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Online Coupling of Bead Injection Lab-On-Valve Analysis to Gas Chromatography: Application to the Determination of Trace Levels of Polychlorinated Biphenyls in Solid Waste Leachates

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Online sorptive preconcentration exploiting renewable solid surfaces, so-called bead injection (BI), in the miniaturized lab-on-valve (LOV) platform is for the first time hyphenated to gas chromatography (GC) for automated determination of trace level concentrations of organic environmental pollutants. Microfluidic handling of solutions and suspensions in LOV is accomplished by programmable flow with a multisyringe flow injection (MSFI) setup. The method involves the incorporation of minute amounts (3 mg) of reversed-phase copolymeric beads with hydroxylated surface (Bond Elut Plexa) into the channels of a poly(ether imide) LOV microconduit, thus serving as a transient microcolumn packed reactor for preconcentration of organic species. The analyte-loaded beads are afterward eluted with 80 μL of ethyl acetate into a rotary injection valve and subsequently introduced via an air stream into the programmable-temperature vaporizer (PTV) injector of the GC. The used beads are then backflushed and delivered to waste. The GC separation and determination is synchronized with the preconcentration steps of the ensuing sample. The potentials of the devised BI-LOV–GC assembly with electron capture detector for downscaling and automation of sample processing were demonstrated in the determination of polychlorinated biphenyls in raw landfill leachates and a leachate containing the Aroclor 1260 congener mixture. By sampling 12 mL of leachates to which 50 vol % methanol was added to minimize sorption onto the components of the flow network, the automated analytical method features relative recovery percentages >81%, limits of quantification within the range of 0.5–6.1 ng L^{-1}, relative standard deviations better than 9% at the 50 ng L^{-1} level, and 25-fold decrease in cost of

solid-phase extraction (SPE) consumables as compared with online robotic systems or dedicated setups.

Solid-phase extraction (SPE) has become the preferred routine sample preparation technique for the determination of organic pollutants[1] because of the advantageous features over liquid–liquid extraction and improved capacity and robustness as compared to liquid-phase or solid-phase microextraction counterparts. Yet, sample preparation accounts for over 60–80% of the total analysis time and normally is the main contributor to analytical uncertainty.[2–4] Thus, automation of sample preparation is of great value in order to maximize throughput and minimize costs, time, and analyst risks due to chemicals exposure. In this context, online coupling of SPE to chromatography represents the automation milestone in water analysis of organic pollutants, as the overall analytical protocol can be fully mechanized. Although the online SPE–liquid chromatography (LC) coupling is currently well established, SPE–gas chromatography (GC) hyphenation via mechanized flow-based approaches is far less common.[2] Actually, as reviewed by Hyötyläinen and Riekkola,[3,4] most applications date back to the 1990s, and research on this topic in the last years is quite scarce. This observation can be attributed to the fact that SPE columns for water analysis should be gently dried prior to elution and the final eluate volume needs to be reduced prior to introduction into the GC capillary column, most often by an on-column or programmable-temperature vaporizer (PTV) injector, to avoid column overload.[2–5] As a result, the SPE–GC coupling is technically more complex and difficult to optimize. Online analyzers, so-called Prospekt-2 (Spark Holland, The Netherlands), for automated SPE are currently available,[6–9] yet this robotic system requires the modification of the PTV injector, whereby the GC can merely be used for analysis in

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combination with the robotic SPE analyzer. Prospekt-2 setups allow the renewal of the sorbent cartridge in each analytical cycle. Most researchers have, however, decided to regenerate the sorbent material and reutilize it in several assays as a consequence of the elevated costs of these dedicated cartridges.[6−8]

Indeed, the Achilles's heel of online SPE−GC analyzers described in the literature up to date is the usage of a permanent sorbent column attached to the flow setup, which deteriorates the ruggedness of the system and calls for extensive operational maintenance. In fact, it has been reported that the increasing of back-pressure in real-life sample analyses caused by the progressive tighter packing of the sorbent beads when reusing the material for a given number of assays hinders the effective drying of the sorbent after sample loading.[6] Further, the reuse of columns might result in deterioration of peak shapes of target analytes in GC,[6] most likely due to the concomitant adsorption of matrix ingredients. Hence, online SPE−GC analyzers have been merely utilized for automated determination of trace level of organic pollutants in simple matrixes, e.g., surface waters.[6−8] Analyte carryover effects over 5−15% have been also observed in copolymeric phases between sample runs, hereby tedious cleansing procedures should be applied.[6]

The difficulties associated with flow resistance can be alleviated to a certain extent via various approaches, including the performance of the elution sequence in the backflush mode.[10] Yet, the malfunctions of the sorbent surfaces themselves are not addressed by these means. Therefore, a superb alternative for eliminating any problems associated with the changes of the surface properties of the sorbent materials and/or the creation of flow impedance in a column reactor is to employ a surface renewal scheme, that is, the so-called bead injection (BI) procedure.[11,12] Here, the contents of the packed column are automatically renewed for each analytical run. Such a scheme is readily downscalable in the lab-on-valve (LOV) platform,[13−18] which has opened up a host of prospects within the environmental analytical field.

