Analytical potential of mesofluidic lab-on-a-valve as a front end to column-separation systems

Manuel Miró, Hugo M. Oliveira, Marcela A. Segundo

The lab-on-a-valve (LOV) integrated microdevice has recently attracted much attention as a functional mesofluidic platform for programmable, pressure-driven flow as compared to lab-on-a-chip counterparts. We review the current state of the art of LOV as a versatile front end to column-separation techniques, namely, liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) for automatic mesofluidic handling at the low-microliter level, in-line sample processing and introducing the appropriate form of the analyte into the instrument for separation or detection.

The open architecture of the LOV monolith unit has been to date exploited to accommodate micro solid-phase extraction in a renewable fashion, the so-called bead-injection analysis, encompassing reversed-phase materials and molecularly imprinted polymers, and in-valve microscale affinity chromatography. A plethora of interfaces have been recently devised for reliable injection of minute, well-defined volumes of analyte-containing solutions into LC, GC or CE.

We illustrate these applications with representative examples in environmental and bioanalytical arenas.

Keywords: Lab-on-a-valve; Flow analysis; Bead-injection analysis; Capillary electrophoresis; Gas chromatography; Liquid chromatography; Mesofluidic; Micro affinity chromatography; Micro solid-phase extraction

1. Introduction

Notwithstanding the fact that chromatography has been consolidated as a routine and research tool for assays in a vast number of analytical fields, sample preparation is still the bottleneck for reliable and unbiased analysis. Multi-step conventional sample processing is the most error-prone part of the analytical process and accounts for some two-thirds of the overall time of liquid chromatographic (LC), gas chromatographic (GC) or capillary electrophoretic (EC) assays [1].

The continual quest for novel sample-preparation techniques has led to the development of faster, more cost-effective, miniaturized versions of sample extraction with green chemical credentials to replace conventional methods [1,2]. Liquid-phase microextraction (LPME) techniques, and variants thereof [i.e. single-drop LPME, dispersive LPME, solidified floating organic drop, and hollow-fiber assisted LPME] are gaining ground prior to column-separation systems [3–5] over time-consuming, labor-intensive traditional liquid-liquid extraction (LLE) with the added advantage of reduced exposure of the analyst to harmful organic solvents. However, the predominant sample-processing methods that have been rapidly growing in recent years as a consequence of the improved enrichment factors, and, thus, sensitivity for chromatographic assays as compared to LLE and LPME, are solid-phase extraction (SPE) and miniaturized versions thereof including fibre/needle-based solid-phase microextraction (SPME), in-tube capillary SPME, stir-bar sorptive extraction (SBSE), and micro-solid phase extraction (μSPE) in the format of packed microcolumns, syringes or pipette tips [1,2,6,7].

Much effort has gone into the development of not merely miniaturized but also automated sample pre-treatment approaches to simplify the overall analytical process, capitalizing on robotic systems (e.g., Prospekt, Prospekt-2 and Symbiosis from Spark Holland or OS-2 from Merck for μSPE prior to LC) [8] or flow-based approaches [9–11]. Automation of sample processing exploiting the first and second
generation of flow analysis [i.e. flow injection (FI) and sequential injection (SI)], and related techniques (e.g., multi-commuted or hybrid systems) coupled to chromatographic techniques [viz., LC, ion chromatography, low- to moderate-pressure monolithic chromatography (so-called SI chromatography), GC or CE] has attracted much attention from researchers over the past few years [12–14]. Coupling FI- or SI-based sample pre-treatment to chromatographic systems introduced new ways of improving the overall efficiency of chromatographic methods with minimal operator intervention and offering potential incorporation of unit operations at will for chemical derivatization, sample clean up and/or analyte enrichment as integral or hyphenated modules of the flow network prior to separation and detection.

The aim of this manuscript is to discuss the analytical capabilities of the third generation of flow analysis [i.e. the lab-on-a-valve (LOV) platform integrated into an SI fluidic system] as a front-end to column-separation systems for solution handling and automated execution of appropriate pre-treatments of troublesome samples, encompassing matrix isolation, analyte preconcentration and derivatization reactions. The underlying objective is to introduce the analytes optimally into chromatographic and detection systems in line with the requirements of the assays. To date, no overview has been published on the current state of the art of LOV as a sample-processing unit prior to liquid and gas column-separation techniques.

In this work, we briefly pinpoint the fundamental principles of the mesofluidic LOV concept, whereupon the various interfaces designed so far for coupling LOV with different degrees of automation to discrete sample-introduction chromatographic/electrophoretic instruments (i.e. LC, GC and CE) are described in detail. The potential of LOV as an emerging downscaled tool for simplification of chromatographic assays and implementation of expedient sample-processing procedures is illustrated with representative examples in the environmental and bioanalytical fields reported in the literature over the past decade.

2. Fundamental principles of LOV-based assays

The inception of the third generation of FI originated from the need to downscale reagent-based assays for minimal consumption of samples and consumables [15], but, contrary to lab-on-chip microdevices, with an open architecture to accommodate a plethora of unit operations at will within the same mesofluidic platform on the basis of programmable flow. Readers are referred to comprehensive critical reviews for in-depth insight into the fundamentals and distinct features of LOV for miniaturization and simplification of analytical assays [16–20].

