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Towards Integrated Detection and Graphene-based Removal of Contaminants in Lab-on-a-chip Platform

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ABSTRACT

A novel, miniaturized microfluidic platform for simultaneous detection and removal of polybrominated diphenyl ethers (PBDEs) was developed. The platform consists of a polydimethylsiloxane (PDMS) microfluidic chip for immunoreaction step, a PDMS chip with an integrated screen-printed electrode (SPCE) for detection and a PDMS-rGO (reduced graphene oxide) chip for physical adsorption (removal) of PBDEs residues. The detection was based on competitive immunoassay between PBDE and PBDE modified with horseradish peroxidase (PBDE-HRP) followed by monitoring of enzymatic oxidation of o-aminophenol (o-AP) using Square Wave Anodic Stripping Voltammetry (SW-ASV). PBDE was detected with good sensitivity and a limit of detection similar to commercial colorimetric test (0,018 ppb) but with the advantage of using lower reagents volumes and a reduced analysis time. The use of microfluidic chips also brings the improvement of the linearity and reproducibility of the method in comparison to batch-based measurements using screen-printed electrodes. In order to design detection system suitable for toxic compounds, such as PBDEs, reduced graphene oxide – PDMS composite was developed and optimized and its increased adsorption (based on both the hydrophobicity and the π-π stacking between rGO and PBDE molecules) compared to non-modified PDMS was proved. To the best of our knowledge this is the first approach of electrochemical detection of flame retardants as well as a novel application of rGO-PDMS composite in a biosensing system. Moreover, this system can be easily applied to detect any analyte using appropriate immunoassay and support the operation in such a complex matrix as seawater.

1. Introduction

Lab-on-a-chip (LOC) platforms are under intensive development in last decade due to their versatility, ease of fabrication and multitude of applications in biosensing and analysis. So far, LOCs devices were reported for detection of protein [1], nucleic acids [2, 3], drugs [4, 5], hormones [6, 7] and many different organic compounds. Thus, LOCs are very promising for health diagnostics, safety and security as well as in environmental screening.

Various methodologies of LOCs fabrication have been lately developed involving materials such as paper [8], glass [9, 10], polymers [11, 12] or even proteins (e.g. gelatine [13, 14]). The use of a certain material depends on the desired application, physicochemical properties of analyte that needs to be detected, the need of biocompatibility and possible integration with other devices/materials [11].

One of the most common approaches in LOC development is polydimethylsiloxane-based (PDMS) technology (soft-lithography) where polymeric elastomer is used to fabricate chips of different shapes, patterns and properties [15].

The development of low-volume (in a range of micro- and nano-liters) and portable devices is especially interesting regarding environmental screening of potentially hazardous compounds. Various pollutants present in air, soil or water require regular monitoring and the use of LOCs can bring efficient in-field detection where an access to sophisticated laboratories is limited or linked to high cost and time consumed.

Among others, LOCs are reported for electrochemical detection of phenolic compounds [16, 17] and atrazine [18], optical platforms for pesticide [19] and heavy metals detection [20]. LOCs can be applied not only for analysis of pollutants derived from industry and urban areas but also for toxins with origin from natural environment such as toxins produced by marine microalgae [21-23].

One of the hazardous chemicals requiring the progress in its detection are polybrominated diphenyl ethers (PBDEs), a class of halogenated compounds, similar to polychlorinated diphenyls (PCBs) and commonly used as flame retardants. PBDEs have been introduced in market since the 60s. and used for many years in domestic electronics, toys, furniture, polyurethane foams and building materials [24, 25]. But over the time, the negative effects of long-term exposure to PBDEs have been discovered, nevertheless many of them are still not well known. In spite of all, commercial mixtures of PBDEs were banned from all applications in 2004 (penta-BDE and octa-BDE) or 2013 (deca-BDE) in the United Stated, European Union and elsewhere [26, 27].

PBDEs due to their hydrophobicity can accumulate in various tissues and organs, especially in adipose tissues what might be related with possible liver dysfunctions [28]. Moreover, PBDEs can be found in breast milk and many research showed the dependence between persisting contact with materials containing PBDE (due to dermal absorption, consumption of contaminated food or inhalation) and their further accumulation in the body. Beside small size and hydrophobicity, the chemical structure of PBDEs, containing two aromatic rings, is pretty similar to thyroid hormones such as 3,5-diiodothyronine (T2), 3,3,5-triiodothyronine and 3,3,5,5-tetraiodothyronine (T4) thus, the ability of PBDE to bind with alpha- and beta-thyroid hormone receptors was found. Apart of that, PBDEs are linked to neurodevelopment toxicity and various types of tumours [28, 29].

Despite the need of monitoring the PBDE level, so far they are only a few methods used for their detection. Standard determination of PBDE is based on gas chromatography (GC) that can be combined with electrode capture detection (GC-ECD) or mass spectrometry (GC-MS). The use of these methods is still limited by very expensive equipment, need for qualified workforce and time consumption [30-32]. One of the alternatives is a competitive immunoassay for PBDE detection that bases on competition between PBDE molecules and PBDE-HRP in binding to anti-PBDE antibodies that are coupled to magnetic beads (MB-Ab). Signal is revealed using well known enzymatic reaction that catalyses 3,3',5,5'-Tetramethylbenzidine (TMB) oxidation, leading to a coloured product that can be easily measured using a spectrophotometer. This method is relatively simple and offer attractive range of detection (0,025 – 1,0 ppb of PBDE) both in standard solutions and environmental samples like soil or seawater.

