

# Novel Syringe Pump System for Continual Flow Delivery with Gradient Capability

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## Introduction

Syringe Pumps are effective in the delivery of fluids for microbore and packed capillary chromatography. One particular advantage of syringe pumps is the ability to produce a low flow without pulsation. This is essential to obtain reproducible chromatography at the very low flow rates required for micro chromatography. Syringe pumps have the inherent problem of eventually emptying and therefore must refill periodically. The refill process is usually accompanied by a loss of pressure on the system which can be deleterious to packed bed columns. This report describes a novel system which employs three syringes. Two syringes are used to generate a binary gradient and the third is used to deliver solvent while the other two are undergoing refill. This device, termed the Transitional Liquid Delivery (TLD) slave unit, offers the advantages of gradient generation of a dual syringe pump for very low flow rates, and the continual flow without pressure loss of a reciprocating pump. The third syringe can deliver a wash fluid between runs, or a pre-run equilibration fluid while the other two syringes refill.

A description of the appropriate hardware to facilitate this technique will be presented. Reproducibility data will also be presented showing packed capillary column chromatography of peptides using binary gradients and flow rates of 2 to 5  $\mu\text{L}/\text{min}$ . Decreased sample run time data will be shown. A discussion of enhanced run speed and sample turnaround time will be included.

## Experimental

Data was collected using the following equipment:

- Fluid Delivery:** Eldex MicroPro Gradient Pump (HPLC2g) fitted with 2mL syringes and a MicroPro Transitional Liquid Delivery Slave Pump (HPLC2tld).
- Injector:** Valco Injector Valve; 1  $\mu\text{L}$  external loop.
- Column:** Unimicro 200 x 0.32mm C-18; 300Å 5 $\mu\text{m}$  packed capillary.
- Column Oven:** Eldex CH-150 Column Oven
- Detector:** Shimadzu LC4 Variable Wavelength UV Detector with LC Packings UZ View 35 nL flow cell.
- Data Acquisition:** EZ Chrom Elite Data System.

Figure 1: Pressure Decay Profile with Dual 2mL syringe on-line

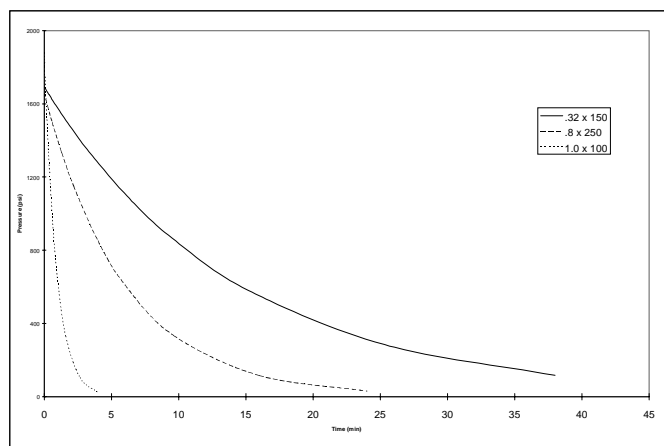
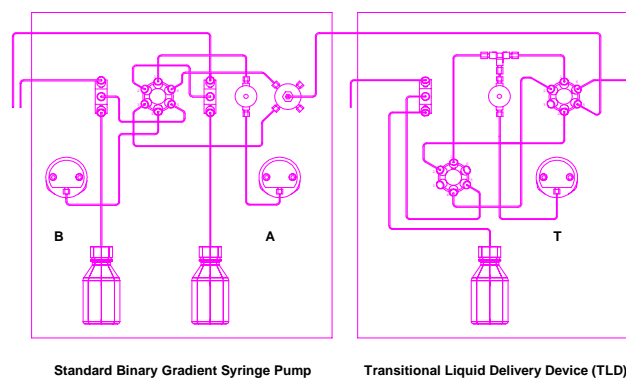


Figure 2: Flow Schematic for Continuous Flow Gradient Syringe System



## Results and Discussion

Typical gradient syringe operation involves: pre-pressurization, equilibration, gradient formation, system depressurization, and syringe refill.

This mode of operation is repeated every run. The system is therefore subjected to continual pressurization and depressurization. As packed bed column technology has evolved, smaller ID columns have become common. These columns use similar linear velocities to those in traditional ID columns, yet have substantially lower cross sectional area. The relative larger volume of the syringes therefore causes a much greater pressure decay times when flow is stopped. Figure 1 shows the decay times associated with typical packed capillary columns connected to a 2 mL binary syringe pump.