The microconduit LOV unit is a single monolithic structure mounted atop of a multiposition valve of a sequential injection network. The LOV approach should be viewed as a judicious advance toward the automation of microfluidic handling of samples and sorbent materials within integrated microbore units.[16] In short, packed column reactors for microscale SPE[14,15,18,19] are in situ generated by aspirating beads with particular surface characteristics and particle sizes, advantage being taken of the fact that the sorbent can be manipulated exactly as when handling liquids. The solid entities can even be automatically transported between different column positions within the LOV, their retention within the columns being facilitated by fitting the column positions with appropriate stoppers or frits, which will keep hold of the beads, yet allow solutions to flow freely. Following sample loading and cleanup protocols, appropriate eluents can be aspirated, and the eluate propelled to the detection device, as sandwiched by air or immiscible liquid segments in order to preserve its integrity.[17] The microfabricated channel system is amenable to incorporate detection facilities, that is, optical devices (namely, diode-array spectrophotometers, charged-coupled devices (CCDs), laser-induced spectrofluorimeters or luminometers)[15,16,20] or electrochemical detectors,[21,22] but also to admit conventional-sized peripheral devices. Hence, LOV might be hyphenated to a plethora of modern detection techniques/analytical instruments, such as electrothermal atomic absorption spectrometry,[19,23−25] cold-vapor atomic absorption spectrometry,[26] electrospray ionization mass spectrometry,[27,28] atomic fluorescence spectrometry,[29−31] inductively coupled plasma mass spectrometry,[32] and most importantly for determination of organic contaminants, to separation systems, such as capillary electrophoresis[33−35] or high-performance liquid chromatography.[36] However, the coupling of LOV with GC has not been to the best of our knowledge yet reported.

This work aims at appraising the analytical performance of BI-LOV as a front end to large volume injection GC exploiting multisyringe flow pumping for execution as demanded in environmental assays of appropriate sample pretreatment, such as matrix isolation and analyte preconcentration prior to introducing the analytes optimally into the GC apparatus for separation. The considered case study herein has been the determination of polychlorinated biphenyls (PCBs) in complex environmental matrixes, that is, solid waste leachates. Landfill leachate is a major source of water and soil pollution with PCBs if not properly monitored.[37,38] Moreover, no method has been published to date dealing with online SPE−GC determination of PCBs in solid waste landfill leachates or environmental waters.

MATERIALS AND METHODS

Chemicals and Stock Solutions. A mix standard containing the PCB-28, PCB-52, PCB-101, PCB-118, PCB-153, PCB-138, and PCB-180 congeners at a concentration level of 10 mg L^{-1} each in isooctane was obtained from Absolute Standards (Hamdem, CT). A solution of 100 mg L^{-1} of PCB-166, employed as internal standard (IS), in isooctane was also from Absolute Standards. A standard solution of 1000 mg L^{-1} of Aroclor 1260 in isooctane was purchased from Supelco (Bellefone, PA). Stock standards were diluted to the desired concentration in acetone for sample spiking or in isooctane or ethyl acetate for direct injection into GC.

Reversed-phase bead material explored for BI-SPE analysis involved copolymeric Oasis HLB (30 μm, Waters, Mildford, MA), copolymeric Lichrolut EN (40−120 μm, Merck, Darmstadt, Germany), copolymeric Bond Elut Plexa (40−55 μm, Varian, Palo Alto, CA), and spherical octadecyl chemically modified silica Upti-

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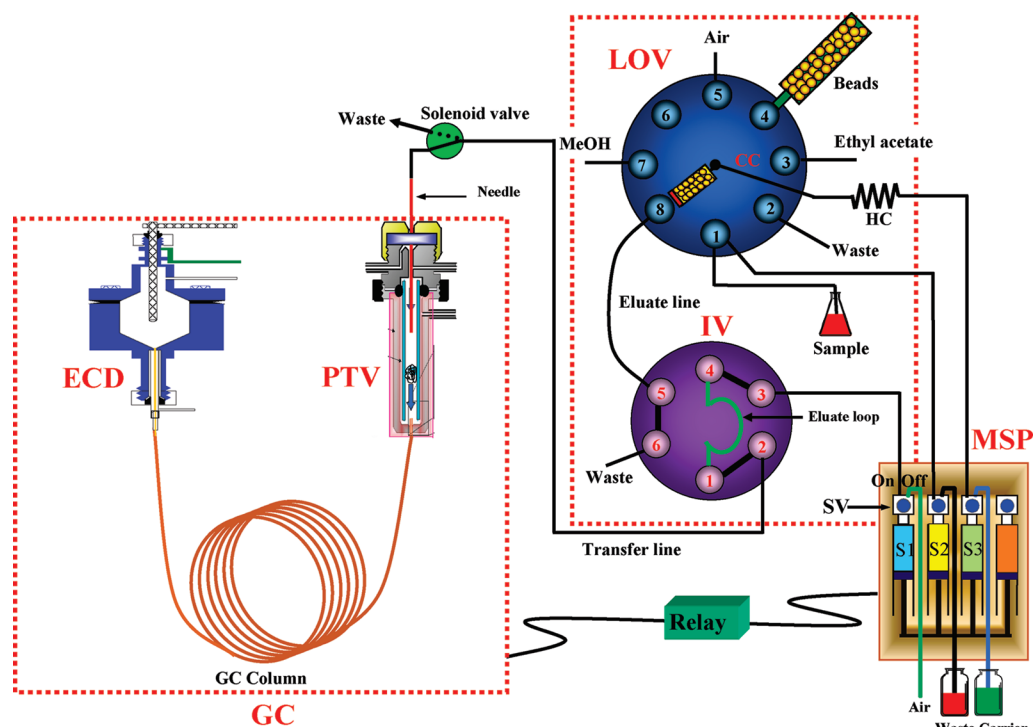
Figure 1. Schematic illustration of the multisyringe flow-based bead injection lab-on-valve setup hyphenated to large volume injection gas chromatography for preconcentration and determination of trace level concentrations of PCBs in solid waste leachates: LOV, lab-on-valve; IV, injection valve; MSP, multisyringe piston pump; HC, holding coil; PTV, programmed temperature vaporizer; SV, solenoid valve; GC, gas chromatograph; ECD, electron capture detector.

Clean C18 (50 μm, Interchim, Montluçon, France). Suspensions of 1:5 (w/v) of the reversed-phase beads were prepared in pure methanol.

Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q water generator (Millipore, Billerica, MA). Methanol for GC residue analysis (99.9%), acetone (99.8%), isooctane for GC residue analysis (99.9%), ethyl acetate (99.5%), and disodium ethylenediaminetetraacetic acid (EDTA) were all purchased from Scharlau (Barcelona, Spain). Toluene (99.5%) was supplied by Panreac (Barcelona, Spain).

Samples. Solid waste landfill leachate samples were collected in July 2008 in glass bottles and stored refrigerated. Two samples (nos. 1 and 3) were collected on different days from an urban waste landfill located in Santa Margalida (Mallorca, Illes Balears, Spain). Sample no. 2 was collected from a landfill of bulky and electronic equipment in Manacor (Mallorca). Before analysis, samples were filtered through a 0.45 μm cellulose filter and diluted to 50% (v/v) with methanol as an organic modifier to prevent adsorption of PCBs on the PTFE tubing of the flow manifold and glassware as well; otherwise PCBs could not be recovered quantitatively, and cross-contamination might occur. Sample no.

2 was also modified with 0.1% (w/v) disodium EDTA and 0.75% (v/v) concentrated ammonia to prevent precipitation of metal species.

Multisyringe Flow Injection Lab-on-Valve System. A multisyringe piston pump (MSP) with programmable speed (MicroBU 2030, Crison, Alella, Spain) equipped with three high-precision bidirectional syringes (S1, S2, and S3) (Hamilton, Switzerland) connected in block to a single stepper motor, was utilized as a multiple fluid driver. S1 and S3 with a capacity of 10.0 and 5.0 mL, respectively, contained air and the carrier solution (50% (v/v) methanol/water), respectively. S2 with a capacity of 5.0 mL served for rinsing the sampling line between consecutive samples or standards. A three-way solenoid valve (SV) (N-Research, Caldwell, NJ) was mounted atop of each syringe, enabling the communication with the liquid reservoirs or atmosphere in the OFF position, or with the flow manifold whenever activated to ON. The flow network was built from PTFE tubing of 0.8 mm i.d., excepting the 285 cm long holding coil (HC), which was made from 1.5 mm i.d. PTFE tubing. The multisyringe flow injection bead injection lab-on-valve (MSFI-BI-LOV) assembly hyphenated to PTV-GC is schematically illustrated in Figure 1.

The dedicated LOV microconduit (Sciware, Palma de Mallorca, Spain), fabricated from poly(ether imide) (Ultem) for chemical resistance to a wide range of organic solvents and encompassing eight integrated microchannels (1.2 mm i.d./14.0 mm length, excepting the bead reactor channel made of 1.7 mm i.d.), was mounted atop of an eight-port multiposition selection valve (Valco Instruments, Houston, TX). The central port of the integrated LOV sample processing unit, connected to S3, is made to address the

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peripheral ports of the unit (1–8), for sequential aspiration of the various constituents for the BI-based SPE process, via the central communication channel (CC) in the selection valve. One of the LOV channels (port 8) served as a microcolumn position for the renewable beads. To contain the sorbent within the cavity of the LOV microbore module and prevent them from escaping, the outlet of the column was furnished with a polyethylene frit of 10 μm (Mo Bi Tec, Göttingen, Germany). The suspension of reversed-phase beads was contained in a 1.0 mL plastic syringe, which was mounted vertically on port 4 of the integrated microsystem. The eluent and cleansing solvent reservoirs were attached to peripheral ports 3 and 7, respectively, whereas ports 2 and 5 were employed for sorbent disposal after each analytical assay and air aspiration for liquid segmentation, respectively. The specially designed dual channel (port 1) was utilized for sample introduction into the flow system, the outgoing channel being connected to S2, thereby permitting a thorough rinsing of the sampling tubing between samples to prevent cross-contamination effects. For quantitative injection of a metered eluate zone, a rotary injection valve (IV, Valco) furnished with a 150 μL eluate loop was connected at the outlet of the BI-SPE column. The LOV unit and the injection valve were connected via a 100 μL PTFE tubing. The 40 cm long transfer line to GC ends with a stainless steel capillary tubing of 6 cm × 127 μm i.d./794 μm o.d. (Supelco, Bellefonte, PA) permanently mounted in the PTV injector. A discrete solenoid valve was implemented within the transfer line for ease of rinsing of the transfer line between assays.

The operational procedures for the multisyringe flow-based LOV analyzer were fully computer-controlled by the software package AutoAnalysis 5.0 (Sciware) based on dynamic link libraries (DLLs). In our particular configuration, the principal protocol of the software was loaded with custom-built DLLs designed for the automatic control of the multisyringe pump and selection and injection valves. The multisyringe module is furnished with four digital outputs, each capable of providing 12 V/0.5 A. One of the digital outputs is connected to a relay, which is utilized for activation of GC via the Autoanalysis 5.0 software following injection of eluate into the PTV interface.

Gas Chromatography. Separation and detection of PCBs was performed with a CP-3800 gas chromatograph equipped with full electronic flow control (Varian, Walnut Creek, CA). The GC is composed of a 1079 PTV injector operating under solvent vent mode, a CP-Sil 8 capillary column (30 m × 0.25 mm × 0.25 μm, 95% methyl/5% phenyl polysiloxane, Varian), and an electron capture detector (ECD). Large volume injection was carried out into a glass wool packed gooseneck-type liner (54 mm × 3.4 mm

