Talanta 84 (2011) 846–852

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 7 February 2011
Accepted 14 February 2011
Available online 19 February 2011

Keywords:
Lab-on-valve
Bead injection
Solid-phase extraction
Automation

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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doi:10.1016/j.talanta.2011.02.011

(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

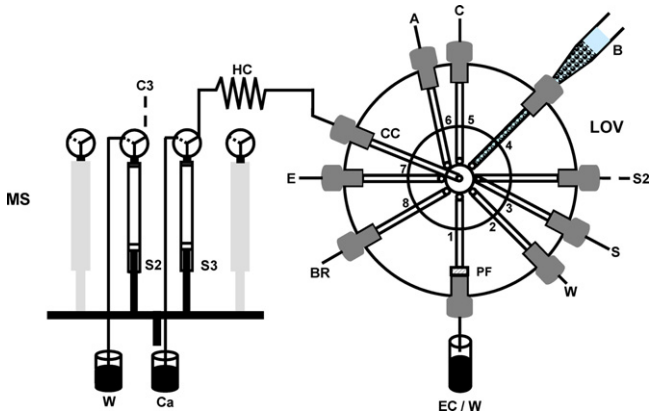

Fig. 1. Illustration of the LOV manifold used for automatic handling of bead suspensions and performing μSPE of propranolol in a bead injection mesofluidic format. MS, multisyringe; S_i, syringes (2500 μL capacity); LOV, lab-on-valve; HC, holding coil; CC, central channel; W, waste; Ca, carrier solution; EC, eluent collector; S, sample; B, bead suspension; C, conditioning solvent (methanol), A, air; E, eluent; BR, exceeding beads removal; PF: polyethylene frit. For the sake of simplicity the connection between S2 and the LOV dual-channel (3) was omitted and represented by a dashed line. For the extraction of aqueous propranolol by MIP β-receptors, the LOV channel 8 was connected to water reservoir and the exceeding beads were discarded through the waste channel (2).

at 45°. Polytetrafluoroethylene tubing (Omnifit, Cambrigde, UK) with 0.8 mm i.d. was used to connect the LOV ports to the solution reservoirs. The connections between the syringes and respective solution reservoirs and the HC (5000 μL capacity) were made of 1.5 mm i.d. tubing of the same material.

All the programmable flow sequences were executed by a personal computer running lab-made software written in Quick Basic 4.5 (Microsoft, Redmond, WA, USA). The parameters controlled by the software through an RS232 interface were the direction (aspiration/propulsion) and speed (flow rate) of fluid handling unit (multisyringe module), the position of commutation valves, and also the selection (one at a time) of the different ports on the MPV.

2.3. Strategies employed for mesofluidic handling of beads

Procedures commonly used for μSPE under BI-LOV format comprise four main operations that can be defined as (1) sorbent packing and conditioning, (2) sample loading and matrix removal, (3) elution and (4) bead discarding. In the present work customized protocols for operation (1) were evaluated (Table 2 and Table S1), followed by application of operation (4), aiming at bead recollection for mass evaluation. For propranolol extraction, operations (2) and (3) were added (Table S2), in order to perform the retention and elution of the analyte. For the sake of simplicity, the steps respecting to the refilling of S3 were omitted in the following description of the operating procedures.

Table 2
Summary of the protocol sequences for the two strategies proposed for microcolumn packing of bead suspensions under LOV format.

Step description	Strategy	
	Direct aspiration[a]	In-line resuspension[b]
Aspiration of MeOH/water	√	√
Resuspension of beads	×	√
Beads settling	×	√
Aspiration of bead suspension	√	√
Packing and conditioning of the microcolumn	√	√

√, step included; ×, step not performed in this strategy.
[a] This strategy was applied to methanolic and glycerolic suspensions.
[b] This strategy was applied to methanolic suspensions, with partial or full packing of LOV microconduit.

