. DIW, deionized water; LWCC, liquid-waveguide capillary cell; IV, injection valve; AsAc, ascorbic acid; R1, molybdate solution; R2, oxalic acid solution; and R3, ascorbic acid solution.

Operation of the system was essentially the same as previously reported (Table 1 in [24]) except steps 10–12 (the mixing solution steps). For these tests the total mixing duration was identical (8 min) for both mixing chambers to permit direct comparison of the two different mixing approaches with respect to this specific application.

3. Results and discussion

3.1. Studying of mixing efficiency of secondary mixing chambers

Fig. 4(A) shows the apparent mixing of phenol red solution and deionized water in a syringe with no secondary mixing chamber and no external agitation of any kind. The phenol red solution mixes only as a result of sinking and the resulting eddy diffusion. The syringe was photographed after 0, 1, 2, 3, and 16 min. Mixing by gravity sinking alone is clearly both slow and ineffective and a secondary mixing chamber or agitation is necessary to mix solutions within the syringe.

We first tested the efficacy of the mixing coil. Photographs of the solution in the syringe after 0, 1, 2, 3, and 14 mixing cycles are shown in Fig. 4(B). It appears that the solution is reasonably well mixed after only 2 cycles of mixing. However, when the mixed solution is injected into a spectrophotometer, measuring light absorption at 428 nm, we see that the photograph is misleading. A completely mixed solution would yield a uniform flat-topped signal. As shown in Fig. 5(A) the solution was not completely mixed even after 16 cycles.

We then tested the 5-mL pipette tip mixing chamber using the spectrophotometer to measure the efficiency of mixing. Fig. 5(B) shows the signals recorded after 1, 2, 3, 4, 5, 15, and 27 mixing cycles. This system was able to achieve the flat-top peak expected of complete mixing after only 5 mixing cycles. We believe a major reason this system is more efficient is that the syringe contents are inverted during each cycle when the solution is put in at the top but removed from the bottom of the secondary mixing chamber. The result is analogous to manually mixing the solution in a capped volumetric flask by repeatedly flipping the flask upside down. The use of a pipette tip in this manner can decrease system cycle time to 3–4 min for fast chemical reactions.

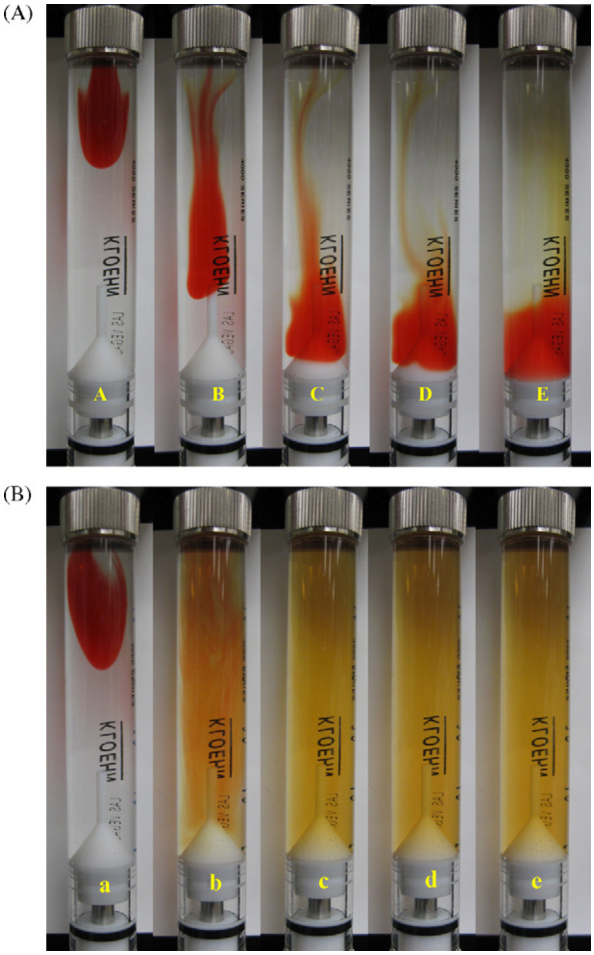

Fig. 4. (A) Temporal evolution of mixing in the syringe of a syringe pump with no agitation and no external secondary mixing device: (a) 0 min, (b) 1 min, (c) 2 min, (d) 3 min, and (e) 16 min. (B) Temporal evolution of mixing in the syringe of a syringe pump using a mixing coil as a secondary mixing device: (a) 0 cycle, (b) 1 cycle, (c) 2 cycles, (d) 3 cycles, and (e) 14 cycles.