i.d. × 5 mm o.d.) using the following temperature program: The temperature was initially set to 70 °C (held for 1.5 min), then increased to 280 at 150 °C min^{-1} (held for 10 min), and finally returned to initial temperature. The split valve was kept open for 1.5 min with a split ratio of 60 (purge flow of 78 mL min^{-1}) for solvent elimination, whereupon it remained closed for 3.0 min to proceed with the transfer of analytes into the GC capillary column. The column temperature program involved a first step of 60 °C (held for 5 min), increased to 170 °C (held for 10 min) at 30 °C min^{-1}, and finally raised to 250 °C (held for 5 min) at 3 °C min^{-1}. He (99.999%) was employed as carrier gas. Just before transfer of the liquid eluate into the PTV injector, the He pressure was automatically reduced to 1.0 psi (6.9 kPa) using the pressure pulse option of the GC instrument, in order to ensure the quantitative and reproducible introduction of the eluate into the PTV injector via the multisyringe flow setup. Subsequently, the inlet pressure was automatically increased to ensure a constant flow of 1.3 mL min^{-1}. The ECD was kept at 300 °C with a makeup of 29 mL min^{-1} of N_2 (99.999%). Setting and control of GC parameters, data acquisition, and processing were conducted using the Galaxie software package (Varian). Both Autoanalysis 5.0 and Galaxie softwares were installed in the same PC, whereby the flow setup and GC could be controlled simultaneously by a single PC.

Analytical Procedure for Automated BI-LOV Preconcentration and Determination of PCBs. The operational details of the BI-LOV–GC method based on flow programming for the determination of trace level concentrations of PCB congeners using reversed-phase copolymeric beads (Bond Elut Plexa) as sorptive media are detailed in the Supporting Information (Table SI-1). A complete measuring cycle runs through a given number of steps, namely, bead packing and conditioning, sample loading, drying of sorbent by an air purge, analyte elution, transfer of eluate into the PTV injector, and finally bead disposal and system conditioning.

The basic features of the analytical procedure can be summarized as follows:

Step 1: Bead Packing into LOV and Conditioning. S3 is set to aspirate consecutively 300 μL of air, 300 μL of methanol, and finally 300 μL of ethyl acetate into HC. A metered portion of the copolymeric bead suspension is next aspirated slowly (viz., 0.5 mL min^{-1}) into HC. The central port is then connected to port 8, and S3 is programmed to propel the sequentially aspirated plugs into the microcolumn channel. During this step, the beads are transferred to the column and moistened consecutively with ethyl acetate, methanol, and carrier (methanol/water (1:1)) for sorbent preconditioning.

Step 2: Sample Loading and Bead Drying. To prevent dispersion of the sample segment into the carrier solution, the LOV central port is directed to aspirate 500 μL of air from port 5 followed by aspiration of 4.0 mL of sample through port 1, which volume is stored into HC, prior to be being pumped by flow reversal to the packed microcolumn for PCB uptake at 0.5 mL min^{-1}. This procedure is repeated threefold, whereby a total amount of 12 mL of sample is loaded. The air plug is finally delivered to the column for drying of the beads prior to initiate the elution step.

Step 3: Elution. In order to prevent the introduction of aqueous segments into the GC transfer line the eluent zone was followed

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by an air plug. On that account, an amount of 250 μL of air is first aspirated into HC, whereupon a minute, well-defined organic eluent volume (80 μL) is aspirated. The eluent zone is divided in two parts, each being halted into the column for 15 s for effective stripping out of PCBs. It should be stressed that both eluate and transfer lines were filled with air prior to elute the retained analytes.

Step 4: Transportation of Eluate. Once analyte elution is completed, the rotary injection valve is automatically activated to the load position and the eluate zone is transported to the valve loop followed by the air segment stored in the HC. The injection valve is next switched to the inject position, and the eluate is forthwith delivered to the GC by a gentle stream of air provided by S1. The GC is at this moment activated, and the temperature programs of the injector and column oven are initiated. The GC separation is actually synchronized with the BI-LOV sorptive procedure, implying that a sample is analyzed while the ensuing one is being processed in the flow system. Total sample preparation time in LOV and transportation of eluate into PTV takes ca. 45 min, thus closely matching the time frame for both the chromatographic run and column/injector re-equilibration to the initial conditions, which amounted to 50 min.

Step 5: Bead Discarding and System Conditioning. Since the polymeric beads are extremely easy to handle within the integrated microconduits, the packed column reactor is facilely backflushed after being moistened with methanol and delivered to waste (port 2) with carrier solution. Further, the transfer line to GC is cleansed with an air-segmented volume of ethyl acetate, which is dispensed to waste via the additional solenoid valve integrated into the manifold. Hence, the flow system is ready to initiate a new analysis cycle with a fresh portion of beads, thus eliminating any possibility of cross-contamination between consecutive runs.

RESULTS AND DISCUSSION

Configuration of the MSFI-LOV−GC System. A crucial aspect in online hyphenation of SPE to GC is to possess means for reliable delivery of eluate into the injector. Thus, a vast number of researchers have opted for exploitation of syringe pumps as liquid drivers for accurate handling of minute volumes of eluate.[6,8,10,39−41] As a consequence of the stringent demands of PTV-GC as to the maximum solvent volume to be accommodated into the injector in a single step to prevent column flooding (<100−200 μL), heart-cut injections have been proposed[8] despite the partial loss of the preconcentration capabilities gained during analyte sorption. Most common is the introduction of the entire volume of eluate into the GC.[6,8,10,39−41] Yet, the use of a solvent stream as carrier leads to increased dispersion of eluate in the transfer line with the consequent undue dispersion of eluted analytes. To overcome this drawback, we selected an air-sandwiched type elution as earlier described in BI-LOV systems coupled to discontinuous operating detectors[18,23,24] for accurate injection of discrete volumes of eluate. Yet, direct air segmentation into the transfer line was proven inappropriate as a consequence of the buildup of back-pressure by the capillary tubing tailored to the PTV injector which compressed the air segments and rendered irreproducible solvent transfer. Thus, an ancillary rotary valve furnished with an eluate loop was in this work implemented into the flow network to feed the injector with the eluted analytes via a pressurized air stream provided by S1 (see Figure 1).