Microcolumn packing and conditioning. Four different protocols (I–IV, Table S1) for performing the automated aspiration of bead suspension into the LOV and packing of the integrated sorptive microcolumn, based on two different strategies, were evaluated (Table 2). They were characterized by direct aspiration of a methanolic (I) or glycerolic (II) bead suspension or by effecting a resuspension step to generate fluidized-bed conditions before aspiration into the LOV of a bead suspension in MeOH (III and IV). In the latter, the sorbent bed was physically confined to the volume of microchannel 1 after discarding the excess of beads previously aspirated. In all cases, the flow rates for bead aspiration and column packing were fixed at 0.5 and 2.0 mL min^{-1}, respectively:

(I) *Direct aspiration of methanolic suspensions*. 500 μL of MeOH and 125 μL of bead suspension were sequentially aspirated into the HC. Syringe 3 was refilled, and after flow reversal, the LOV microcolumn was generated and further conditioned with MeOH and water (see Table S1).
(II) *Direct aspiration of glycerolic suspensions*. After aspiration of 750 μL of water, 125 μL of glycerolic suspension were inserted into the HC and the column was packed by flow reversal. Next, a plug of 625 μL of MeOH was aspirated into the HC for sorbent conditioning. When a sorbent mass of 10 mg was targeted, the sorbent suspension aspiration step was adjusted to 86 and 72 μL for Oasis HLB and MIP β-receptors, respectively. For the propranolol extraction with MIP, the volumes were adjusted and extra conditioning steps were added (Table S3).
(III) *Aspiration of methanolic suspensions after a resuspension step*. In this case, after the aspiration of 525 μL of MeOH and the repositioning of the syringe piston, a burst of 125 μL was delivered to the bead suspension reservoir. For a period of approximately 10 s, S3 was refilled while the beads were settling in the container placed at microchannel 4. Thereafter, 125 μL of sorbent were aspirated. Then, the flow direction was reversed and the LOV microcolumn was generated and conditioned. For experiments for which the sorbent mass targeted was 10 mg, the volumes of suspension aspirated were modified to 58, 95, 75 and 34 μL, when Oasis HLB, MIP β-receptors, Lichrolut EN and Discovery DSC-MCAX were used.
(IV) *Aspiration of methanolic suspensions after a resuspension step with full packing of LOV microchannel 1*. In this strategy only two extra steps were added to the above described (III). After sorbent aspiration, trapping within LOV and bead conditioning, S3 was refilled and the exceeding beads remaining stored in the MPV rotor and central channel were discarded to waste.

Beads disposal. The sorbent packed inside the microchannel 1 was wetted with 100 μL of MeOH, previously stored in the HC. Next, the beads were aspirated back into the HC and immediately disposed into waste by 1275 μL of carrier solution that was collected into a 2 mL capacity vial for subsequent sorbent mass determination. Finally, the LOV column cavity was rinsed with 200 μL of carrier solution (MilliQ water).

2.4. Microcolumn weighing procedure

As mentioned above, sorbent microcolumns packed by different automatic fluidic protocols were collected (after discarding procedure) by placing 2 mL glass vials (Supelco) in port 2 in the course of step (e) (Table S1). The vials were previously cleaned by a N_2 stream and weighed (AG285 balance, Mettler-Toledo, Columbus, OH, USA). The liquid content of the vials was evaporated overnight by oven

Table 3
Analytical protocol for automatic in-line SPE of propranolol using SupelMIP β-receptors and Discovery DSC-MCAX in BI-LOV format.

Operation	MIP β-receptors	Discovery DSC-MCAX
Conditioning	500 μL of 1% (v/v) HCOOH in ACN + 500 μL H_2O + 1400 μL of 5 mmol L^{-1} NH_4COO	500 μL of MeOH + 500 μL of 2% (v/v) CH_3COOH
Sample loading	1000 μL of 2.00 mg L^{-1} propranolol standard	1000 μL of 2.00 mg L^{-1} propranolol standard
Washing	1000 μL of 5 mmol L^{-1} NH_4COO	1000 μL of 2% (v/v) CH_3COOH
Analyte elution	1500 μL of 1% (v/v) HCOOH in ACN	1000 μL of 5% (v/v) NH_4OH in MeOH

drying with temperatures between 80 and 90 °C. After cooling, the vials were weighed again. The sorbent amount in the microcolumn was calculated as the difference between the mass obtained for the vial containing the dried sorbent and for the mass of the same empty vial. For quality control purposes, control charts of the mass of individual empty vials were constructed ($n > 10$) and outliers (SD > 5%) were eliminated from the experiment.