3.2. Repeatability, $S_{1/2}$, and carryover

Repeatability of the system was tested by injecting a diluted phenol red indicator solution (0.21 mL of 0.04 g/L) and mixed it with a 4.79 mL DIW to a final volume of 5 mL. The relative standard deviation of 15 replicates was 0.48%. The value of $S_{1/2}$ was determined by quadruplicate injections of increasing sample volumes of dye solution. The peak signals obtained are shown in the inset of Fig. 6. The peak heights were plotted against the injected volume in Fig. 6, and an $S_{1/2}$ value calculated from the volume corresponding to one-half of the absorbance of 0.027 g/L phenol red solution. As shown in Fig. 6, the value of $S_{1/2}$ was 1.63 mL. Moreover the plot of peak height against injected volume yielded a straight line. The linear relationship indicates that our mixing chamber completely mixes the injected solution over a considerable range of injected volume. It does much better in this regard than conventional flow injection techniques using a mixing coil [26,27].

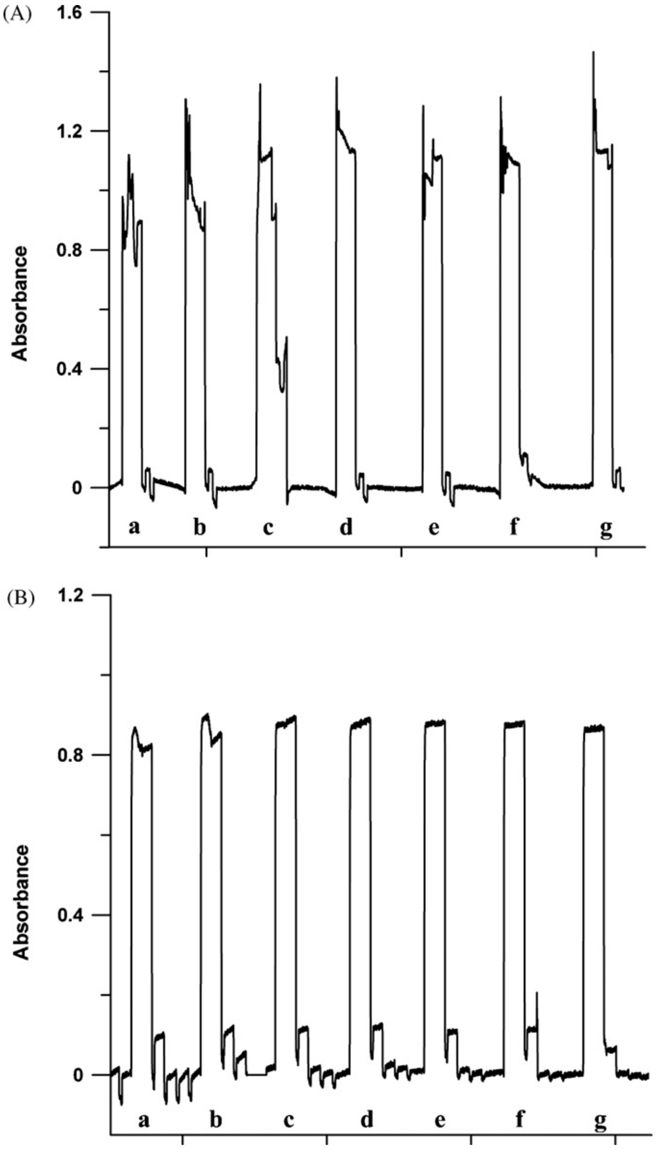

Fig. 5. (A) Recorded mixed solution absorbance using a syringe pump and a mixing coil as a secondary mixing device: (a) 1 cycle, (b) 2 cycles, (c) 3 cycles, (d) 4 cycles, (e) 8 cycles, (f) 12 cycles, and (g) 16 cycles. (B) Recorded mixed solution absorbance signals of using a 5-mL pipette tip as a secondary mixing device: (a) 1 cycle, (b) 2 cycles, (c) 3 cycles, (d) 4 cycles, (e) 5 cycles, (f) 15 cycles, and (g) 27 cycles.