Taking advantage of well-developed antibodies and competitive reaction, we modified the colorimetric kit into electrochemical by performing electrochemical detection of HRP-PBDE and replacing TMB with o-aminophenol (o-AP) as a substrate for electrochemical reaction. We believe that electrochemical reaction brings an opportunity to perform the assay with better sensitivity and moreover may lead to improved automation, especially when it's integrated in LOCs device. Presented LOC system and detection approach is shown in Fig. 1.

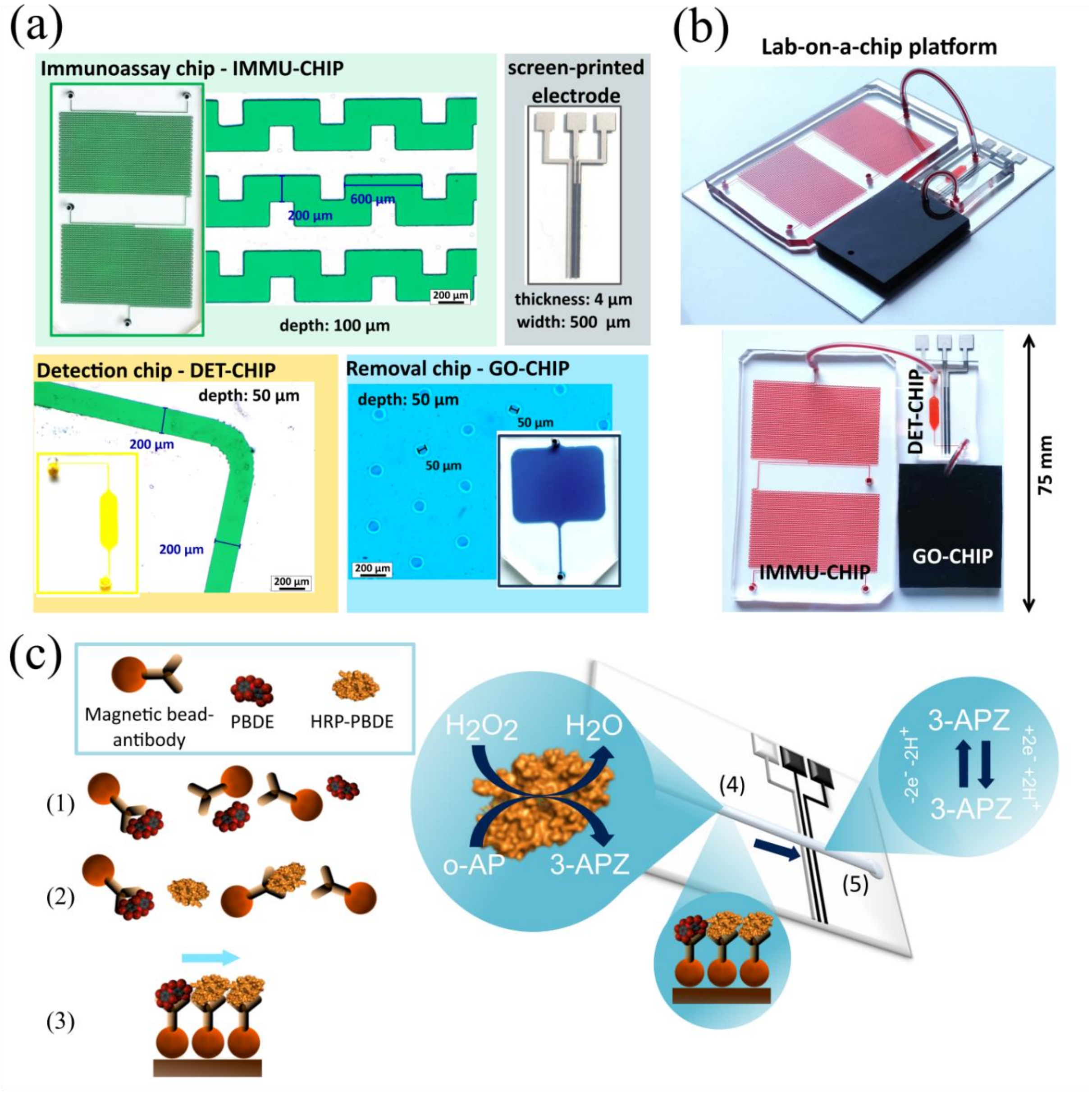


Figure 1 Lab-on-a-chip system for detection and removal of PBDEs (a) components of the LOC device with visualisation of the microfluidic structures (10x magnification objective); (b) side and top view of assembled LOC device on polycarbonate substrate with screen-printed electrode (SPCE); (c) assay procedure: (1) on-chip mixing and incubation of magnetic beads coupled to anti-PBDE antibodies (MB-Ab) with samples containing PBDE, (2) on-chip mixing and incubation of MB-Ab with HRP-PBDE, (3) immobilization of immunocomplex in chip using external magnet and washing of unbound molecules, (4) incubation with substrate solution (o-AP + H2O2), (5) electrochemical detection of the product of enzymatic reaction (3-APZ, 3-aminophenoxazone).

The role of nanomaterials in LOCs and other devices is increasing significantly in recent years for both sensing and removal of contaminants. In this context, we take advantage of adsorption/removal capabilities of reduced graphene oxide (rGO) toward certain contaminants. As a proof of concept, a composite of PDMS and rGO (PDMS-rGO) was developed and used to fabricate a microfluidic chip where samples after measurements are injected and any organic traces, such as an excess of PBDE are immobilized/adsorbed onto the channels walls thus, improving the whole safety of the system. Such kind of non-covalent interactions between PBDEs and carbon materials (based on π-π stacking) were already used for novel extraction methods [33, 34] as well as micromotor-based removal of PBDEs [35]. Furthermore, various composites of PDMS with carbon materials were already developed but most of them based on sophisticated protocols and are optimized in order to obtain conductive materials rather than materials of highly adsorptive surface [36, 37].