In short, the microfabricated LOV channel system (typically 1.6 mm i.d.) is a single monolithic structure made of polymethylmethacrylate (Perspex), hard polyvinylchloride (PVC), polyetheretherketone (PEEK) or polyetherimide (Ultem), meeting the demands of assays and mounted atop a multi-position selection valve of an SI network (Fig. 1). Designed to incorporate all necessary laboratory facilities for a variety of homogeneous and

![Figure 1. Sequential injection lab-on-a-valve (SI-LOV) assembly as coupled to column-separation systems using various interface modes. SP, Syringe pump; IV, Injection valve; HC, Holding coil; D, Detector; μAC, Microaffinity chromatography.](image)
heterogeneous-phase chemical assays, hence the name lab-on-a-valve, it was originally conceived [15] to facilitate solution metering at the low-μL level, mixing sample with reagent(s), incubation of stacked zones by means of stopped-flow approaches and sample dilution. However, more recently, it has proved suitable to implement microsolid-phase extraction (μSPE) procedures [21] and gas-liquid separation of evolved volatile species within the microfabricated channel system [22]. Not least, the LOV platform houses a multipurpose flow-through cell [15,16] for real-time monitoring of the development of chemical reactions and separation processes. The integrated microconduit system has been so far furnished with optical detection facilities, including USB charge-coupled devices, laser-induced spectrofluorimeters or luminometers and miniaturized atomic fluorescence spectrometers [19], where communications to the detector and/or the light source are made via optical fibers (see Fig. 1), and where the position of the fibers can tailor the path length of the flow-cell [15]. Invalve electrochemical detection has recently proved feasible using the flow-through cell to house:

- miniature ion-selective electrodes for potentiometric assays [23];
- three-electrode voltammetric units for scanning voltammetry of multiple redox species [24]; or,
- anodic/cathodic stripping analysis of trace elements [25].

The microconduit LOV unit is also amenable to operate with conventionally sized peripheral devices, including a wide variety of optical detection techniques and modern analytical instruments {e.g., electrothermal atomic absorption spectrometry [16,19,21], inductively coupled plasma-atomic emission spectroscopy or mass spectrometry [21], X-ray fluorescence spectrometry [26], and electrospray ionization mass spectrometry [27–29]}. However, over the past few years, coupling the mesofluidic platform to chromatographic or capillary electrophoretic systems has increasingly attracted attention in a variety of areas of research endeavor, and merits a comprehensive report on the role of LOV for accelerating and miniaturizing bioseparations and sample handling in multi-residue analysis.

3. Interfacing LOV with column-separation techniques

A survey of recent literature in the field has revealed that diverse designs and configurations have been reported where LOV is exploited as a front end to LC, GC or CE. The mesofluidic platform can be coupled to column separation systems in off-line, at-line, on-line or in-line fashions, terms that have been misused in several works. Fig. 1 shows the varied coupling strategies and Table 1 sets out representative interface modes in real-world applications.

Off-line coupling includes manual injection of the sample pre-processed in SI-LOV into the chromatographic/electrophoretic equipment [30]. The beauty of this approach is the ease of reconstituting the analyte-containing media after pre-treatment in either the mobile phase for reversed-phase LC or an organic volatile solvent for GC. This is aimed to minimize potential LC band-broadening effects, overlapping peaks, co-elution with void volume and peak asymmetry, although off-line coupling lacks automation.

At-line procedures are performed with a programmable robotic station or dedicated/commercial autosampler [31], and no manual transfer of samples is required. The pretreated sample from LOV is delivered to a vial in a discontinuous mode with regard to the separation process [i.e. there is a need to interrupt the separation procedure during the sampling step (see Fig. 1)]. Previously developed flow-through methods for sample processing are easily automated in at-line LOV format, as it does not require a dedicated flow interface.

On-line coupling refers to a further degree of automation, whereby the LOV and chromatographic/electrophoretic components are connected in a continuous-flow format via a flow interface {e.g., rotary injection valves for LC or GC or the internal flow-through cell of LOV itself for CE (see Fig. 1) [32–34]}. The sample injected or processed in LOV is transported to the interface, exploiting programmable flow inherent to SI, from which it is introduced into the separation system by pressure-driven flow in CE [34], mobile phase in LC [32] or gas stream in GC [33]. In contrast to at-line coupling, on-line methods foster repeated injections in CE, while separation is still in progress (i.e. there is no need to wait to detect the analytes with longer retention times before injecting the next sample) [13]. In on-line chromatographic assays, LOV sample processing might be synchronized with separation by LC or GC, whereby two samples could be concurrently handled in a fully automated mode.

In-line coupling refers to the specific scenario where the separation process is accommodated within the microminiaturized LOV module by incorporating a small sorptive column {e.g. in-valve microaffinity chromatography (μAC) for selective uptake, preconcentration and/or separation of biomolecules [27–29,35–37] (see below for further details)}. The most significant drawback of in-line coupling, compared to at-line or on-line methods, is its inability to handle samples in parallel because the overall analytical process encompassing sampling, derivatization, separation and occasionally detection [27,35] is confined in the integrated LOV unit.