2.5. Automatic μSPE and chromatographic determination of propranolol

Solutions containing 2.00 mg L^{-1} of propranolol prepared in 5 mmol L^{-1} ammonium acetate at pH 6.7 or 2% (v/v) CH_3COOH were extracted by MIP β-receptors and Discovery DSC-MCAX sorbents, respectively. The automatic μSPE-BI protocol (Table S2) is summarized in Table 3 and was designed on basis of the information endorsed by sorbent suppliers. The eluate was diluted 1:1 with 0.1% (v/v) TFA before analysis. A given volume of diluted eluate (50 μL) was analyzed by liquid chromatography aimed at the potential determination of mixtures of β-blockers and/or β-agonists following class-specific molecularly imprinted-SPE or mixed-mode SPE.

Chromatographic assays were performed on a liquid chromatography setup Merck/LaChrom 7000 series (Hitachi, Tokyo, Japan). It was composed of a high-pressure pump (L-7455), an UV–Vis detector (L-7100) and an interface (D-7000). The system control and data acquisition were performed by D-7000 software. Analyses were conducted in the isocratic elution mode using 50% (v/v) MeOH:aqueous 0.1% (v/v) TFA at a flow rate of 1.50 mL min^{-1} and a monolithic column (100 mm × 4.6 mm i.d., Chromolith RP-18e, Merck, Darmstadt, Germany) as analytical column connected to a guard column (5 mm × 4.6 mm i.d.) of the same material. Eluates were manually injected through a Rheodyne 7725i injector (Rohert Park, CA, USA) equipped with a 50 μL loop.

Retention time and spectra were used for propranolol identification. Peak area at the maximum absorbance wavelength (288 nm) was used as analytical signal. A linear response ($r^2 \geq 0.998$) was found for calibration curves established over the range of 0.25–1.5 mg L^{-1}.

3. Results and discussion

3.1. Mesofluidic-based strategies for handling bead suspensions

The sorbents used in the present work (Table 1 and Fig. S1) were chosen considering their physicochemical properties and the potential application in reversed-phase SPE protocols under an LOV fluidic format. Therefore, SupelMIP β-receptors, Lichrolut EN and Discovery DSC-MCAX selected in this work. MIP β-receptors and Lichrolut EN are irregularly-shaped polymeric materials. The former is a molecularly-imprinted polymer for the selective extraction of drugs with affinity for β-receptors and the latter is a PS-DVB sorbent of large surface area suitable for reversed-phase extraction of polar organic compounds at trace levels. Discovery DSC-MCAX, composed of functionalized C8-silica-based non-spherical particles bearing benzene sulfonic acid moieties, is a mixed-mode solid-phase that retains molecules by hydrophobic interactions and cation-exchange mechanisms. For comparison purposes, Oasis HLB, a spherical polymeric sorbent previously used in BI-LOV protocols [27,30], was also applied. Considering the differences in the backbone structure, particle size and physicochemical properties, these sorbents can be regarded as a relevant sample of commercially available products for reversed-phase SPE.

The sorbents studied were suspended using methanol or 75:15:10 glycerol/methanol/water (in wt%) as solvent. These suspensions were handled by different flow programs that involved two different strategies (Table 2): (i) the direct aspiration of the beads from the container or (ii) the aspiration after a resuspension step, where a fluidized sorbent bed was generated. Concerning the amount of beads packed in each protocol sequence, two different approaches were tested. The first approach involved the aspiration of a fixed volume of suspension from the container (125 μL), while the second involved the sampling of a variable suspension volume for a fixed amount of ca. 10 mg of sorbent. Thus, it was possible to evaluate the precision of the mesofluidic column packing at different nominal volumes of bead suspension uptake.

3.1.1. Direct aspiration of the beads from methanolic and glycerolic suspensions

In BI-LOV methods described so far, the physicochemical properties of bead materials were taken into consideration for choosing the suspension milieu. Hence, the hydrophilic (polysaccharide-based materials) are generally suspended in aqueous solutions [35,37] while hydrophobic (reversed-phased) materials are frequently suspended in alcoholic media [26,30]. Considering the hydrophobic character of the sorbents used in this work, the first approach for handling of the beads involved direct aspiration of suspensions prepared in 100% MeOH (see Table S1). For 125 μL of bead suspension, lump formation was noticed in the second or third aspirate replicate of MIP β-receptors and Lichrolut EN beads. This resulted in a poor precision in sorbent packing with relative standard deviation values (RSD) of 67.6 and 24.2%, respectively (see Table 4). For spherically-shaped beads (Oasis HLB), this strategy provided better within-run precision (namely, 3.8%).