Several PTV conditions, namely, purge (vent) time, purge temperature, and purge flow, were explored for appropriate performance of the injector. Among them, the former was proven to be the most relevant parameter. Early vent end times tended to yield irreproducible results due to incomplete solvent elimination and lack of instant inlet pressure equilibration from the (negative) pulse of 1 psi at the injection to the working pressure (~15 psi) required for maintaining a constant GC flow of 1.3 mL min^{-1}. This problem was solved by setting the vent time to 1.5 min while affixing the injector temperature and split ratio to 70 °C and 60, respectively. No losses of the most volatile PCBs were observed under the vent time selected for solvent evaporation.

Notwithstanding the fact that the use of sorbent-packed liners has been proposed for uptake of the eluate to prevent early introduction of analytes into the GC capillary column,[10] the liners were, however, in this work simply packed with glass wool. The latter is less retentive than sorbent materials, e.g., carbofrit, which avoids eventual carryover effects in the injector while assuring efficient collection of eluate. It should be stressed that a good discrimination between PCBs and solvent is accomplished in the venting step as a consequence of the low volatility of the target compounds.

Selection of BI-SPE Sorbent and Eluent for LOV Analysis. Four different reversed-phase sorbent materials, namely, Oasis HLB, Lichrolut EN, Bond Elut Plexa, and spherical Upti-Clean C18, were evaluated as regards their ability for retention and elution of the target analytes, as well as their ease for handling in LOV in a renewable mode. They offer different chemical functionalities, and to our best knowledge, merely copolymeric *N*-vinylpyrrolidone−divinylbenzene (Oasis HLB) beads have been successfully tested in a BI-LOV operation.[36,42] In fact, automated manipulation in aqueous media of hydrophobic and high-density sorbents (e.g., C18 covalently modified silica gel or PTFE) in LOV had been proven rather cumbersome to date because of their ease of settling down within the flow conduits.[18,43] The sorption/elution performance of the overall sorbents was initially tested in combination with three eluents of different polarity (viz., isooctane, toluene, and ethyl acetate) by retention of a given amount of the suite of PCBs followed by consecutive elution in three to four fractions of 80 μL each. Isooctane was proven not suitable for quantitative stripping out of any of the selected PCBs; even with a total solvent volume of 320 μL (4 × 80 μL), the amount of either of the analytes eluted was lower than 70% (data not given). On the other hand, ethyl acetate featured enhanced elution strength, with more than 80% of the PCBs being eluted in a single 80 μL

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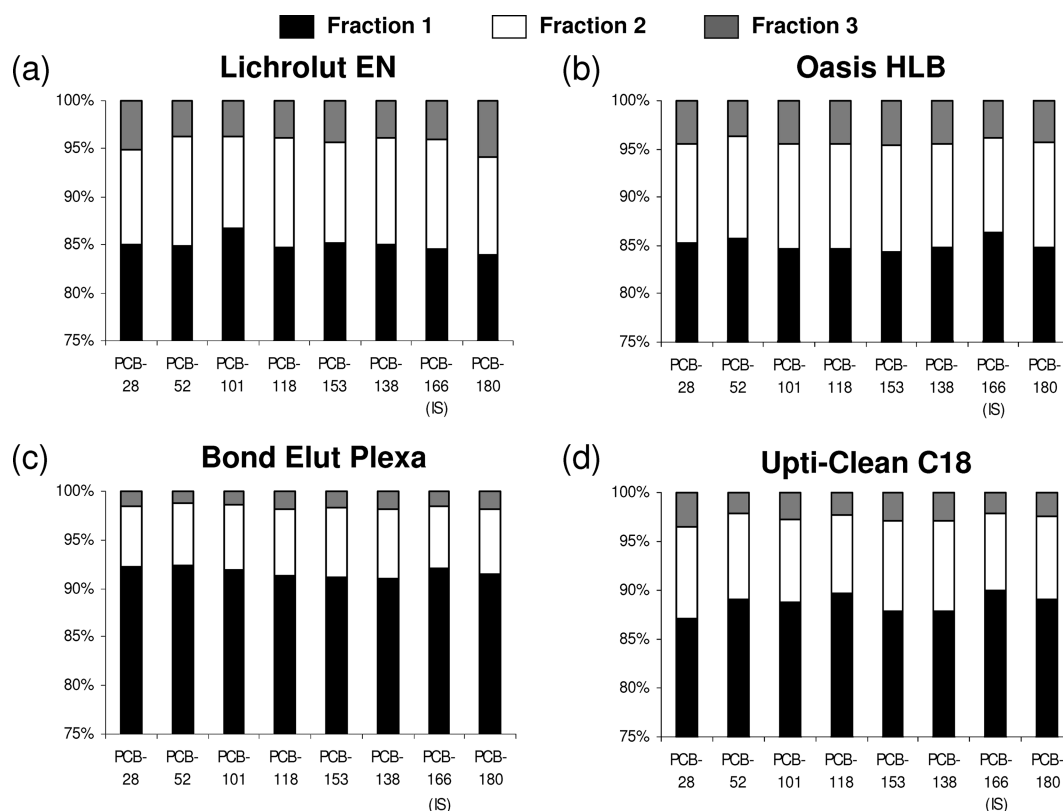
Figure 2. Normalized elution profile of different sorbents with three 80 μL fractions of ethyl acetate in BI-LOV ($n = 3$): (a) Lichrolut EN, (b) Oasis HLB, (c) Bond Elut Plexa, and (d) Upti-Clean C18. Beads were loaded with 1.0 mL of standard containing 50% (v/v) methanol spiked at the 5 μg L^{-1} level. N.B.: y-axis scale 75−100%.

fraction (Figure 2). This eluent was particularly advantageous in combination with the copolymeric Bond Elut Plexa sorbent (Figure 2c), where an elution efficiency >90% was achieved in the first fraction. The improved performance of ethyl acetate might be attributed to its partial solubility with remains of water left onto the beads due to incomplete air-drying of the sorbent under moderate/low pressure (see the Materials and Methods section). In fact, ethyl acetate has been recommended by Hyötyläinen and Riekkola in several reviews dealing with online SPE−GC coupling, because of the formation of a volatile azeotrope with water.[3,4] Toluene provided initially similar results as those attained with ethyl acetate, but its usage resulted into the deterioration (partial dissolution) of the Ultem material of the LOV after a few cycles and was therefore discarded.