4. Automated sample processing

In this section, we provide insight into the unique features of LOV as a mesofluidic platform for downsizing
<table>
<thead>
<tr>
<th>Coupling mode, chromatographic technique and detection system</th>
<th>Target analyte</th>
<th>Sample matrix</th>
<th>Manual sample treatment</th>
<th>Automated LOV sample treatment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-line LC-ESI-MS-MS</td>
<td>Female steroid hormones</td>
<td>Urine</td>
<td>Enzymatic hydrolysis with β-glucuronidase</td>
<td>Off-valve SPE</td>
<td>[30]</td>
</tr>
<tr>
<td>At-line CE-UV</td>
<td>Linear alkylbenzene sulfonates</td>
<td>Treated effluent, estuarine water, wastewater and sea water</td>
<td>pH adjustment and centrifugation</td>
<td>Off-valve SPE</td>
<td>[31]</td>
</tr>
<tr>
<td>On-line CE-UV (indirect)</td>
<td>Anions (sulfate, chloride, thiosulfate, nitrite, nitrate, citrate, fluoride, phosphate, bicarbonate, acetate)</td>
<td>n.a.(^a)</td>
<td>–</td>
<td>–</td>
<td>[34]</td>
</tr>
<tr>
<td>On-line CE-UV-FL and in-valve UV</td>
<td>Pancreatic islet proteins</td>
<td>Cellular perfusion liquids</td>
<td>Dilution in HEPES buffer at pH 7.5</td>
<td>In-line derivatization with fluorogenic reagent (fluorescamine)</td>
<td>[38]</td>
</tr>
<tr>
<td>On-line LC-UV</td>
<td>NSAIDs</td>
<td>Surface water, raw wastewater, treated wastewater and urine</td>
<td>Filtration and pH adjustment</td>
<td>In-valve SPE (BI)</td>
<td>[32]</td>
</tr>
<tr>
<td>On-line LC-UV</td>
<td>UV filters</td>
<td>Swimming pool and seawater</td>
<td>Acidification and filtration</td>
<td>In-valve SPE (BI)</td>
<td>[40]</td>
</tr>
<tr>
<td>On-line LC-UV</td>
<td>Chlorotriazine herbicides and degradation products</td>
<td>Crude soil extract, surface water and groundwater</td>
<td>Ultrasonic extraction of soil</td>
<td>Off-valve SPE (BI)</td>
<td>[41]</td>
</tr>
<tr>
<td>On-line LC-UV</td>
<td>Riboflavin (vitamin B(_2))</td>
<td>Milk, energetic drink, pig liver</td>
<td>Acidic/enzymatic digestion of pig liver</td>
<td>In-valve SPE (BI)</td>
<td>[42]</td>
</tr>
<tr>
<td>In-line μAC-UV and μAC-ESI-MS</td>
<td>Biotin-containing conjugates and beta-galactosidase activity</td>
<td>Lysates from cultured human skin fibroblasts</td>
<td>Incubation with biotin-containing conjugates</td>
<td>Off-valve removal of free biotin by ion-exchange</td>
<td>[27]</td>
</tr>
<tr>
<td>In-line μAC-ESI-MS</td>
<td>Binding constants (Kd) between tPex5 proteins and synthetic peptides</td>
<td>Five-component mixture of peptides with variable affinity constants</td>
<td>–</td>
<td>In-valve BI</td>
<td>[28]</td>
</tr>
<tr>
<td>In-line μAC-ESI-MS</td>
<td>Phosphomannomutase and phosphomannose isomerase activities</td>
<td>Lysates from cultured human skin fibroblasts</td>
<td>In-vitro enzymatic reaction of primary product with yeast transketolase</td>
<td>In-valve BI</td>
<td>[29]</td>
</tr>
<tr>
<td>In-line μAC-UV</td>
<td>Mouse immunoglobulins (IgG)</td>
<td>Mixture of BSA and IgG proteins</td>
<td>–</td>
<td>In-valve BI</td>
<td>[35]</td>
</tr>
<tr>
<td>In-line μAC-UV and in-line μBIS-UV</td>
<td>Human IgG, rabbit IgG and horse IgG</td>
<td>n.a.(^a)</td>
<td>–</td>
<td>In-valve BI</td>
<td>[36]</td>
</tr>
<tr>
<td>In-line μBIS-FL</td>
<td>Biotinylated single-stranded DNA</td>
<td>n.a.(^a)</td>
<td>–</td>
<td>In-valve, on-column derivatization with fluorogenic reagent (Oligreen)</td>
<td>[37]</td>
</tr>
</tbody>
</table>

\(^a\) Applied to standard solutions; NSAIDs, Non-steroidal anti-inflammatory drugs; BI, Bead injection; PTV, Programmable temperature vaporizer; ECD, Electron-capture detector; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; μBIS, Micro-bead injection spectroscopy; μAC, Micro-affinity chromatography; FL, Fluorimetry; ESI-MS, Electrospray ionization-mass spectrometry.
Table 2. Working operational conditions of lab-on-a-valve (LOV)-based solid-phase extraction (SPE) protocols coupled to column separation systems