Polysaccharide-based beads (Sepharose® and Sephadex®), often used in BI-LOV protocols [4], are spherical and have a density similar to that of water. Thus, homogeneous suspensions might be readily prepared whereby column packing into the LOV microchannels is reported to be highly reproducible [20,31,32]. In contrast, the reversed-phase sorbents used in this work, except for Oasis HLB, were non-spherical. On the other hand, the introduction of the reversed-phase beads into the LOV unit from a homogeneous bead suspension was investigated. To this end, the viscosity and density of the suspension solvent were increased by the addition of glycerol. This alcohol was selected considering its high-viscosity and compatibility with both organic and aqueous solvents commonly used in solid-phase extraction procedures. Preliminary experiments were made preparing glycerol/water solutions in the range of 50–75% (w/w). Considering our previous knowledge with another MIP material [29], different suspensions containing 100 mg of MIP β-receptors per mL of solvent were prepared and homogeneity was evaluated by naked eye. The use of 75% (w/w) glycerol seemed to provide a homogeneous suspension. However, when

Table 4
Average sorbent mass (mg) obtained in Bead-Injection microcolumn packing by application of different mesofluidic protocols[a].

Sorbent	Microcolumn packing protocol					
	Direct aspiration			Fluidized suspension		
	Methanol (I)	Glycerol (II)		(III)		(IV)
	Fixed volume	Fixed volume	Variable volume[b]	Fixed volume	Variable volume[b]	Filled channel
Oasis HLB	28.8 ± 1.1 (3.8%, n = 8)	13.2 ± 0.8 (6.1%, n = 8)	10.2 ± 0.7 (6.9%, n = 9)	23.6 ± 2.2 (9.3%, n = 8)	10.0 ± 1.3 (13.0%, n = 8)	17.2 ± 0.7 (4.1%, n = 8)
MIP β-receptors	6.8 ± 4.6 (67.6%, n = 5)	14.5 ± 0.5 (3.5%, n = 10)	8.6 ± 0.4 (4.7%, n = 7)	15.1 ± 0.9 (6.0%, n = 5)	9.7 ± 0.3 (3.1%, n = 7)	11.1 ± 0.2 (1.8%, n = 10)
Lichrolut EN	18.2 ± 4.4 (24.2%, n = 10)	n.a.	n.a.	20.1 ± 2.1 (10.4%, n = 10)	10.9 ± 1.5 (13.8%, n = 7)	16.7 ± 0.4 (2.4%, n = 8)
Discovery DSC-MCAX	32.9 ± 2.6 (7.9%, n = 8)	n.a.	n.a.	23.2 ± 1.7 (7.3%, n = 7)	9.3 ± 1.0 (10.7%, n = 8)	24.5 ± 0.4 (1.6%, n = 8)

n.a., not available, please see text for additional information.
[a] Values in parenthesis correspond to relative standard deviation and number of replicates.
[b] In this case different volumes were used to attain a sorbent mass of ca. 10 mg.

Fig. 2. Mass of sorbent weighed for consecutive packing procedures of beads suspended in 75:15:10 glycerol/MeOH/H_2O (in wt%): □, Lichrolut EN; ♦, Discovery DSC-MCAX; ○, SupelMIP β-receptors; △, Oasis HLB.