To the best of our knowledge this is the first approach showing the LOC device with an electrochemical approach for PBDE detection. Likewise a novel rGO-PDMS composite is used for the first time in LOCs device for biosensing/removal purposes.

2. Experimental

2.1 Reagents

Polybrominated Diphenyl Ethers (PBDE) colorimetric immunoassay was purchased from Abraxis (Pennsylvania, USA). PBDE stock solution was purchased from AccuStandard Inc. The screen-printed inks (carbon sensor paste C2030519P4 and silver-silver chloride paste C2130809D5) were purchased from Gwent Group, United Kingdom. Polycarbonate sheets were purchased from Vink Plastics S.L.U., Spain. PDMS (Sylgard® 184 Elastomer kit) was purchased from Ellsworth Adhesives Iberica, Spain. Graphene Oxide stock solution (5 mg/mL) was purchased from Angstron Materials (Dayton, USA). Negative photoresist (SU8-50) and appropriate developer was purchased from Microchem, USA). Flexible photolithography masks were provided by Microlitho, UK. 4 inch silicon wafers were purchased from NOVA Electronic Materials. Hydrogen peroxide, o-aminophenol, HPLC grade methanol, phosphate buffered saline (PBS), 3-aminopropyltriethoxylane (APTES), L-ascorbic acid and microscopic glass slides were purchased from Sigma. 0.04 M, pH 5,7 Britton-Robinson (BR) buffer was prepared as described elsewhere [39]. All commercial reagents were of analytical grade and handled according to the material safety data sheets suggested by the suppliers.

2.2 Instruments

Screen-printed carbon electrodes (SPCE) were fabricated by screen-printing technology using a screen-printer (DEK 248, UK). Electrochemical measurements were carried out using computer-controlled Autolab PGSTAT-12 (302 N-High performance) (potentiostat/galvanostat) with general-purposes electrochemical software operating system (GPES version 4.9.007, from Eco Chemie B.V., Utrecht, The Netherlands). Microfluidic chips with integrated SPCE electrode were connected to the Autolab PGSTAT-12 with a specially adapted electrical edge connector. Syringe pump (Pump 11 Elite; Harvard Apparatus, USA) was used as a driving unit for microfluidic system. Syringe pump was equipped with 0,5 mL glass syringes (Gastight®, Hamilton, USA). Centrifuge SIGMA 2-16PK was used for rGO purification and magnetic stirrer (IKA lab disc white) during reduction process. Plasma cleaner PDC-002 was used as a source of oxygen plasma for chips fabrication. Optimization of microfluidic chips operation was made using optical microscope (Olympus IX71, Germany) coupled with a CCD camera (Olympus DP72, Germany) with the bright field mode, 10 x magnifications.

2.3 Experimental methodology

Unless otherwise stated, all optimization experiments regarding microfluidic chips were carried on 5 independent chips. Optimization of electrochemical measurements based on the use of 5 independent screen-printed electrodes. Final calibration curves were obtained based on 3 repetitions. When colorimetric method was used, each sample was run in duplicate according to manufacturer´s procedure. All errors of the results obtained were expressed as a relative standard deviation (RSD).

2.4 Fabrication and operation of Lab-on-a-Chip (LOC) devices

Fabrication of screen-printed electrodes based on already reported design and procedures, using polycarbonate sheets as a substrate [43]. Microfluidic mold was prepared using previously reported methodology of photolithography process [15, 43] where a 4-inch silicon wafer was spin coated with a negative photoresist SU8-50 and patterned by photolithography using a flexible mask.

PDMS was mixed with its curing agent in the mass ratio 10:1. After intensive stirring for 5 minutes, an excess of air bubbles was removed using vacuum. Afterwards, PDMS was poured onto silicon wafer with a given design of microfluidic chip and kept on the hotplate, 70ºC, for 4-5 hours until completely polymerized. After polymerization, proper shapes of PDMS were cut out from the template, washed in miliQ water, followed by isopropanol and dried on the hotplate 70ºC for 10 minutes. In order to modify the surface, oxygen plasma treatment was performed. Meanwhile, polycarbonate sheets (either with or without screen-printed electrodes) where washed with miliQ water, followed by isopropanol, ethanol, plasma treatment and incubated in 2% APTES solution in order to silanize the surface (1h). Afterwards, both parts (PDMS and polycarbonate) are simply attached to each other forming a permanent bonding that is strengthen by incubation on the hotplate 70ºC for 1 hour. Prior to assay, chips were connected with each other using polymeric tubes.

General scheme of the system as well as dimensions of microfluidic channels are shown in Fig. 1. The system consists of 3 microfluidic chips: IMMU-CHIP, DET-CHIP and GO-CHIP.

IMMU-CHIP consists of channels with repeatable curvatures and 3 inlets (MB-Ab, PBDE, PBDE-HRP). The role of curvatures is to support the diffusion between mixed reagents. In this chip an immunoreaction between PBDE/PBDE-HRP and MB-Ab occurs. The PDMS layer is attached either to glass or polycarbonate.

DET-CHIP consists of single channel and the chamber (10 mm length, 3 mm width and approximate volume of 15 µL) where immunocomplex (MB-Ab-PBDE/PBDE-HRP) is immobilized as underneath the chip an external magnet is placed. This chip is bonded to polycarbonate with SPCE. The DET-CHIP can be employed as a part of LOCs system as well as separate chip just for various electrochemical measurements.

GO-CHIP is fabricated using rGO-PDMS composite. The design used to fabricate this chip is based on a broad channel with pillars along its surface so as to increase surface-area-to-volume ratio and improve the adsorption performance through the microfluidic channel. Fabrication process is based on patterning of SU-8 photoresist.