<table>
<thead>
<tr>
<th>Target analyte</th>
<th>Solid phase (type, size, quantity, packing mode)</th>
<th>Sorbent conditioning</th>
<th>Sample loading (volume/flow rate)</th>
<th>Sample matrix removal</th>
<th>Elution procedure (solvent, volume, flow rate)</th>
<th>Introduction into chromatographic system</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female steroid hormones</td>
<td>C18 Hydra, 30–40 μm, 50 mg, non-renewable packed column</td>
<td>2 mL of MeOH + 1 mL of carrier&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 mL/1.2 mL/min</td>
<td>2 mL of carrier + 1 mL of air</td>
<td>150 μL of MeOH at 0.3 mL/min</td>
<td>Off-line, with eluate reconstitution</td>
<td>[30]</td>
</tr>
<tr>
<td>Linear alkylbenzene sulfonates</td>
<td>C18 Hydra, 30–40 μm, 50 mg, non-renewable packed column</td>
<td>1 mL of MeOH + 1 mL of water</td>
<td>50 mL/3 mL/min</td>
<td>1 mL of air</td>
<td>100 μL of MeOH at 3 mL/min</td>
<td>At-line, with partial introduction of eluate through a vial placed in CE carrousel</td>
<td>[31]</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Oasis HLB, 30 μm, 4.5 mg, BI renewable column</td>
<td>150 μL of 95% (v/v) MeOH + 150 μL of 10 mmol/L HCl</td>
<td>18&lt;sup&gt;b&lt;/sup&gt; mL or 1&lt;sup&gt;c&lt;/sup&gt; mL/3 mL/min</td>
<td>1 mL of 10 mmol/L HCl</td>
<td>150&lt;sup&gt;d&lt;/sup&gt; μL of 95% (v/v) MeOH at 0.5 mL/min</td>
<td>On-line, by filling loop of LC injection valve (entire eluate)</td>
<td>[32]</td>
</tr>
<tr>
<td>UV filters</td>
<td>Oasis HLB, 30 μm, ca. 6 mg, BI renewable column</td>
<td>650 μL of 95% (v/v) MeOH + 1350 μL of 10 mmol/L HCl</td>
<td>9 mL/8 mL/min</td>
<td>750 μL of 10 mmol/L HCl</td>
<td>600 μL of 95% (v/v) MeOH at 6 mL/min</td>
<td>On-line, by filling loop of LC injection valve (heart-cut approach)</td>
<td>[40]</td>
</tr>
<tr>
<td>Chlorotriazine herbicides and metabolites</td>
<td>Oasis HLB, 30 μm, 2.7 mg + SupelMIP triazines&lt;sup&gt;e&lt;/sup&gt;, 59 μm, 7.0 mg, BI renewable column</td>
<td>500 μL of MeOH + 500 μL of water</td>
<td>10&lt;sup&gt;f&lt;/sup&gt; mL or 1&lt;sup&gt;e&lt;/sup&gt; mL/1 mL/min</td>
<td>800 μL of water&lt;sup&gt;g&lt;/sup&gt;,&lt;sup&gt;f&lt;/sup&gt; + 800 μL of toluene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>170 μL of MeOH at 0.3 mL/min</td>
<td>On-line, by filling loop of LC injection valve (entire eluate)</td>
<td>[41]</td>
</tr>
<tr>
<td>Riboflavin (vitamin B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>SupelMIP riboflavin, 58 μm, 11.2 mg, BI renewable column</td>
<td>975 μL of 50% (v/v) MeOH + 565 μL of water</td>
<td>1 mL/1 mL/min</td>
<td>2 mL of water</td>
<td>312.5 μL of 50% (v/v) MeOH + 1% CH&lt;sub&gt;3&lt;/sub&gt;COOH at 0.5 mL/min</td>
<td>On-line, by filling loop of LC injection valve (heart-cut approach)</td>
<td>[42]</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>Bond Elut Plexa, 40–55 μm, 3 mg, BI renewable column</td>
<td>300 μL of EtAc + 300 μL of MeOH + 300 μL of 50% (v/v) MeOH</td>
<td>12&lt;sup&gt;g&lt;/sup&gt; mL/0.5 mL/min</td>
<td>500 μL of air</td>
<td>80&lt;sup&gt;g&lt;/sup&gt; μL of EtAc at 0.3 mL/min</td>
<td>On-line, by transfer of the entire air-segmented eluate to PTV injector</td>
<td>[33]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Water acidified with phosphoric acid (pH 4.5).
<sup>b</sup>Environmental samples.
<sup>c</sup>Urine.
<sup>d</sup>Stopped-flow of 10 s per aliquot of 50 μL.
<sup>e</sup>Soil extracts.
<sup>f</sup>Waters.
<sup>g</sup>Corresponding to 6 mL of sample diluted 1:1 in MeOH.