As regards their physical manipulation in LOV as renewable sorptive surfaces, all the four sorbents could be handled within the microfabricated conduits of the integrated LOV platform in amounts varying from 2.5 (Oasis HLB) to 4 mg (spherical C18) per column in the conditions given under the Materials and Methods section. However, Lichrolut EN and Upti-Clean C18 sorbents were difficult to renew whenever sample volumes ≥10 mL were preconcentrated. This observation was attributed to the nonspherical shape of Lichrolut EN beads and the hydrophobic nature of the C18 material. On the other hand, the lipophilic/hydrophilic copolymeric Oasis HLB sorbent had already shown its good performance in LOV because of its good water wettability.[36,42] This holds true for Bond Elut Plexa beads, which consist of a hydrophobic polystyrene−divinylbenzene copolymer core, covered by a hydrophilic hydroxylated surface. In terms of
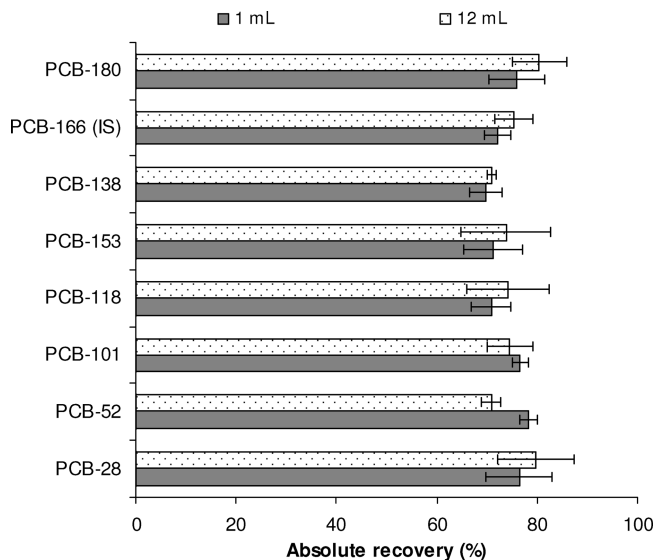

Figure 3. Comparative evaluation of absolute recoveries in BI-LOV as obtained by loading on Bond Elut Plexa either 1.0 or 12 mL of standard solutions spiked with 2 ng of each PCB congener (including the IS) containing 50% (v/v) methanol ($n = 3$).

PCBs retention capacity, all sorbents performed equally well, without appreciable analyte breakthrough when increasing the loading volume from 1 to 12 mL, in spite of the small amount of sorbent packed and the progressive loading of the SPE column with the methanol in the standards, as exemplary illustrated in Figure 3 for Bond Elut Plexa beads.

Table 1. Analytical Performance of the Online BI-LOV–GC Method for Determination of Trace Level Concentrations of PCBs

	linearity $(r^2)^a$	LOQ (ng L^{-1})^b	recovery (% RSD)–sample no. 1^c		recovery (% RSD)–sample no. 2^d	
			10 ng L^{-1}	50 ng L^{-1}	10 ng L^{-1}	50 ng L^{-1}
PCB-28	0.9993	4.1	107.4(12.8)	103.1(6.8)	91.3(9.3)	92.0(4.6)
PCB-52	0.9992	6.1	99.7(5.7)	87.9(4.4)	89.5(10.2)	94.7(5.0)
PCB-101	0.9999	2.0	99.1(5.2)	90.7(5.9)	81.4(9.1)	90.0(4.8)
PCB-118	0.9992	1.2	100.9(7.6)	90.3(5.4)	87.6(9.4)	97.0(4.3)
PCB-153	0.9992	1.4	103.1(4.2)	88.7(4.2)	86.4(2.0)	91.7(2.3)
PCB-138	0.9989	1.0	116.2(11.5)	100.2(8.8)	98.4(8.5)	102.2(7.1)
PCB-180	0.9977	0.5	112.5(7.8)	93.9(4.9)	90.7(7.9)	99.8(1.2)

