

## Micro-Sequential Injection: A multipurpose Lab-on-Valve for the Advancement of Bioanalytical Assays

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With the advent of microSequential injection, the versatility and potential of flow injection has been greatly expanded into bioanalytical research. The Lab-on-Valve (LOV) is a first generation integrated manifold that has been microfabricated onto a selector valve. Microassays are made practical since the sample/reagent injection ports, mixing coils, reaction columns and flow-through detector are centralized minimizing all flow paths. The LOV system has served as the hub for all fluidic operations including its integration to an autosampler, mass spectrometer, and CE instrument. Readily available software makes these microchemistries and instrument integration easy to achieve allowing researchers to focus on the critical development of novel microchemistries and methodologies. These important advantages to microSequential injection are demonstrated through examples in immunoassays, bead injection, on-line fermentation monitoring, mass spectrometry and capillary electrophoresis.

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The Lab-on-Valve (LOV) is a first generation integrated manifold<sup>1</sup> that has been microfabricated onto a selector valve. Microassays are made practical since all key elements such as the sample/reagent ports, mixing coils; reaction columns and flow-through detector are centralized, minimizing all flow paths.

This approach has several important implications: First, pre-existing *sequential injection* methods can be quickly adapted onto a Lab-on-Valve (LOV) manifold to develop microassays that substantially reduced reagent consumption and waste generation. This is a critical feature for many bioassays where miniscule quantities of sample/reagent are only available. Furthermore, instrumentation becomes compact and streamlined making it ideal for on-line monitoring. Second, the LOV with its large bore channels and short paths is ideal for microfluidic manipulations of suspended matter. For example, *Bead Injection*<sup>2</sup> using renewable microaffinity columns or living cells can be performed within this manifold. Third, the LOV manifold uses conventional fittings and hardware making integration into existing hardware simple to implement.

These important advantages to microSequential injection have been demonstrated through examples in immunoassays, bead injection, on-line fermentation monitoring, mass spectrometry and capillary electrophoresis.

### Experimental

#### Instrument

The Lab-on-Valve (LOV) manifold is made of Plexiglas in order to observe its internal structure. The LOV manifold is designed to replace the headpiece of a Cheminert Valco VICI valve. Thus the center rotor of the valve now serves to direct the flow "to and from" the integrated elements found on the LOV. A typical arrangement would use a conventional 6-port valve where the center port is connected to a syringe pump (via holding coil). The syringe pump serves as the main fluidic motor to aspirate and dispense micro-aliquots of reagent and sample at the manifold. This single device can be used to perform absorbance

spectroscopy as well as fluorescence detection as seen in Figs. 1 and 2. Noteworthy is that the fiber optics are fabricated into 1.6mm OD stainless steel rods. The fiber optics can be placed into the flow-through detector cell and sealed into place using standard ¼-28 (~M6) flangeless fittings.

These fiber optics serve two purposes. First, they allow for the introduction of light and for the detection of the transmitted signal. Second, they serve to define the internal geometry of the region of detection. As highlighted in Fig. 1, three stainless fiber optic probes are set for light transmittance and detection for absorbance measurements. A dark dye has been injected to define the flow path from the valve's rotor slot through the flow cell. The two-fiber optics that are immediately orthogonal to one

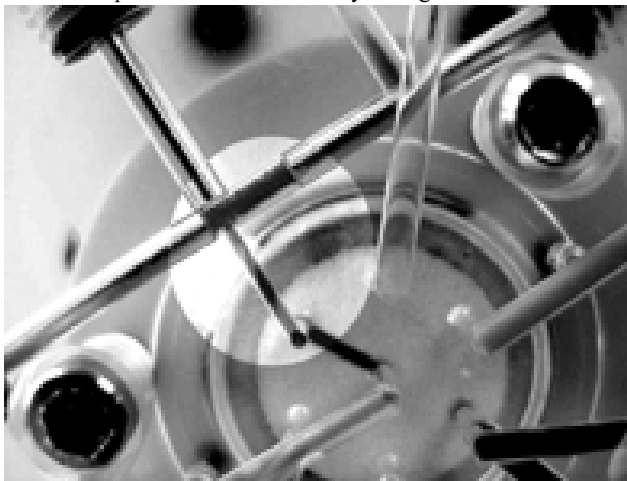


Fig. 1 Lab-on-Valve is configured for absorbance measurements across a 5mm path with the fiber optic cables aligned along the flow path

another remained fixed at the entrance of the flow-through cell. However the fiber optic rod at the end of the flow cell can slide to change the internal volume of the detection chamber. Thus the

path length for absorbance measurements can be varied from 1.5-mm to 12.0-mm giving an internal displacement volume of 3-24 microliters. For absorbance, the fiber optic rod that is orthogonal to the other two simply serves to block the flow and redirect it through the flow chamber.