<sup>h</sup>Stopped-flow of 15 s per aliquot of 40 μL.

BI, Bead injection; PTV, Programmable-temperature vaporizer; EtAc, Ethyl acetate.
and automating sample processing in homogeneous or heterogeneous phase prior to chromatographic or electrophoretic separations. Tables 1–3 survey the main real-world applications in the environmental and bio-analytical arenas, including practical operational details of LOV-based sample pretreatment, coupling mode to LC, CE and GC and relevant analytical figures of merit.

### 4.1. Homogeneous phase

SI and LOV should be regarded as advanced platforms for reproducible, expedient sample presentation to column-separation systems and for pressure-assisted CE using syringe pumps, as nicely demonstrated by the seminal work in 2002 [34]. Wu et al. interfaced a conventional LOV micromachined unit with a dedicated CE system using the flow-through cell component of the LOV into which the separation silica capillary and cathode were inserted [34]. The outflow channel of the LOV was furnished with an open/close solenoid valve for controlled pulsed-flow hydrodynamic injection, as facilitated by pressurizing the flow chamber with the aid of the syringe pump. Conductivity bias in electrokinetic injection for samples of varied ionic strength is thus entirely circumvented. The hydrodynamic injection of a sample zone of low conductivity prior to the application of voltage would lead to electrokinetic sample preconcentration by head-column field amplification/sample stacking with no need for manual manipulation of the capillary injection site [34]. In-line activation, flushing and rejuvenation of the capillary and the exchange or refreshment of the background electrolyte buffer using the same instrumentation can be deployed as desired by resorting to computer-controlled operating protocols. Pressure bursts of well-defined volumes of electrolyte to decrease residence times or washing out of the capillary as soon as the target peaks were recorded fostered high-throughput separations [34,38]. A field-deployable SI-CE instrument based on similar principles was recently reported for unattended monitoring of anions and cations in creek streams [39].

Dual optical detection attaching an optical fiber CCD-spectrophotometer to the LOV flow-through cell, besides the in-line capillary detector for recording of isolated species, has been exploited for real-time observation of automatic CE hydrodynamic injection [38]. Demonstrating an assay of anionic surfactants, Ruiz-Jiménez

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**Table 3. Analytical figures of merit of lab-on-a-valve (LOV) mesofluidic methods involving chromatographic/electrophoretic separations**

<table>
<thead>
<tr>
<th>Target analyte</th>
<th>Working range</th>
<th>LOD</th>
<th>Precision (RSD%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female steroid hormones</td>
<td>n.g. (0.33–5.4 µg/L)</td>
<td>0.18–1.8 ng/L</td>
<td>&lt;11</td>
<td>[30]</td>
</tr>
<tr>
<td>Linear alkylbenzene sulfonates</td>
<td>4 ng/L–10 mg/L</td>
<td>1–15 ng/L</td>
<td>&lt;5.4</td>
<td>[31]</td>
</tr>
<tr>
<td>Anions (sulfate, chloride, thiosulfate, nitrite, nitrate, citrate, fluoride)</td>
<td>0.3–3.0 mmol/L</td>
<td>n.g.</td>
<td>&lt;14</td>
<td>[34]</td>
</tr>
<tr>
<td>phosphate, bicarbonate, acetate)</td>
<td>0.014–1.4 mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic islet proteins</td>
<td>3.43–6.87 µmol/L</td>
<td>n.g.</td>
<td>&lt;6.8</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>0.39–1.96 µmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>260–781 nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>0.4–40 µg/L</td>
<td>0.02–0.67 µg/L</td>
<td>&lt;11</td>
<td>[32]</td>
</tr>
<tr>
<td>UV filters</td>
<td>5–160 µg/L</td>
<td>0.45–3.2 µg/L</td>
<td>&lt;13</td>
<td>[40]</td>
</tr>
<tr>
<td>Chlorotriazine herbicides and degradation products</td>
<td>0.1–10 µg/L</td>
<td>0.02–0.04 µg/L</td>
<td>&lt;5.5</td>
<td>[41]</td>
</tr>
<tr>
<td>Riboflavin (vitamin B2)</td>
<td>0.45–5.0 mg/L</td>
<td>0.05 mg/L</td>
<td>&lt;5.5</td>
<td>[42]</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>2–100 ng/L</td>
<td>0.5–6.1 ng/L</td>
<td>&lt;9</td>
<td>[33]</td>
</tr>
<tr>
<td>Beta-galactosidase activity</td>
<td>46.1 (nmol of product)/h/mg of protein</td>
<td>n.g.</td>
<td>n.g.</td>
<td>[27]</td>
</tr>
<tr>
<td>Binding constants (Kd) between tPex5 proteins and synthetic peptides</td>
<td>10⁻⁷–10⁻²⁵ mol/L⁵</td>
<td>n.g.</td>
<td>n.g.</td>
<td>[28]</td>
</tr>
<tr>
<td>Phosphomannomutase and phosphomannose isomerase activities</td>
<td>2.0 and 2.8 (µmol of product)/min/mg of protein</td>
<td>n.g.</td>
<td>&lt;10</td>
<td>[29]</td>
</tr>
<tr>
<td>Mouse immunoglobulins (IgG)</td>
<td>n.g.</td>
<td>6 mg/L</td>
<td>n.g.</td>
<td>[35]</td>
</tr>
<tr>
<td>Human IgG, rabbit IgG and horse IgG</td>
<td>0.10–1.00 g/L</td>
<td>5 mg/L</td>
<td>n.g.</td>
<td>[36]</td>
</tr>
<tr>
<td>Biotinylated single-stranded DNA</td>
<td>0–0.993 mg/L</td>
<td>11.1 µg/L</td>
<td>n.g.</td>
<td>[37]</td>
</tr>
</tbody>
</table>

n.g., not given.