^{a} Six-point calibration (2, 5, 10, 20, 50, and 100 ng L^{-1}). ^{b} Calculated as the signal for which the signal-to-noise ratio is 10 using a spiked landfill leachate sample at the 10 ng L^{-1} level. ^{c} Sample no. 1: landfill leachate ($n = 4$). ^{d} Sample no. 2: bulky and electronic waste disposal leachate ($n = 4$);0.1% (w/v) disodium EDTA and 0.75% (v/v) concentrated NH$_3$ were added to this sample in order to prevent precipitation of metal ions.
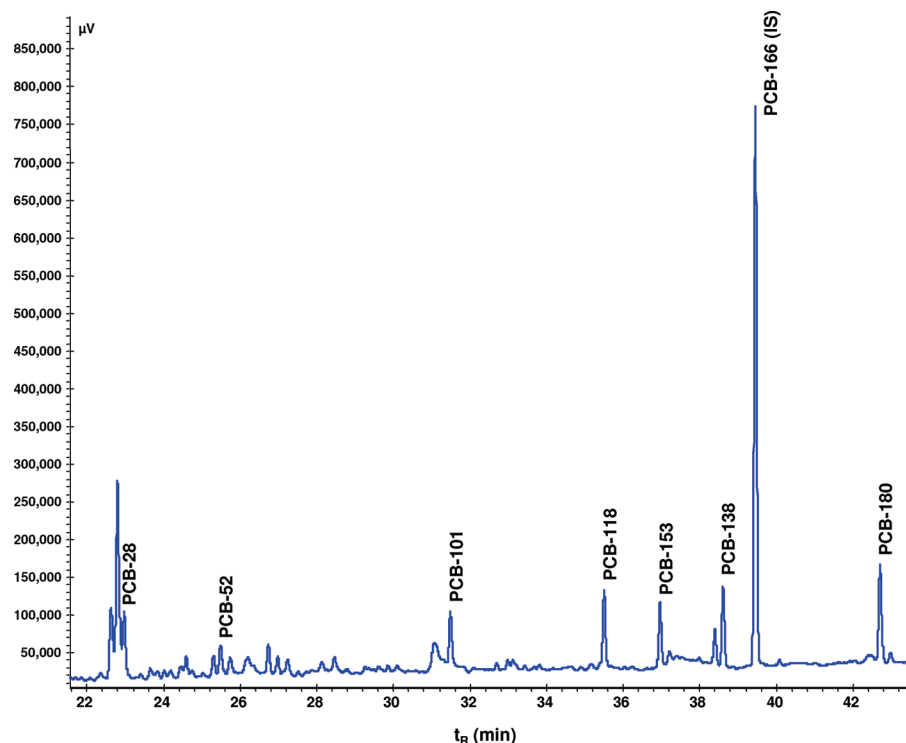
Figure 4. Chromatogram of a landfill leachate sample (sample no. 1) spiked at the 10 ng L^{-1} level of each PCB congener and 50 ng L^{-1} of IS (PCB-166).

The combination of Bond Elut Plexa beads and elution with a single plug of 80 μL of ethyl acetate was selected as a compromised approach for sensitive and expeditious BI-LOV–GC analysis. Absolute recoveries, as compared to manually injected standards, were >70% (Figure 3), and fast mass transfer of PCBs from hydroalcoholic media is warranted as a consequence of the hydrophilic surface of the beads. Analyte recoveries could be improved by increasing the elution volume, yet this would entail further optimization of the PTV parameters and more complex operational protocols. It should be borne in mind that nonquantitative recovery of analytes in BI-LOV–GC procedures should not be in detrimental of the method's trueness and precision, because of the high reproducibility in fluid handling in automated flow systems along with the fact that a surrogate IS is added to both samples and standard solutions, calibration is performed under identical experimental conditions than sample analysis, and

the entire SPE column is withdrawn and replaced by fresh beads after each single assay to eliminate potential analyte carryover sources.

Analytical Performance of the BI-LOV–GC System. The analytical performance of the proposed procedure using Bond Elut Plexa beads in a renewable fashion is summarized in Table 1 including linearity, quantification limits (LOQ), and recoveries at the 10 and 50 ng L^{-1} levels. The online BI-LOV–GC method features an excellent linearity, with correlation coefficients >0.9977 for the entire suite of PCB congeners, taking into account that the SPE column is merely utilized in a single analytical run. The LOQ values, calculated at a peak-to-peak signal-to-noise ratio (S/N) of 10 for a spiked landfill leachate sample at the 10 ng L^{-1} level (Figure 4), ranged from 0.5 to 6.1 ng L^{-1}, thereby sufficing for determination of PCBs in leachates at environmentally relevant concentrations. In fact, the enrichment factors under the optimized experimental conditions were

Table 2. Comparison of Expected and Found Concentrations of PCB Congeners in a Landfill Leachate Spiked with Aroclor 1260 at the 500 ng L^{-1} Level

	expected concn (ng L^{-1})^a		found concn (ng L^{-1})^b		Student t test	
	mean	SD	mean	SD	calcd t	statistically different?^{c}
PCB-28	2.1	0.2	<LOQ			
PCB-52	2.39	0.09	<LOQ			
PCB-101	24.0	1.5	22.6	0.9	1.37	no
PCB-118	3.7	0.6	3.2	0.5	1.21	no
PCB-153	75.5	3.4	76.7	1.7	0.55	no
PCB-138	57.0	3.3	59.8	1.6	1.27	no
PCB-180	59.6	2.9	62.2	3.5	0.97	no

^{a} Calculated from the concentration of each PCB in the Aroclor spike as determined by GC ($n = 3$). ^{b} $n = 3$. ^{c} 95% confidence level; $t_{critical} = 2.78$.

>52. Further, the method's selectivity and LOQs could be readily improved by coupling the miniaturized BI-LOV system to gas chromatography/high-resolution mass spectrometry (GC/HRMS) whenever needed.

Trueness and precision of the method were studied using two real-life samples, namely, leachates from an urban solid waste landfill (sample no. 1) and an electronic equipment disposal site (sample no. 2), which were analyzed and proven not to contain detectable amounts of PCBs. Both samples were spiked at the 10 and 50 ng L^{-1} levels, and the analytical recoveries and relative standard deviation were thus calculated. As compiled in Table 1, relative recoveries varied from 81% to 116% for both samples, and relative standard deviations were found to range from 2.0% to 13% and 1.2% to 8.8%, for 10 and 50 ng L^{-1} level spikes, respectively. It should be noted that the proposed analytical method features under the selected experimental conditions

better accuracy and reproducibility than those recommended by the EPA method 1668a for PCBs determination, where the quality control criterion establishes that the precision and recovery in standard solutions should be better than 40% RSD and within the range from 60% to 140%, respectively.[44]

Because none of the analyzed samples contained PCBs, another landfill leachate (sample no. 3) was spiked with Aroclor 1260, one of the most common PCB mixture still in use in some old electrical transformers and capacitors, in order to analyze a more environmentally realistic sample in terms of matrix complexity and congener's profile and, hence, evaluate the reliability and ruggedness of the hyphenated LOV−GC system. The Aroclor mixture was first characterized by direct injection in the GC (see first two columns in Table 2) and then spiked into the leachate sample. The spike level was set to 500 ng L^{-1}, as total Aroclor, because this is the maximum concentration level permitted by the U.S. EPA National Primary Drinking Water Standards.[45] It should be here highlighted the fact that the landfill and dump leachate contamination of water sources might pose serious risks to public health. A chromatogram of the spiked landfill leachate is presented in Figure 5, and experimental results are compiled in Table 2. A t test of comparison of means[46] confirmed the inexistence of significant differences between the expected and found concentrations for the overall analytes at the 0.05 significance level.