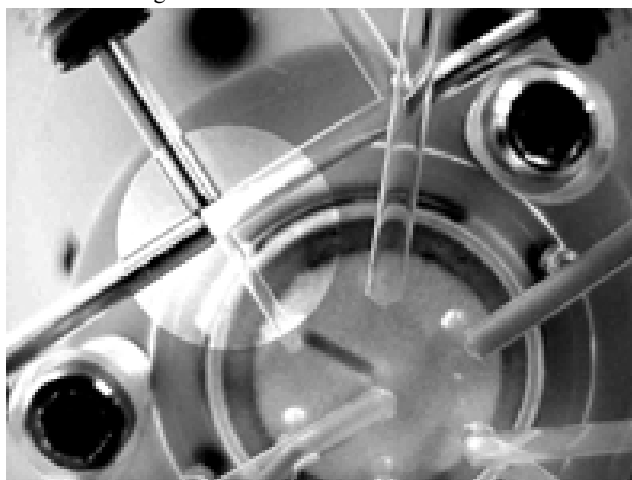


Fig. 2 Lab-on-Valve is configured for fluorescence measurements across a 1.5-mm path with the fiber optic cables orthogonal to one another.

However in the case of fluorescence, this fiber optic is activated for detection as shown in the highlighted region of Fig. 2 above.

The flow-through detector chamber can have other uses as well. For example, electrochemical analyses can be performed by substituting the fiber optic rods with electrodes (cathode, anode, auxiliary or reference). The detector chamber can also be adapted for capillary electrophoresis in a similar fashion.

The LOV design permits precise manipulations of a few microliters of sample or reagent in a very reproducible fashion. Since all port bore sizes are large (1.61 mm diameter) they allow standard 1/16" (1.6-mm) OD tubing to be used as inserts. Thus the bore ID is variable with readily available 1/16" OD tube inserts having 65 $\mu$ m to 1.4mm internal diameters. Larger tube bores (ID: 0.5-0.8mm) are effective in preventing clogging for bead injection, while smaller bores conserve reagents for standard colorimetric assays.

Sample introduction is performed using a customized port that has a dual inlet. This permits the introduction of a new sample without direct intervention by the syringe pump. Typically this port would be connected to a small peristaltic pump and to an autosampler to completely automate sample introduction.

## Discussion

### Affinity separations

Bead Injection Electrospray Mass Spectrometry (BIEMS) has been introduced as a new method<sup>4</sup> for automated affinity purification combined with on-line analysis of an eluted conjugate. Strong binding between biotin and streptavidin serves as the basis where avidin coated Sepharose beads is used to capture a biotin derivative. The LOV manifold automates the assemble of a fresh bead column which is used to capture it biotin conjugate. The method achieves fast capture-purification-release for MS analysis using only a few microliters of sample. This has been demonstrated for assays of the enzyme  $\beta$ -galactosidase in human cell lysates. The overall goal of this work is to use the BIEMS system to screen newborn baby's blood for defective enzymatic activities, which are indicative of the presence of genetic diseases. In contrast to batch enzymatic assays that require several hours for the capture-release of a conjugate, the BIEMS using continuous flow achieves capture and elution in under five minutes.

### Fermentation Monitoring

The LOV system has been used to monitor aerobic fermentations<sup>3</sup> of *E. Coli* and *S. Cerevisiae* cell cultures. Due to its compact size, the analyzer was situated on-line and used colorimetric methods to monitor important nutrient levels in the fermentation broth. Stopped-flow SI mode was used to produce linear calibrations of ammonia (3-1200 ppm), glucose (35-1000 ppm), glycerol (20-120 ppm) and free iron (80-400 ppm). Sampling was performed at five-minute intervals over the course of several days. The assays only required a few microliters of sample, which is of particular importance to a small-scale experimental fermentation. Moreover, the large bore size found in the LOV prevented clogging for fermentation debris making this  $\mu$ SI system very reliable.

### Functional cellular assays

Ligand/reagent binding to a cell receptor site is not indicative of a subsequent physiological response, an important failure of most binding assays used to date. The identification and characterization of drug candidates require that the efficacy of a drug be determined. Such assays are referred to as functional assays, and are designed to classify a drug as an agonist or antagonist depending on whether a biological response is invoked or inhibited. Several different functional assays have been carried out using various cell lines and fluorescent probes.<sup>5</sup>

The LOV coupled to a fluorescence microscope for detection has proved to be an ideal tool for automating these assays.

A model experiment based on a functional assay of muscarinic receptor agonists with CHO cells was performed. The CHO cells selected for this research express type-1 muscarinic acetylcholine receptor, which signals through a G-protein coupled pathway. In order to make the cells suitable for BI assay, they are cultured on microbeads, incubated with the fluorescent intracellular calcium probe fura-2-am and suspended in a vial. The SI instrument automatically aspirates and traps a small volume of this suspension into a jet ring chamber then delivers agonist to the trapped beads. After recording the calcium stimulation response that results, the beads are flushed to waste and the assay is repeated with a fresh batch of beads. Using this method, the pharmacological properties of muscarinic receptor agonists are measured. Dose response curves constructed from the bead injection assay can discriminate a partial agonist response (i.e. pilocarpine) from a full agonist response (i.e. acetylcholine) allowing these drugs to be rated for pharmacological purposes.

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