*Values found in samples.

⁻Values found in samples.

⁻Sample introduction by electrokinetic injection for chloride and sulfate anions.

⁻Sample introduction by head-column field amplification for chloride anion.

⁻Sample introduction by head-column field amplification for sulfate anion.

⁻Values for insulin, proinsulin and c-peptide, respectively.

⁻Mean value found for real samples.

⁻Peptide in the sample medium.
and Luque de Castro [31] were able to quantify two parameters concurrently, namely, total content and individual species of linear benzene sulfonates. Not least, preliminary derivatization of analytes for proper separation and/or detection of optically-inactive species using classical SI methodology would be readily surveyed by in-valve optical detection. This was nicely illustrated by Wu et al. [38] for in-line fluorogenic labeling of islet proteins and optimization of the derivatization protocol in terms of sample-to-reagent volume ratio and degree of zone overlap via programmable fluidic control. Fig. 2 illustrates the configuration of LOV with dual optical detection and in-line sample derivatization in homogeneous phase as a front end to CE for monitoring of small proteins.

Coupling LOV with hybrid-flow approaches [e.g., multi-syringe FI analysis (MSFIA)] opened new avenues in on-line hyphenation of SPE (see also below for further details) to reversed-phase LC or GC for appropriate processing of the eluate in homogeneous phase [32,40–42], or delivery of minute, well-defined volumes of eluate via air segmentation into large-volume [programmable-temperature vaporizer (PTV)] GC injector [33], respectively. Fig. 3 shows the MSFIA-LOV-PTV-GC set-up for determination of trace-level concentrations of polychlorinated biphenyls in solid-waste leachates. It should be noted that conventional on-line SPE-reversed-phase LC coupling relies upon column-switching methods [43], wherein the sorbed species are eluted with the mobile phase itself, rather than with a methanolic/ethanolic eluent of improved elution strength, because of undue band broadening of the most polar compounds whenever the latter eluent is used. However, the marriage of MSFIA with LOV has proved to ensure both optimal conditions for retrieval of concentrated species and efficient LC band focusing. This has been accomplished by automatic post-SPE dilution of up to 175 µL of the polar organic eluent with a water stream provided concurrently by one of the liquid drivers of the multi-syringe device (see Fig. 4 for the system configuration), thereby rendering a final eluate plug that closely matches the initial chemical composition of the mobile phase [32,40–42].

4.2. Heterogeneous phase

Besides the successful miniaturization achieved on applications concerning homogeneous chemistry, LOV is particularly useful for handling heterogeneous, solid-liquid systems as a front end to separation techniques. This has been aimed at implementing µSPE protocols (Table 2 and references therein) or monitoring interactions between biomolecules, including on-column detection [36,37,44].

The coupling of LOV-SPE to LC, GC or CE has been devoted to assays in complicated matrices, namely those belonging to environmental, food or biological samples. As illustrated in Tables 2 and 3, over the past few years,
Figure 3. Lab-on-a-valve (LOV) platform coupled to programmable temperature vaporizer-gas chromatography (PTV-GC) for preconcentration and determination of trace-level concentrations of polychlorinated biphenyls in solid-waste leachates. LOV, Lab-on-a-valve; MPV, Multi-position selection valve; IV, Injection valve; MSP, Multi-syringe pump; HC, Holding coil; PTV, Programmable temperature vaporizer; SV, Solenoid valve; GC, Gas chromatograph; ECD, Electron-capture detector (Reproduced from [33] with permission of the American Chemical Society).
the marriage of LOV and SPE has gained widespread acceptance in uptake and preconcentration of a broad spectrum of analytes, including emerging pollutants in environmental samples [31–33,40,41] or biologically relevant molecules in urine [30,32] or food [42], prior to their separation and quantification with CE-UV [31], LC-ESI-MS² [30], LC-UV [32,40–42] or GC-ECD [33]. In some instances, a conventional, packed-bed column filled with octadecyl chemically-modified silica gel (see Table 2) has been attached at a peripheral port of the monolithic LOV device. By taking advantage of the ease of handling micro amounts of bead suspension through the precisely machined channels of the LOV device, a plethora of tailor-made protocols capitalizing on the so-called bead-injection (BI) approach [45] have been designed, comprising column packing, sorbent conditioning, sample loading and matrix removal, analyte elution and column removal with sorbent disposal (see Table 2 for experimental details). The BI approach offers two main advantages, not matched by any other automatic, flow-based SPE scheme:

- the automatic renewal of sorbent, without any intervention of operator or replacement of devices or physical parts of the system, so as to circumvent the progressive deactivation and tighter packing of permanent in-line SPE cartridges; and,
- the accurate metering of sorbent and eluate quantities by resorting to bi-directional programmable flow, as precisely controlled by the syringe pump, along with the possibility of introducing all eluate or merely a fraction of it via heart-cut injection protocols [42] in the separation technique for further processing.