The comparison of the BI-LOV−GC approach with previous online SPE−GC methods reported in the literature reveals that, on one hand, the recoveries and RSD values obtained in this work are similar or better than those of commercial robotic Prospekt-type instruments or in-house systems, where the sorbent is usually reused and synthetic aqueous matrixes, most often ultrapure water, in lieu of real-life samples are employed for method validation.[6−8,10] On the other hand, the expenses in BI-LOV in
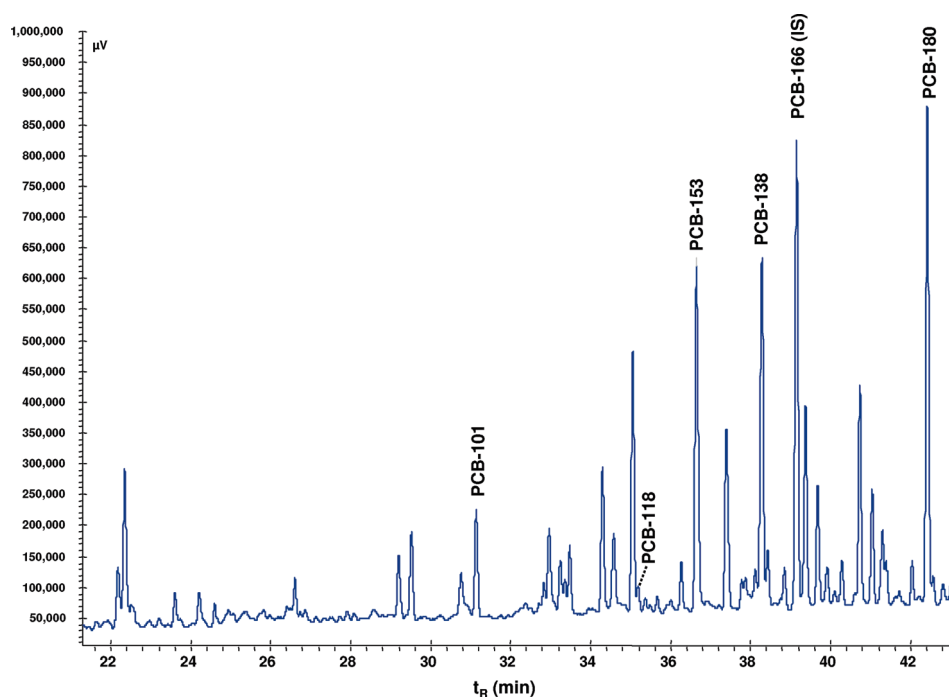

Figure 5. Chromatogram of a solid waste leachate sample (sample no. 3) spiked with 500 ng L^{-1} of Aroclor 1260 and 50 ng L^{-1} of IS (PCB-166).

Table 3. Comparative Evaluation of Sorbent Costs for Analytical Methods Involving SPE

extraction fashion	typical format	approximate cost per 100 analyses (euro)
off-line	syringe barrel/disk (C18 or polymeric)	150–400[a]
online Spark Holland systems	Prospekt or Prospekt-2 cartridges	300–500[a,b]
online reusable precolumn	SPE precolumn (C18 or polymeric; 2.1–4.6 mm × 20 mm)	150–400[a,c]
online BI-LOV–GC (this work)	LOV microcolumn (3 mg Plexa)	~6

[a] Estimated range depending on sorbent material employed (C18, polymeric, etc.). [b] Cartridge is renewed after every sample/standard analysis. [c] Assuming the reutilization of a single precolumn for 100 analyses.

terms of sorbent material are 25-fold lower than those of off-line and online SPE counterparts (see Table 3). In fact, some researchers using robotized systems decided to reuse cartridges to reduce costs,[6] in spite of risks of sample cross-contamination.

Finally, it is also worth mentioning that the GC equipment operated for more than 200 standards and samples analyses without column performance deterioration. This can be partly attributed to the restricted access material (RAM)-like nature of Bond Elut Plexa beads that prevents nonvolatile macromolecules from being retained and further eluted and transferred to the GC injection port and column head.

CONCLUSIONS

Online hyphenation of BI-LOV with GC has been in this paper for the first time demonstrated. This new approach fostered the implementation of SPE and determination of PCBs from landfill leachate samples in a fully automated fashion with the advantages over traditional online SPE–GC methods of simplicity, versatility, and inexpensiveness as compared to robotic systems (e.g., Prospekt, Prospekt-2) and negligible cross-contaminations as a consequence of the renewal of the sorbent material in each analysis cycle. The automated BI-LOV–GC method is highly sensitive (LOQs between 0.5 and 6.1 ng L^{-1} for 6 mL sample (12 mL after 1:1 dilution with MeOH)), accurate (recovery percentages >81%), and reproducible with RSD values lower than 13% for real leachate samples. Indeed, this first work opens new horizons for hyphenation of LOV microfluidic analysis with GC, including the potential interface of BI-LOV with GC/(HR)MS for water and beverages analysis or the coupling of flow-through dynamic fractionation schemes[25,47] to GC via the LOV platform for availability studies of organic pollutants in environmental solid substrates.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on May 13, 2009 with an error in Table 2. The corrected version was published ASAP on May 22, 2009.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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