2.5 Optical evaluation of on-chip immunoreaction performance

The motivation of using microfluidic chip for mixing (IMMU-CHIP) is the possibility of the process automation as samples just by passing through the channel will be mixed with each other supporting (1) PBDE binding to MB-Ab as well as (2) PBDE-HRP binding. The design of IMMU-CHIP was previously used and its effectiveness (not reported here) in biochemical reactions was confirmed.

Using optical microscope, the process of mixing was under observation, so as to match the best parameters and make sure that all chemicals used in the assay are compatible with materials used for LOC device. As under microscope all the reagents seem to be transparent, Trypan blue (10%) was used to facilitate the distinction of two different solutions mixed with each other. Besides mixing process, the washing step was assessed in order to find an optimum flow rate and buffer volume (Fig. S1 in the ESM).

2.6 Optimization of on-chip magneto-immunoassay

IMMU-CHIP was fabricated as in (2.4) and washed by priming 1 mL of washing buffer (water + 0,05% Tween-20) prior to use. A dedicate inlet was connected with the syringe containing reagents of magneto-immunoassay for PBDE detection.

Two types of optimization experiments were performed. The first one checked the occurrence of non-specific adsorption of PBDE to PDMS and how it is changing with respect to the flow rate. A solution of 1.0 ppb PBDE was flushed through the whole chip and collected in the Eppendorf tube at the outlet. Afterwards, colorimetric test was performed following the standard procedure (“Colorimetric procedure…” in the ESM). Results were expressed as a % of signal related to the control sample of 1.0 ppb of PBDE.

The second experiment checked the possible adsorption of magnetic beads and HRP-PBDE onto the microchannel. First inlet was connected to PBDE blank solution (0,0 ppb), second to MB-Ab and the third one to HRP-PBDE. Solutions were injected and mixed in chip with each other using different flow rates, collected in Eppendorf tube and preceded as in standard procedure for colorimetric detection of PBDE. Results were expressed as a % of signal obtained by control sample containing MB-Ab, HRP-PBDE and 0,0 ppb of PBDE.

2.7 Preparation of rGO-PDMS composite

Already reported approach of GO reduction via L-Ascorbic Acid (L-AA) was employed [44]. L-AA was added to 200 mL GO solution to reach the concentration of 50 mg/mL. Samples were adjusted to desired pH using NaOH solution and incubated for 48 hours with continuous magnetic stirring (600 rpm). After reduction, solution was transferred to 15 mL centrifugal tubes and centrifuged for 1 hour, 12000rpm, 4ºC. Supernatant was discarded, while pellet washed with miliQ water. This step was repeated twice using miliQ and twice using 96% (v/v) ethanol. Purified solution of rGO was used for further experiments promptly.

Two components of PDMS kit (elastomer and curing agent) were mixed in volume ratio 10:2. After stirring, a desired amount of rGO was added and vigorous stirring was continued for about 10 minutes. Afterwards, rGO-PDMS was poured onto silicon wafer with the design of a given chip. Wafer with rGO-PDMS was kept for 1h in RT in order to remove air bubbles entrapped within polymer before its polymerization. Then, wafer was kept on the hotplate and incubated for 4-5 hours, 70ºC until rGO-PDMS is completely polymerized. Microfluidic chips were fabricated using exactly the same procedure as mentioned above.

Analysis of rGO-PDMS composite (the effect of pH, flow rate, rGO concentration) was performed regarding the ability of adsorption of standard solution of PBDE (1.0 ppb). All parameters had been chosen in such way to maximize the adsorption yield. Experiments based on passing PBDE sample through rGO-PDMS chip (single channel of 5 cm length, 500 µm width and 100 µm depth) and afterwards determining the concentration of PBDE using colorimetric kit. As this kit is a competitive assay, the relative adsorption was counted using the following formula (Eq. 1):

$$ADS=A1(1.0ppb)-A0(1.0ppb)/B0(0,0ppb) \qquad (1)$$

where A1(1.0ppb) is the absorbance of sample used in chip, with initial concentration of 1.0ppb; A0(1.0ppb) is the absorbance of control sample with initial concentration of 1.0ppb and B0(0,0ppb) is the absorbance of control sample where 0.0 ppb solution was used as a blank.

2.8 Electrochemical detection

Electrochemical detection of HRP is inspired by already reported works [39, 45]. According to competitive immunoassay, the level of HRP is inversely proportional to the level of PBDE. HRP catalyses the redox reaction where hydrogen peroxide is converted to water molecules, while a given substrate is oxidized. In this approach (Fig. 1c) o-AP was chosen as a substrate of enzymatic reaction due to compatibility with other reagents used in this assay as well as electrochemical properties. Oxidation of o-AP leads to formation of 3-aminophenoxazone (3-APZ) that can be reduced onto SPCE surface and detected in several electrochemical techniques such as Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV), Chronoamperometry (AMP) and Square Wave Anodic Stripping Voltammetry (SW-ASV). As the PBDE detection kit based on HRP-PBDE molecule is optimized for a relatively narrow range of PBDE concentration, SW-ASV was chosen as this technique is reported as much more sensitive than others. Nevertheless, some examples of PBDE detection using CV and DPV are shown in Figure S2 in the ESM. The following parameters were applied: deposition potential (-0,6 V); deposition time 20 s; equilibration time 15 s; potential range from 0,0 to -0,6 V; step potential 4 mV; amplitude 25 mV; frequency 25 Hz.

Detection in microfluidic chip was preceded by measurements based on bare SPCE so as to find the optimal detection protocol. Briefly, immunoassay was performed as usual, after washing an immunocomplex, samples were reconstituted in 10 µL of PBS buffer and 100 µL of substrate solution (o-AP + H2O2 in BR buffer) and incubated for 30 min. After incubation, 50 µL of sample was pipetted onto SPCE surface and electrochemical measurements were performed. Following this procedure, incubation time, o-AP concentration, H2O2 concentration and deposition time in SW-ASV were optimized.