The transfer of the entire volume of eluate into the separation system is essential to reach low limits of detection (LODs), especially when using low-sensitivity detectors (e.g., UV spectrophotometers coupled to LC). This approach, along with the handling of a well-defined volume of sample > 10 mL and in-line dilution as detailed above, fostered the determination of non-steroidal anti-inflammatory drugs [32] and chlorotriazine herbicides and metabolites thereof [41] in environmental samples at the low-µg/L level (see Table 3). Low LODs were also attained through the automatic, on-line transfer of all eluate to the PTV-GC-ECD system, as proposed by Quintana et al. [33] for determination of polychlorinated biphenyls in raw solid-waste leachates at the 2–100 ng/L range.

In other situations, where the enrichment factor is not an issue, a heart-cut approach was adopted. For example, in the determination of riboflavin (vitamin B2) in troublesome foodstuffs [42], commercially-available molecularly imprinted polymers (MIPs), tailored for supramolecular recognition of riboflavin, were utilized in a BI fashion for clean up of crude protein-rich samples and selective uptake of the target vitamin with no need for any ancillary sample treatment, followed by elution with 312.5 µL of 50% (v/v) methanol/water + 1% (v/v) acetic acid. Nonetheless, merely 67 µL at the eluate heading were injected into the liquid chromatograph, providing results in agreement with certified values of standard reference materials (i.e. infant milk (NIST 1846)) but also pig liver (BCR 487) extract, for which the MIP was not originally devised. In this particular work, the automatic sorbent renewal fostered by BI proved essential for attaining unbiased results, as severe carry-over effects were observed when reusing MIP beads in successive analysis of raw-milk-based samples. This application, along with the pioneering work of Boonjob et al. [41], demonstrated that manipulation of non-spherical, heterogeneously-sized materials is feasible inside LOV conduits in a repeatable fashion, despite the state of the art so far, where the need for suspensions containing spherically-shaped particles with a narrow size distribution has been frequently emphasized [16,21]. To this end, a long-lasting stable suspension of triazine-selective MIP chunks in a bead container attached to LOV was achieved by tailoring the composition (density and viscosity) of the suspending media via modulation of the methanol or acetonitrile/water ratio (in this case, 60% (v/v) methanol/water was selected) so as to prevent beads floating or settling in the LOV microchannels [41]. Also, for riboflavin-selective MIPs, the chunks were dispersed prior to their aspiration into LOV by providing a burst of elution solvent through the particles deposited at the bottom of the container [42]. This promoted formation of an adequate, reproducible dispersion during the time interval required for bead withdrawal, providing repeatable column packing (RSD = 1.8%, n = 10).

A large number of works exploiting AC in a miniaturized BI-LOV fashion were reported recently [27–29,35–37]. Frontal AC (FAC) is commonly employed to assess the interactions between proteins and analytes by continuous infusion of the sample through a column containing a covalently-attached protein, and by monitoring the elution of analytes. The breakthrough time per analyte is correlated inversely with its binding strength. Estimation of binding constants is feasible once the column capacity is known.

FAC was successfully miniaturized for determination of binding constants between synthetic peptides and truncated Pex5 proteins from human or from Trypanossoma brucei, demonstrating it to be a useful tool in drug development against parasitic diseases [28]. The LOV apparatus used in this study featured full automation along with repetitive reloading of beads with immobilized protein at a well-defined column capacity, infusion of a precise sample volume, and continuous delivery of the eluted solution into an MS detector.

A similar arrangement was devised to monitor the efficiency of capturing biotin-containing conjugates on streptavidin-agarose beads and ascertaining the efficiency
of their release upon perfusion of free biotin [27]. In this case, initial measurements were performed off-line, as fractions of eluate were collected and later processed at an ESI-MS detector.

Further studies were devoted to the assessment of biotin-containing conjugates elution rates, profiting from direct coupling of the ESI-MS detector to the affinity column assembled inside the LOV conduits. Affinity capture-elution protocols were also automated using this configuration for evaluation of enzyme activities in cell lysates [27,29]. A synthetic molecule, combining three parts, designated “handle”, “linker” and “substrate”, was converted to a “handle-linker product” by the target enzyme and both entities were selectively retained on streptavidin-coated beads if the “handle” was biotin.

After elution, the substrate and product molecules were determined by ESI-MS, thereby facilitating concurrent estimation of the activities of several enzymes.

