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Universal approach for mesofluidic handling of bead suspensions in lab-on-valve format

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ABSTRACT

In the present report, new protocols are introduced for automatic mesofluidic handling of irregularly shaped and non-uniformly sized bead materials for renewable micro-solid phase extraction (μSPE) under the lab-on-valve (LOV) format. To this end, two alternative strategies were studied comprising either (i) the direct aspiration of bead suspension placed at a container attached to LOV device or (ii) the aspiration of beads after a resuspension step, allowing the formation of a fluidized bed inside the beads' container. Suspensions with homogeneously dispersed beads were also tested in the first strategy above, as prepared by increasing the viscosity of the suspension milieu with 75:15:10 glycerol/MeOH/H_2O (in wt). The bead injection protocols were applied to four commercial reversed-phase sorbent materials with different sorptive surfaces: Oasis HLB, SupelMIP β-receptors, Lichrolut EN and Discovery DSC-MCAX, and the mass of sorbent packed in each microcolumn was assessed. Direct aspiration of methanolic suspensions gave rise to bead stacking and clogging of the LOV microconduits, resulting in a source of results with poor precision (RSD: 3.8–67.6%). The use of glycerolic suspensions was merely effective for repeatable sampling and packing of Oasis HLB and SupelMIP β-receptor beads without sorbent settlement along time. The resuspension strategy was able to handle all the materials tested with acceptable precision (RSD: 1.6–13.8%). Enhanced precision was attained (RSD <4.1%) when the sorbent bed was physically restricted to the volume of the LOV microchannel cavity. Different volumes of suspension aiming at a target mass of sorbent of 10 mg were successfully handled (RSD: 3.1–13.8%), showing the reliability of the bead resuspension approach for varied nominal bead sampling volumes. The proposed bead handling protocols were applied to μSPE of propranolol taken as a model of β-blocker from aqueous solutions by SupelMIP β-receptors and Discovery DSC-MCAX beads with high repeatability (RSD <6%) and absolute recoveries between 69 and 74% in a bead-injection mode.

1. Introduction

Over the past few decades, flow injection analysis and related techniques have been a tool for new developments in the analytical chemistry field [1,2]. In this context, the handling of solid suspensions in a fully automatic fashion, where the solid-phase (in form of micrometric beads) could be renewed in each individual analytical cycle, and defined as bead injection (BI) [3], emerged as a disruptive concept in the automation of chemical assays and sample preparation [4]. In BI, the solid-phase suspended in a given solvent is a dynamic part of the system that is treated as a homogeneous solution, in a deep contrast with the classical use of solid-phase reactors in flow analysis, where the packed microcolumn is viewed as a permanent part of the manifold that should be replaced occasionally [5]. The BI concept started a new era for the automation of sample processing based on flow analysis, making possible the development of new SPE methodologies that overcome the decrease in performance caused by surface deterioration along the time. Not the least, BI allows the simultaneous monitoring of both effluent and solid phase itself (optosensing) in real time, which leads to complementary and enhanced insight into the SPE procedure in a single assay [6]. Though effective alternatives capitalized on flow injection have been reported [7–9], launching and evolvement of the BI technique were associated with the introduction of programmable flow; firstly by sequential injection analysis (SIA) [10], where flow cells with special configurations [11] including jet-ring cells [3,12], magnetic flow-through cells [13–15], rotating rods [11,16] and frit restriction-based containers [11,17] were assembled to the manifold, and more recently, with the introduction of lab-on-valve

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(LOV), the so-called third generation of flow injection analysis [18,19].

As a result of the progress towards automation, miniaturization and integration of overall analytical processes, the LOV module comprises a monolithic structure with microconduits machined in a polymethylmethacrylate or polyetherimide unit, which is mounted atop the multiposition valve in SIA modules. Due to the mesofluidic scale of the assays, integrated detection and compatibility with real-world samples [20], LOV is becoming an attractive analytical tool, with a particular impact in the bioanalytical field [21,22]. The open architecture and simple design, associated with the flexibility provided by programmable flow operation, converted LOV in the preferential way to develop new BI protocols. Despite the wide range of BI-LOV applications reported to date, renewable sorptive surfaces have been particularly exploited in methods involving color development and optical measurements [23,24], but also SPE protocols for handling of environmental samples [23,25]. The use of BI-LOV as front end to chromatographic techniques is also a current topic of growing interest [26–31].

Reliable manipulation of bead suspensions within the flow manifold is the major challenge in mechanized BI protocols for repeatable trapping of beads in microcolumns with subsequent minimization of the uncertainty measurement of the overall analytical method. Previous works about this topic [3,4,23,32] are consensual about the requirements that should be fulfilled by the bead materials in order to obtain homogeneous suspensions, usually prepared in an aqueous, hydro-alcoholic or alcoholic solvent [32]. Spherical shape, uniform size distribution and water-wettability (for reversed-phase materials) have been identified as imperative characteristics. To this end, the materials commonly used in BI have their backbone structures based on polystyrene-divinylbenzene (PS-DVB), polyvinylpirrolidone, or agarose. With the use of high density polytetrafluoroethylene [33] or silica-type [34] chunks, aspiration of sorbent material into the LOV was proven troublesome as a consequence of the stacking of the sorptive surfaces at the bottom of the reservoir and within the LOV central communication channel. This shortcoming was alleviated to a large extent by promoting a continuous recirculation of the suspension by a peristaltic pump [33,34]. Another strategy proposed for ensuring homogeneity of the sorbent suspension was the continuous stirring of the bead-containing reservoir [35]. Although both strategies provided improved precision in bead sampling and in-line microcolumn formation, they are associated with the use of additional instrumentation that increases the complexity of the manifold.