Afterwards, electrochemical detection was performed using DET-CHIP. Parameters of detection were chosen based on optimization in static measurements. In this case, when substrate solution is injected into the microfluidic channel it passes through the chamber where magnetic beads with immunocomplex are immobilized, so the enzymatic reaction is performed in flow and product is passing further along channel up to the electrode surface where it is electrochemically deposited and detected. Besides that, an optimum flow rate was analysed in order to find the best sensitivity and limit of detection.

3. Results and discussion

3.1 rGO-PDMS composite

Graphene oxide and other derivative carbon materials are already reported as effective adsorbents of different types of molecules. In this work we tried to combine the ability of rGO to adsorb PBDE with convenient properties of PDMS chips by obtaining a LOC device with increased adsorption performance (Fig. 2). There are already reported similar approaches where graphene materials and PDMS are used as a composite but they are devoted to its electrical properties and none of them were applied for adsorption/removal applications in LOCs [36, 38]. Moreover, as presented rGO-PDMS composite does not need to be conductive, a much simpler protocol of fabrication could be applied, where the effect of rGO adsorption is still maintained/visible and attractive for Lab-on-a-Chip applications.

Furthermore as the adsorption mechanism is based on π-π stacking, rGO-PDMS composite can be employed for various biomolecules such as DNA or proteins as both of them are rich in structures containing aromatic rings. As a proof of concept we performed analogical test like for PBDE but measuring the adsorption of model protein (HRP) and the effect of rGO adsorption can be clearly observed (Fig. S3 in the ESM).

We found pH as an important factor facilitating rGO reduction, thus the efficiency of PDMS adsorption. Experiments based on PBDE adsorption onto rGO-PDMS channel (Fig. 2e) showed that an increase of pH lead to an increase of adsorption, however this effect is well visible until the pH value reach 7. When it is higher there is no significant improvement anymore.

It is not surprising that an increase of rGO content (concentration) in PDMS will make adsorption stronger as more rGO will be exposed into the channel surface. However on the other hand any compound added to PDMS may affect its ability to polymerize. We found (Fig. 2d) that when rGO concentration is lower than 1% (w/w) there is no effect on adsorption improvement, leading even to its slightly decrease (comparing to bare PDMS). Concentration about 4,5% (w/w) seems to be optimal as brings the highest adsorption of PBDE (around 70%). When the concentration is higher than 4,5% but lower than 10%, the adsorption capacity significantly decrease, however the composite keeps its physical properties, such as durability, hardness etc. If the concentration of rGO is higher than 10% (v/v), polymerization is hardly affected by the presence of many air bubbles (that always appear during the polymerization of PDMS but are released