AC has also been utilized as immuno-SPE material for selective uptake of one specific analyte or group of molecules by perfusion of sample through the affinity sorbent, where retention of analytes takes place, followed by a change of the perfusion media (pH, ionic strength, competitive ligands) with concomitant elution of retained analytes. This type of protocol has also been implemented in the LOV format using protein A-coated sepharose beads for selective separation of mouse immunoglobulin (IgG) [35]. In this case, miniaturization and in-valve integration of optical detection at the outlet of the affinity column provided a 60-fold decrease in the

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**Figure 4.** Multi-syringe flow-injection lab-on-a-valve (MSFI-LOV) manifold for determination of riboflavin in foodstuff. LOV, Lab-on-a-valve; MSP, Multi-syringe pump; LC, Liquid chromatograph; S, Syringe; V, Three-way commutation valve; A, Air; CS, Conditioning solvent (50% (v/v) MeOH/H₂O); BS, Bead suspension in conditioning solvent; C, Carrier solution (H₂O); D, Diluent (H₂O); W, Waste; CC, Central channel; EL, Eluent (50% (v/v) MeOH/H₂O + 1% (v/v) CH₃COOH); B, Channel for discarding beads; Sa, Sample/standard solution; HC, Holding coil; P, Chromatographic pump; IV, Injection valve; MC, Monolithic chromatographic column; D, Diode-array detector (Reproduced from [42] with kind permission from Springer Science+Business Media).
LOD attained (6 mg/L) when compared to a large conventional column mounted atop the LOV device and equipped with the same detection system. Miniaturization also fostered shortening of the assay cycle from 30 min to 2 min, while the volume of waste was reduced from 30 mL to 2 mL.

Most importantly, LOV has proved itself an excellent tool for implementing protocols that are not amenable to conventional AC equipment. Generally, interactions between bioligands and the stationary phase in AC are followed by monitoring of eluted compounds. Ruzicka and co-workers proposed to survey biomimetic interactions in a different way by on-column probing after in-valve packing of affinity sorbent and during perfusion of sample and eluent. This configuration was first described in one of the works mentioned above [27], wherein the elution kinetics of biotin-containing conjugates was also followed by measuring the decrease in absorbance at 330 nm. The affinity column was assembled in-line by creating a minute column (volume ~6 µL), trapped inside one of the channels of the LOV device, in such a way that the bead surface was probed by two optical fibers facing each other to provide light-absorbance measurements.

Dual-phase detection was also explored for separation, binding, and elution of IgGs on protein G-coated sepharose beads [36]. Here, the optical fibers were placed at the outlet of the affinity column to probe eluted analytes or facing the bead column for on-column interrogation and monitoring of analyte capture. The two approaches were complementary and essential for bioligand-interaction studies, as improved LODs (by about 10 times) were attained in column-effluent measurements, while the remaining analytes trapped on the beads could be easily detected by on-column probing. Diagnosing this problem correctly is essential to avoid carry-over effects or the appearance of dummy peaks upon changes of eluent composition.

The preconcentration and fluorimetric detection of biotinylated, single-stranded DNA (b-ssDNA) on streptavidin-coated sepharose beads in LOV might be regarded as an appealing approach to assay DNA elongation [37]. The optical fibers were configured at right angles, the bead column (volume ~2.2 µL) was assembled between them and perfused by b-ssDNA solution followed by a DNA fluorescence probe (Oli-Green) solution. The signal obtained was proportional to the mass of DNA retained in the column. In this case, on-column detection was selected over eluent probing because it allowed detection of strongly-bound or irreversibly-bound molecules, as occurs with biotinylated DNA and streptavidin-derivatized surfaces.

However, on-column LOV probing for absorbance measurements has some drawbacks, namely:

- high background signals, due to the intrinsic absorbance of beads giving rise to reduced signal-to-noise (S/N) ratios;
- limited capacity of the column; and,
- minute optical path length, which leads to lower sensitivity, compared to direct measurements of eluate [36].

Another source of error is the inhomogeneous distribution of analytes through the sorptive column, with a larger concentration in a narrow zone at the head of the sorbent bed [46,47]. If there is variability in gradient distribution between standards and real samples and the optical fibers do not probe the overall effective retention microzone, the quantification of target biomolecules would be biased.

5. Conclusions and future trends

The examples presented here have undeniably demonstrated that LOV is increasingly attracting interest as a programmable, flow-based, mesosfluidic platform for a vast number of sample treatments prior to column-separation methods. The coupling mode (i.e. on-line, in-line, at-line or off-line) reflects the degree of automation attained, which should be offset by the expected cost of analysis and the investment required upon purchasing and maintaining the equipment. Besides, we expect several unit operations, encompassing membrane-based sampling and sample preparation [e.g., (micro)dialysis, gas diffusion or pervaporation], gas-liquid separation or even advanced oxidation processes for analyte derivatization or degradation of organic compounds, to gain momentum from the association between LOV and separation techniques (LC, CE and GC), which leaves room for numerous analytical applications and a wide open road for potential usage in a plethora of areas of research endeavor. Another promising, yet unexploited, area is the coupling of short reversed-phase or dynamically-coated, ion-exchange, monolithic columns or discs to LOV devices for either SPE or low-to-moderate-pressure chromatographic separation of a small number of analytes in troublesome matrices following LOV-based sample processing.

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