processed by the automatic fluidic system, the masses of sorbent obtained were not reproducible (RSD% > 20), in disagreement with visual inspection because there were still very small bead aggregates in the suspension. This source of impaired results was circumvented by wetting the beads with methanol before the addition of the glycerol solution, ensuring a subsequent homogenous solvation of the sorbent by glycerol and water molecules. Thus, glycerolic suspensions were prepared by suspending 100 mg of sorbent in 200 μL of methanol followed by the addition of 800 μL of 87.5% (w/w) glycerol, resulting in the original 75% (w/w) glycerol in the final solvent composition. The four sorbents in test were suspended by this procedure and packed into LOV microchannel 1 by the fluidic protocol (II) described in Table S1. In this particular case, 125 μL of bead suspensions were collected and packed by the carrier solution. The volume for conditioning the beads was increased aiming at complete removal of the glycerol from the surface of the beads (Table S1). The sorbent mass weighed for Oasis HLB and MIP β-receptors (Table 4) deployed a precision suitable for use in μSPE protocols, with RSD values of 6.1 and 3.5%, respectively. On other hand, Lichrolut EN and Discovery DSC-MCAX beads migrated throughout the time (<10 min) either to the top or bottom of the suspension, respectively, because of their lower or higher density when compared to the suspension milieu. This migration was observed by naked eye and by the increase or decrease of the mass packed throughout the time (Fig. 2). Hence, for these two materials, 75% (w/w) glycerol was not a suitable solvent to prepare homogenous suspensions.

For aspiration of smaller volumes of suspension containing ca. 10 mg of sorbent (58 μL for Oasis HLB and 95 μL for MIP β-receptors), the RSD values (4.7–6.9%) were similar to those previously obtained in the aspiration of 125 μL of glycerolic suspension (3.5–6.1%) (see Table 4), demonstrating the appropriate precision of the propulsion unit for bead handling and the reliability of programmed flow protocol.

3.1.2. Aspiration of beads in methanolic suspensions after a resuspension step

The second strategy tested in the present work was based on the introduction into the mesofluidic protocol sequence of a bead resuspension step (see Table 2) before sorbent uptake. Taking into account that beads in 100% MeOH were permanently settled at the bottom of the reservoir, this procedure involved bead flushing by a burst of solvent (125 μL), resulting in the resuspension of the particles within the solvent medium, followed by resettlement within a short time frame of 10 s. Since the collection of beads

began well before complete bead settlement, compactness associated with the mass of sorbent accumulated at the bottom of the container was overcome, and thus a reproducible, well-defined amount of beads could be aspirated through LOV microchannel 4.

The four sorbents under evaluation were subjected to three different operating procedures that included a resuspension step with 125 μL of methanol (Table S1, (III–IV)). The first two approaches included the aspiration of a fixed volume of 125 μL for all sorbents or the aspiration of a variable volume, respectively, aiming at a sorbent mass of 10 mg, as performed before with glycerolic suspensions. In the third approach, after aspirating 125 μL of suspension, the surplus of beads over the volume capacity of microchannel 1 were discarded to waste, restricting the sorbent bed to the volume of the LOV microchannel cavity. In contrast to direct aspiration of methanolic suspensions, the formation of lumps and clogging of the channels was not noticed for any of the resuspension approaches, which made repeatable packing of all sorbents in an automatic mesofluidic format feasible.

Repeatability values of the bead packing procedures for fixed volume (125 μL) or fixed mass (10 mg, suspension volumes varying from 34 to 95 μL) experiments (see Table 4), were similar in the range of 6.0–10.4% and 3.1–13.8%, respectively. The approach based on restriction of the sorbent bed to the overall capacity of the LOV microchannel rendered enhanced precision as compared with the approaches described above for the suite of investigated sorbent materials with RSD values comprised between 1.6 and 4.1%. These results are in agreement with our previous observations using a similar strategy for uptake of water-compatible MIP into LOV [29]. However, in this case, it is not possible to change the amount of beads loaded without physical resizing of the LOV microconduit. A possible way of resizing the microchannel, without manufacturing a new device, would be the implementation of a rigid polyetheretherketone (PEEK) rod of appropriate dimensions inside the channel [27], which serves simultaneously for decreasing the channel volume and as bead stopper as well. Notwithstanding, it should be considered that the use of PEEK tubing could also result in some restrictions upon the maximum flow rates allowed through the operation sequence as reported earlier [30].