Therefore, the current state-of-art of BI-LOV excludes a diversity of sorbent materials with physicochemical properties able to broaden the application scope of this technique, particularly to automated μSPE for processing of samples of high matrix complexity. Hence, the objective of the present work was the development of a universal BI-LOV approach, able to cope with non-spherical and non-uniformly sized distributed beads applied routinely to solid phase extraction protocols. For this, different strategies for in-line bead suspension handling and column packing will be assessed regarding their precision through evaluation of the sorbent mass packed. Moreover, the impact of the proposed strategies on the analytical performance of SPE protocols will be evaluated by taking propranolol as a model β-blocker with further quantification by HPLC.

2. Experimental

2.1. Reagents and solutions

Chemicals were of analytical grade and used with no further purification. All aqueous solutions were prepared in ultra pure water (resistivity >18 MΩ cm) obtained from a MilliQ (Millipore, Bedford, MA, USA) system. Methanol (MeOH) and acetonitrile (ACN) HPLC grade, supplied by Merck (Darmstad, Germany) were also used as solvents.

Table 1
Physicochemical properties of the sorbents used in the present work as per manufacturer specifications.

Sorbent	Shape	Particle size/μm	Specific surface area/$m^2\ g^{-1}$
Oasis HLB	Spherical	30	800
SupelMIP β-receptors	Irregular	56[a]	n.a.
Lichrolut EN	Irregular	40–120	1200
Discovery DSC-MCAX	Irregular	50	480

n.a.: not available.
[a] Average value.

Four sorbents with different physicochemical characteristics (see Table 1) were tested: Oasis HLB (Waters, Milford, MA, USA), Lichrolut EN (Merck), SupelMIP β-receptors (Supelco, Bellefonte, PA, USA) and Discovery DSC-MCAX (Supelco). Methanolic and glycerolic sorbent suspensions were prepared by adding 1000 μL of MeOH or 200 μL of MeOH followed by 800 μL of 87.5% (w/w) aqueous glycerol (Sigma–Aldrich, St. Louis, MO, USA) to 100 mg of sorbent, respectively. For Discovery DSC-MCAX, 200 mg mL^{-1} methanolic suspensions were also prepared.

The stock solution (500 mg L^{-1}) of propranolol (Sigma–Aldrich) was daily prepared by dissolving the appropriate amount of solid in 10.00 mL of MeOH. Working standards for direct injection into the liquid chromatograph were prepared by diluting the stock solution in mobile phase. For SPE of the β-blocker, a 2.00 mg L^{-1} propranolol standard solution was prepared in 5.0 mmol L^{-1} ammonium acetate (Sigma–Aldrich) at pH 6.7, or 2% (v/v) CH_3COOH in water (Sigma–Aldrich) as per sorbent type for analyte uptake. 1% (v/v) HCOOH in ACN and 5% (v/v) NH_4OH in MeOH were used as eluents for MIP β-receptor and Discovery DSC-MCAX, respectively. An isocratic mobile phase composed of a 1:1 volume ratio of MeOH and 0.1% (v/v) trifluoroacetic acid (TFA) (Sigma–Aldrich) was used for chromatographic separation and determination of propranolol, adapted from Cabrera et al. [36]. The mobile phase was filtered through 0.45 μm Millex-HV filters (Millipore) and degassed by ultrasound irradiation during 15 min before use.

2.2. Lab-on-valve manifold

The flow system used for the mesofluidic handling of bead suspension and automatic μSPE of propranolol in a BI fashion (Fig. 1) comprised a BU4S multisyringe module (MS) (Crison Instruments, Allela, Spain), as propulsion unit equipped with two 2500 μL glass syringes (Hamilton, Bonaduz, Swizterland), labelled as S2 and S3. The access to the solutions reservoirs (position off) or LOV (position on) was controlled by the three-way commutation valves (NReseach, Caldwell, NJ, USA) placed at the head of each syringe. The propulsion unit was connected to a customized mesofluidic platform (Ideia.M, Porto, Portugal) containing a central channel and eight peripheral ports with channels of 1.5 mm i.d. engraved in a polyetherimide block (Fig. 1). This monolithic device was mounted atop of an eight-port multi-position selection valve (MPV, Crison Instruments). The access to the complete array of peripheral ports, one at a time, was provided by the central channel (CC), which was connected to S3 by the holding coil (HC). For the bead suspensions handling experiments only S3 was used and ports 3, 6 and 7 were closed. During automatic μSPE of propranolol, all ports were used and S2 was connected to the dual port 3, facilitating sample exchange. The beads were retained at the outlet of microchannel 1 by a 1 mm thick polyethylene frit with a pore diameter of 20 μm (Supelco). The bead container was attached to port 4 of the LOV