To create a system with continual flow for packed capillary chromatography, a dual syringe HPLC gradient pump was connected to a single syringe with special valving, the transitional liquid delivery (TLD) slave pump (patent pending). The gradient syringes first prepressurize to operating pressures followed by an equilibration period. Following the gradient generation, the TLD syringe is pressurized to the system pressure and a high pressure valve switches, taking the gradient system off line and putting the TLD syringe on line. The TLD then pumps solvent for a fixed amount of time. During the TLD solvent flow, the gradient syringes refill and subsequently repressurize to the current system pressure. The valve then switches back to place the gradient syringes on line with no loss of pressure during the transition. Figure 2 is a schematic of the fluid path and system components.

Figure 3 is a superimposed pressure trace of two complete cross over processes. It consists of the dual gradient equilibration and generation followed by the TLD cycle.

Chromatographic reproducibility under this mode of operation was examined by repeated injection of trypsinized  $\alpha$ -chymotrypsinogen. %RSD data is shown in Table 1 along with data generated on a standard gradient syringe system without TLD slave operation. Chromatographic conditions along with the repetitive peptide chromatograms are shown in Figure 4.

## Conclusions

This preliminary work suggests that a gradient syringe system can be coupled to a modified single syringe to create a reciprocating syringe system which will not depressurize between runs, yet has the low flow gradient capabilities of the binary syringe design. Decay time is replaced by immediate column wash or equilibration and run to run turn around times are decreased. Further examination of faster sample turn around time using solid partial technology in packed capillaries for very fast runs (< 5 minutes) is being conducted in our laboratory. Also, the effects of pressurization/depressurization on capillary column efficiency and lifetime are currently being investigated.

Figure 3: Overlaid Pressure Traces

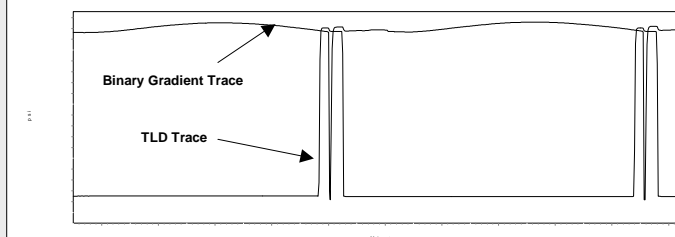
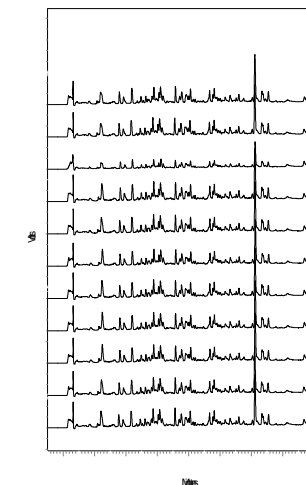


Figure 4: Consecutive Runs



## Conditions:

- Sample:** 6.5 pmole trypsinized  $\alpha$ -chymotrypsinogen in 1 $\mu\text{L}$
  - Flow Rate:** 3  $\mu\text{L}/\text{minute}$
  - Equilibration:** 15 minutes at 6%B
  - Gradient:** 6%B to 76%B in 90 minutes
  - Solvents:** A: 0.05% TFA in water  
B: 0.045% TFA in 60% acetonitrile and 40% water
  - Syringe/Column Temp.:** 33°C
  - Detection:** UV<sub>210</sub>
- TLD pressurizes and delivers B solvent for 5 minutes while gradient syringes refill and pressurize

Table 1: Gradient Reproducibility Data

Mode	N	Peak Number							Time Between Injection (min.)
		1	2	3	4	5	6	7	
Standard Refill Between Run									
2 $\mu\text{L}/\text{min}$ .	17	19.22	28.32	38.96	44.4	60.6	75.02	84.77	125
std dev (min)		0.27	0.14	0.17	0.21	0.16	0.27	0.12	
%RSD		1.39	0.48	0.42	0.47	0.26	0.35	0.14	
Use of Transitional Liquid Delivery Device									
2 $\mu\text{L}/\text{min}$ .	11	16.82	26.33	33.51	40.60	51.69	66.19	81.89	105
std dev (min)		0.15	0.27	0.16	0.15	0.12	0.086	0.082	
%RSD		0.88	1.02	0.48	0.36	0.23	0.13	0.10	