3.2. Solid-phase extraction of propranolol in a bead-injection mesofluidic format

In order to assess the influence of the column packing protocol on the performance of the μSPE-BI procedure, one millilitre of standard aqueous solutions containing 2.00 μg mL^{-1} of propranolol were processed using either MIP β-receptors or Discovery DSC-MCAX sorbents. In both cases, μSPE was performed using either 10 mg of sorbent or the mass that occupies the LOV microchannel capacity, exploiting bead resuspension in a methanolic milieu. In addition, direct aspiration of 10 mg of beads from a 75% (w/w) glycerol suspension was also assessed for MIP β-receptors. The protocol sequences used (Tables S2 and S3) were summarized in Table 3. The analytical procedure involving the storage of beads within the HC and central channel of LOV was proven troublesome for expedient SPE assays in the LOV format. To prevent band broadening effect in the propranolol peak, the eluate was diluted 1:1 with 0.1% (v/v) TFA before injection into the chromatographic equipment.

Using 10 mg of sorbent, the absolute masses of propranolol recovered were 1.37 ± 0.06 and 1.39 ± 0.08 μg for MIP β-receptors and Discovery DSC-MCAX, respectively. These values changed to 1.43 ± 0.03 and 1.46 ± 0.08 μg, respectively, when confining the sorbent bed within LOV microchannel, corresponding to 10.1 ± 0.1 and 22.4 ± 0.9 mg of sorbent, respectively. In addition, for direct aspiration of 75% (w/w) glycerolic suspension of MIP β-receptors beads, the mass of propanol recovered was 1.44 ± 0.03 μg using a sorbent amount of 7.8 ± 0.3 mg. One-way ANOVA analysis was applied to this set of results (5 experiments, 6 replicates of each), providing a calculated F value of 2.46 (critical $F = 2.76$, for a confidence level of 95%). This indicates that the variances between bead handling protocols are not significantly different from the variance obtained in 6 consecutive extractions using a given bead sampling strategy. In addition, there is no evidence of statistically significant differences in absolute recovered masses when applying either of the sorbents tested [38]. The excellent coefficients of variation (RSD <6%) and acceptable absolute recoveries (69–74%) demonstrated that the proposed approaches have potential for the determination of propranolol in real samples. Moreover, it should be emphasized that the multivariate optimization of the μSPE protocol via experimental design procedures, which was not aimed at the present work, could result in an improvement of the recovery levels.

4. Conclusions

In the present work, several strategies for mesofluidic handling in a bead-injection format of irregularly shaped beads with non-uniform size distribution were evaluated. Direct aspiration of the tested sorbents into LOV did not provide repeatable packing, even when the viscosity of the media was modified by addition of glycerol. In fact, the change in viscosity of bead suspending media worked for one of the sorbents (MIP β-receptors beads), requiring further tailoring of glycerol concentration for the other sorbents (Lichrolut EN and Discovery DSC-MCAX) to maintain a homogeneous suspension. Hence, a careful selection of the viscosity and density of the bead suspending solution needs to be effected for individual sorbents, whereby cannot be regarded as a universal approach.

The bead resuspension mode, providing fluidized bed conditions within the sorbent container, was indeed effective to foster a repeatable packing for all sorbents tested. The precision in sorbent packing was even enhanced when the protocol was designed to confine the mass of sorbent within the physical dimensions of the LOV microchannel. Considering the excellent coefficients of variation (RSD <6%) and acceptable absolute recoveries of propranolol from aqueous solutions, the packing conditions offered by the resuspension approach are deemed appropriate for analytical applications, providing a universal approach for bead handling within the LOV format. Future research in this area will be focused on broadening the sorptive materials investigated, as well as on launching new developments in LOV technology towards enhancement of its performance in renewable μSPE-BI applications.

Acknowledgements

This work was financially supported by Fundação para a Ciência e Tecnologia (FCT) and by European Union through FEDER and QREN 2007-2013 programs (project PTDC/AAC-AMB/104882/2008). Funding from the Spanish Ministry of Science and Innovation (project CTM 2010-17214 and integrated action HP2008-0045) and by FCT (Integrated Action E-48/09) is also acknowledged. Hugo M. Oliveira thanks FCT and FSE (III Quadro Comunitário) for PhD grant SFRH/BD/22494/2005. The authors thank Dr. Ferran Hierro from the Microscopy Laboratory at the University of the Balearic Islands for providing the electron microscopy pictures. The gift of MIP material from MIP Technologies is also appreciated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.02.011.

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