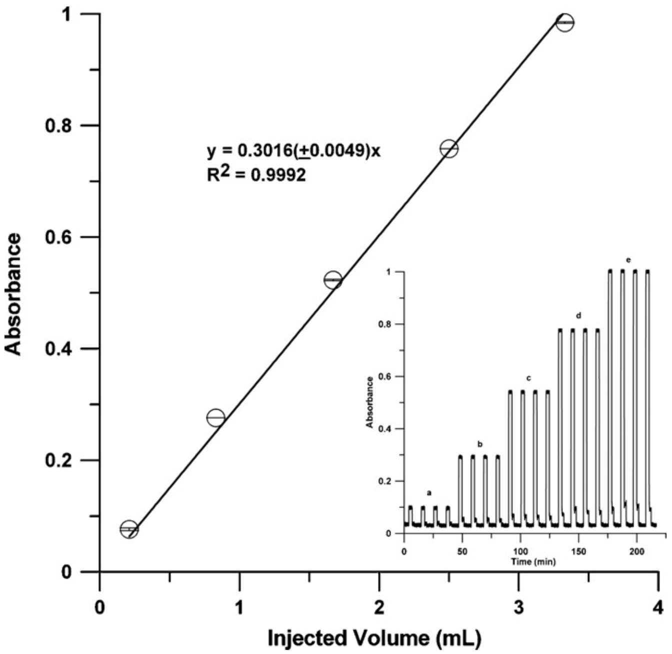

Fig. 6. The influence of injected sample volume upon peak heights obtained by injecting 0.027 g/L phenol red solution. The inset shows quadruplicate peak signals, injecting four times for each volume: (a) 0.21, (b) 0.83, (c) 1.67, (d) 2.5, and (e) 3.33 mL.

We studied carryover in the system by injecting two different volumes of 0.04 g/L phenol red (0.21 mL and 5 mL) and then mixing with DIW. Each volume was alternately injected five times for three cycles. The absorbance signals obtained are shown in Fig. 7. Carryover coefficients were calculated using HLL (high–low–low) and LHH (low–high–high) schemes [28]. Without washing between samples, the carryover coefficient was 7.8%. However, two washes with 5 mL DIW reduced the carryover coefficient to immeasurable levels. In short, with minimal washing the system can achieve essentially zero carryover for both low to high and high to low sampling sequences.

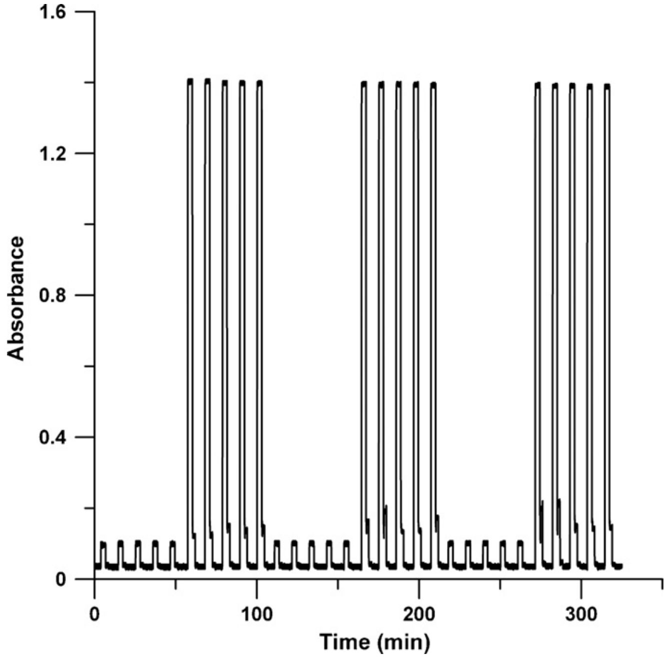

Fig. 7. Testing carryover in the system by alternately injecting high (5 mL) and low (0.2 mL) volumes of 0.04 g/L phenol red indicator solution.

3.3. The effect of mixing upon silicate measurement

We compared the calibration curves for the two mixing chambers keeping the mixing plus reaction time constant. The mixing coil data fit the linear equation $y = 0.1298(\pm 0.0037)x + 0.0075(\pm 0.0223)$, $r^2 = 0.9968$ and the pipette tip fit the linear equation $y = 0.2767(\pm 0.0054)x + 0.0129(\pm 0.0203)$, $r^2 = 0.9992$. The proportion of resulting slopes obtained under identical conditions indicates the pipette tip improves the sensitivity of the system approximately a factor of two. Because the sensitivity of the silicate measurement depends upon reaction time after complete mixing of sample with reagents is achieved, the result implies that the mixing within the pipette tip must be significantly faster than with a conventional mixing coil.

4. Conclusions

This study illustrates the utility of using a typical pipette tip in conjunction with a zero dead volume syringe pump as the secondary mixing chamber in flow analysis systems. The pipette tip can completely mix solutions in a shorter time than the typical mixing coils, resulting in markedly improved sensitivity. The system has no measurable carryover after a minimal wash (two times with 5 mL DIW).

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