MICROFLOW ANALYSIS BASED ON SEQENTIAL AND BEAD INJECTION.

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Abstract.
While use of the continuous forward flow is essential for success of chromatographic separations, its use for automation of the reagent-based assays has numerous drawbacks. This is why the first generation of flow injection (FI) methodology is presently being replaced by sequential injection (SI) that uses reversed/stop/forward flow format, and by bead injection (BI) that employs micro spheres as reagent carriers as well as sensing and separation medium. This communication introduces these two novel concepts as well as the device for their implementation into the toolbox of μTAS technology. The device, nicknamed “Lab-on-Valve” is a multipurpose central sample processing unit (CSPU) served by a conventional sized peripherals. The performance of the system, configured for UV-VIS and fluorescence spectroscopy is characterized by dye injection and its performance is demonstrated on enzymatic reaction rate assay of glucose and Bioligand Interaction assay of anti-mouse IgG with recombinant protein G.

Keywords: flow injection, microfluidic sample processing, renewable biosensor, immunoassays, enzymatic assay, BIA, bioligand interaction assay, drug discovery

1. Introduction.

Flow based assays fall into two categories: chromatography and flow injection. While chromatographic separations benefit from large surface/volume ratios, since they rely on differentiation of migration velocities caused by repetitious surface/solute interactions, the opposite is true for flow injection reagent based assays. Also, while the continuous forward flow is essential for chromatography, its use has serious drawbacks for reagent based assays, as it consumes chemicals, generates waste and necessitates physical reconfiguration of the flow system, whenever the assay protocol is changed, since the sequence of sample processing operations is programmed by means of the succession of the components strung along the sample processing channel. Indeed, μTAS literature is replete with designs of sophisticated mixers, cascade dilutors, serpentine reactors and single purpose flow through cells, strung along a channel in a sequence dedicated to a particular reagent based assay [1, 2]. Thus for a single reagent assay based on a fast reaction a different pattern is micro fabricated then for a multireagent assay based on slower reactions, while yet another, quite different micro manifold is designed if solid/liquid interactions are included in the assay protocol. Considering the vast variety of environmental, industrial, biomedical, biochemical and biological assays, it is
impractical to propose that micro fabrication technology will provide a corresponding variety of sample processing devices.

This is why a different strategy has to be applied, resulting in design of a multipurpose central sample processing unit (CSPU), in which sample metering, dilution, reagent addition, mixing, incubation, separation and product monitoring will be carried out in any desired sequence and to any desired degree. In other words, instead of varying the design of the micro fabricated pattern, sequential injection (SI) and bead injection (BI) will be used to vary sample processing sequences by means of precisely orchestrated fluidic micromanipulations, controlled by a specialized software.

While the principles of SI [3,4] and BI [5,6] as well as their applications [7,8] have been described elsewhere, the downscaling of these techniques and their embodiment into a rigid, monolithic structure has been proposed only recently [9,10]. This communication is focused on description of CSPU and discussion of its performance, evaluated by dye injection, by enzymatic assay of glucose and by bioligand interaction assay (BIA) of FITC labeled anti-mouse IgG with recombinant Protein G attached to Sepharose 4B beads.

2. Principles.

The downscaling of sequential injection to μSI format is based on minimizing the volume of the sample processing path between the detector and injector. This is achieved by integrating sample and reagent ports with the flow through cell into a monolithic structure mounted atop a multiposition valve (Fig.1 and 2).

Fig. 1. Schematics on μSI system showing CSPU within the circle.