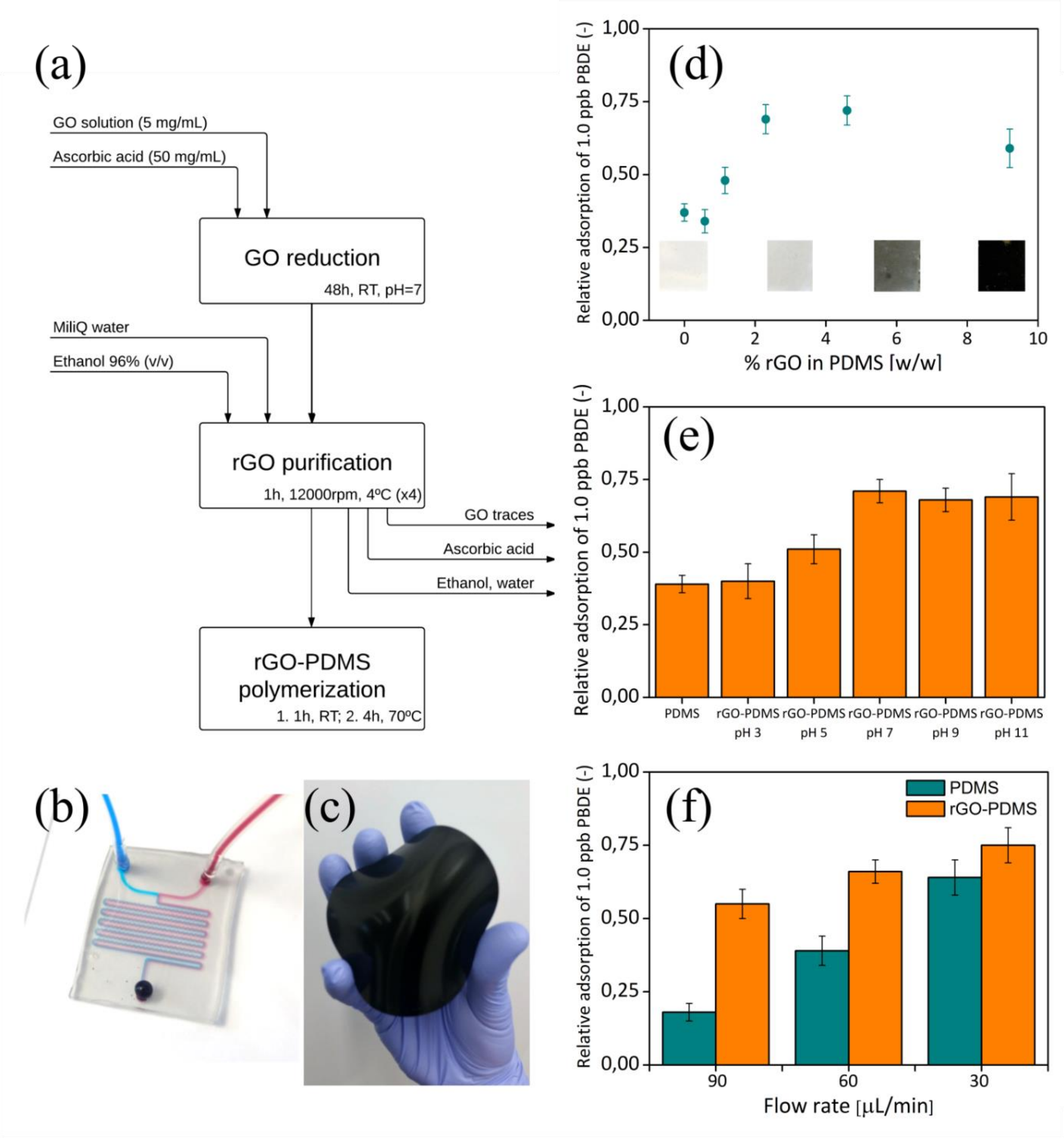


Figure 2 rGO-PDMS composite (a) fabrication process; (b) example of rGO-PDMS-glass chip filled with colour inks; (c) example of flexible solid piece of rGO-PDMS; (d) the effect of rGO concentration (w/w) on PBDE adsorption; (e) the effect of pH during reduction process on PBDE adsorption; (f) the effect of flow rate on PBDE adsorption using rGO-PDMS and bare PDMS.

over time) what makes impossible obtaining a homogenous and flat layer of rGO-PDMS. Such high concentration of rGO-PDMS changes also some physical properties - the polymer is too flexible and spongy. This effect may be related to the presence of rGO but in our opinion is mainly related to the presence of excessive solvent (water from rGO suspension) in PDMS which evaporation during polymerization affects the structure of polymer. Taking into account these observations, we decided to perform last steps of rGO purification in ethanol solution, as it is compatible with PDMS and can evaporate very quickly without affecting the polymerization itself.

Flow rate is a major factor that influence microfluidic processes. We proved (Fig. 2f) that an increase of flow rate will decrease the adsorption yield. However, comparison of PDMS to rGO-PDMS shows that in higher flow rate (90 µL/min) the effect of rGO is well observed (significant increase of adsorption) comparing to lower flow rates (30 µL/min).

Final measurements in LOCs system were equipped with the rGO-PDMS chip fabricated using the design showed in Fig. 1 (GO-CHIP) and following the optimal conditions mentioned above. Due to significantly much bigger contact surface area between the liquid and rGO-PDMS channel, its adsorption capacity is higher comparing to single-channel chip.

We tested PBDE in the range of concentration 1-1000 ppb and found the total adsorption capacity between 100 and 1000 ppb, both in the standard buffer solution and seawater (Fig. S4 in the ESM). We believe that this is sufficient for real samples treatment as the usual level of PBDE in seawater is rather lower than 10 ppb [40, 41, 42]. Moreover, a decrease of the flow rate may bring further improvements regarding adsorption capability. However in this work, the flow rate value is chosen based on demands for other steps performed in IMMU-CHIP and DET-CHIP, as all the operations are performed simultaneously. For example, we tested the PBDE concentration of 10 ppb and after passing through the rGO-PDMS chip with the flow rate of 100 µL/min, none of the PBDE was detected at the outlet of the chip (data not shown).

Apart from adsorption capability, a possible reusability of the platform was tested. We found that solvents introduced to GO-CHIP channel can extract a previously adsorbed PBDE. Acetone showed the best performance, as with the use of this solvent it was possible to perform desorption of PBDE in 4 subsequent cycles and recover nearly 100% of PBDE (Fig. S5 in the ESM). Nevertheless, the ease of fabrication suggest that this platform is more feasible when considered as disposable, rather than reusable.

3.2 Electrochemical detection of PBDE (bare SPE)

The development of Lab-on-a-chip platform for electrochemical detection was preceded by experiments involving just screen-printed electrodes. Fig. 3 shows parameters that were optimized in order to find the best conditions for PBDE determination. One of the most crucial is the concentration of o-AP (Fig. 3a). If it is higher than 0,12 mM it contributes to non-enzymatic oxidation and reduce signal-to-noise ratio, similarly as reported elsewhere 39. For this reason, 0,6 mM o-AP was chosen for further experiments providing satisfactory sensitivity. The effect of the other component, the substrate solution (hydrogen peroxide) upon reaction sensitivity (Fig. 3b), also was studied, thus the concentration of 2*10-4 M was used, as in [39].

Incubation time is the time when redox reaction is catalysed by HRP. 30 min was found as the time providing the highest SNR (Fig. 3c) and it is quite similar to the time of reaction in a commercial colorimetric method (20 min). Further time extension does not lead to sensitivity improvement.

An increase of deposition time (Fig. 3d) leads to proportional increase of SNR, however if the deposition time exceed 60 s, an enormous decrease of SNR is observed due to electrochemical oxidation of the substrate (o-AP) which prevails over the effect of enzymatic reaction (HRP catalysis). Thus, 20 s was chosen as an optimum deposition time providing good sensitivity and reducing the risk of false-positive results.

3.3 Lab-on-a-chip optimization

The next step of the LOC platform development was an evaluation of IMMU-CHIP performance. Fig. 4a shows the process of mixing between the solution of PBDE and MB-Ab suspension. It can be seen that when solutions meet each other in the microfluidic channel they do not penetrate themselves and flow separately. However due to the curvature of the channel, solution is further conveyed from laminar into turbulent flow so the diffusion between them occurs quite efficiently, facilitating immunoreaction. Fig. 4a-3 shows the ending section of mixer chip where the colour of the solution is already uniform, hence, reaction between PBDE and MB-Ab might be occurring.

Fig. 4b presents the results of flow rate optimization, where colorimetric kit was used as a reference technique. Regarding PBDE adsorption, an increase of flow rate is linked with an increase of PBDE recovery what means that no analyte is lost during assay. For the flow rate equal or higher than 100 µL/min, PBDE adsorption is no longer observed. Regarding on-chip immunoreaction, the efficiency of the reaction is not so high when the low flow rate is applied (60% of control signal for the flow rate of 5 µL/min) what is probably related to protein adsorption or the physical loss of magnetic beads. On the other hand when very high flow rates are applied, the efficiency of the reaction is also not satisfactory, probably due to the reduced time that limits the immunoreaction to occur. Thus, analysing both curves it can be seen that the flow rate of about 100 µL/min offers the compromise, as PBDE adsorption is not observed and the reaction between HRP-PBDE and MB-Ab works with the maximum efficiency.

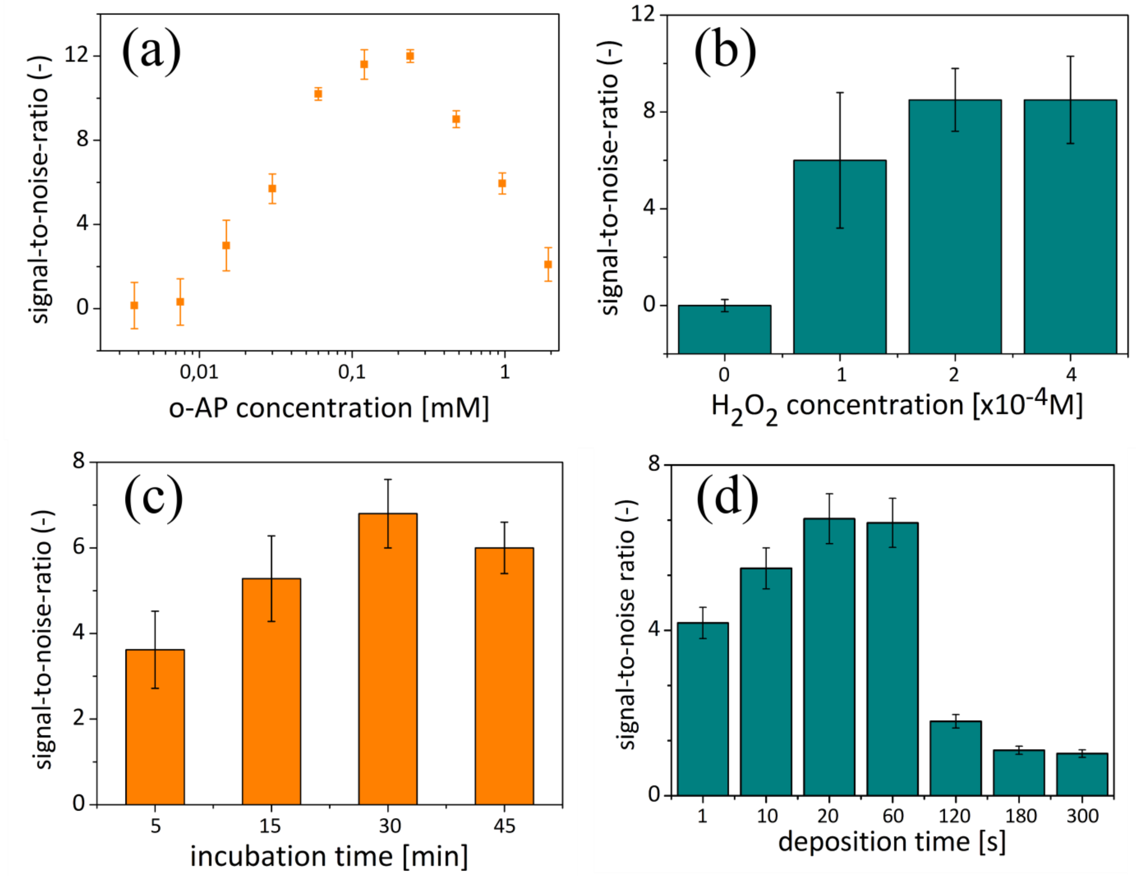


Figure 3 Optimization of electrochemical detection in static conditions using bare SPCE (a) optimization of substrate (o-AP) concentration, (b) optimization of hydrogen peroxide concentration, (c) optimization of incubation time (enzymatic reaction), (d) optimization of deposition time in Square Wave Anodic Stripping Voltammetry. All results expressed as signal-to-noise ratio calculated as a ratio between signal represented by control sample 0,0 ppb of PBDE and substrate solution.

Comparing to electrochemical measurements performed just by drop casting (batch/static measurements), in chip detection (DET-CHIP) requires a big attention to the flow rate, as a main factor affecting sensitivity and limit of detection. The flow of the sample supports particularly the enzymatic reaction, where substrate solution is mixed with immunocomplex and 3-APZ is obtained as well as deposition process, where 3-APZ is accumulated onto the electrode surface. In case of electrochemical stripping (detection step) the flow is stopped and this step is performed in static conditions.

In order to find the optimal conditions some measurements of different flow rates (defined taking into account the constant volume of injected o-AP/H2O2/PBS solution, 30 µL) as well as desired time of enzymatic reaction were carried out. Fig. 4c shows that an increase of flow rate is generally related with a decrease of signal-to-noise ratio. This is obviously caused by the reduced time that is a key factor in enzymatic reactions. Flow rates between 0,5-3 µL/min allowed to perform reaction with the relatively high signal-to-noise ratio, similar as in the optimal condition (30 minutes) of static measurements. The final experiments were performed using the flow rate of 2 µL/min, where the reaction time is only 15 minutes.

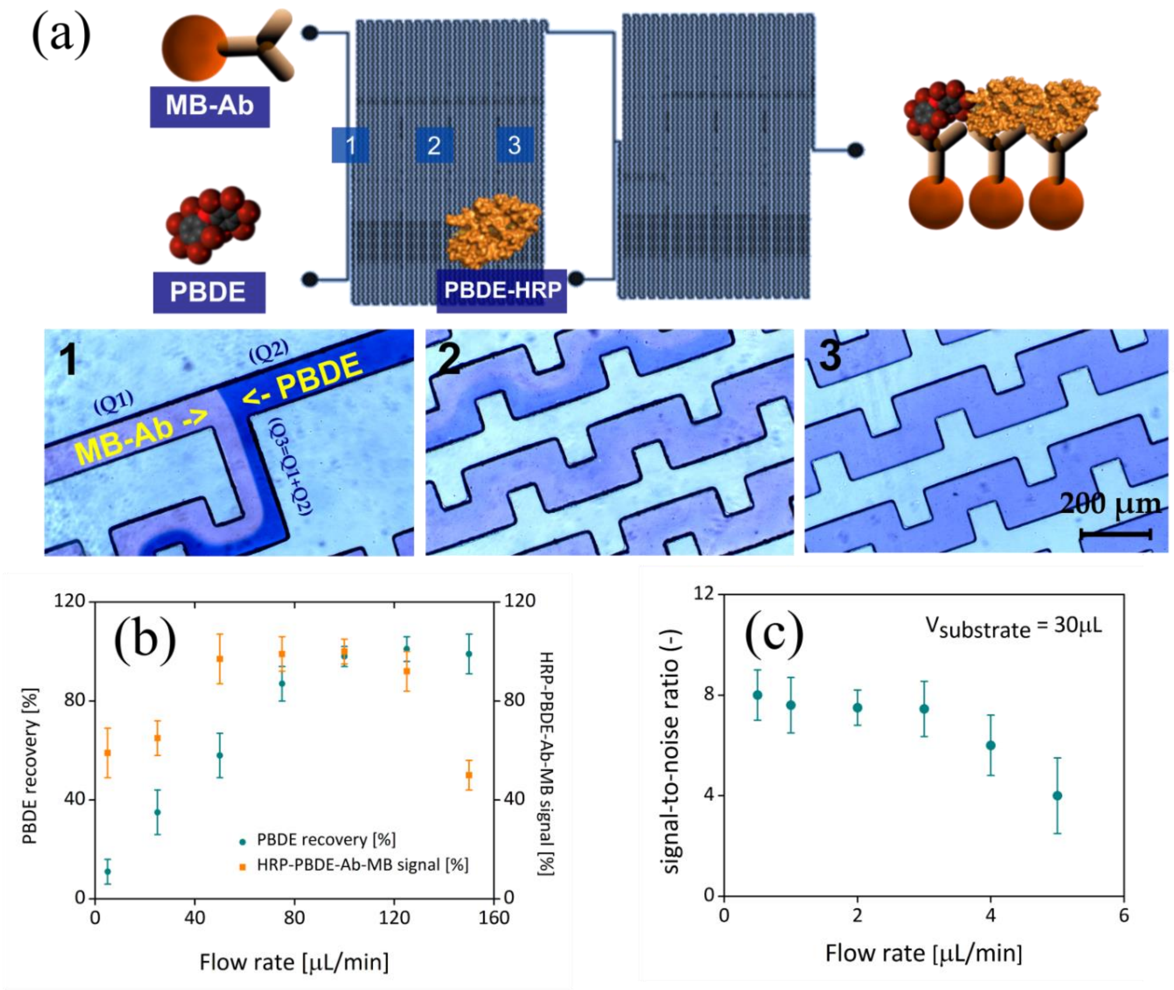
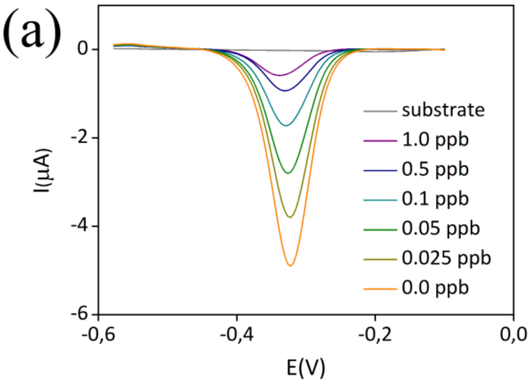


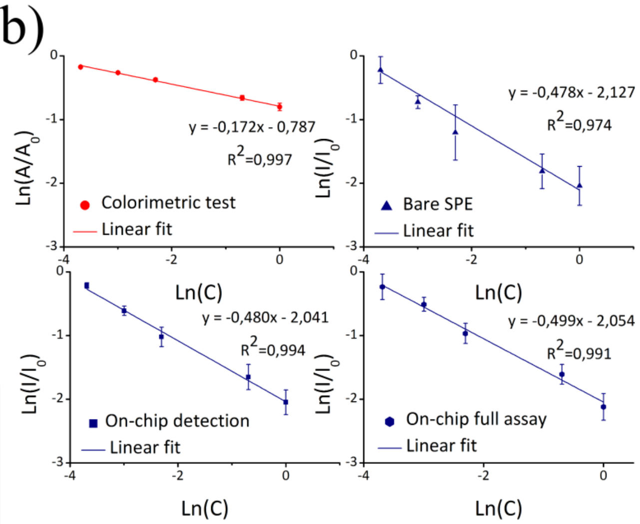
Figure 4 Optimization of LOC operation. (a) Visualisation of mixing in IMMU-CHIP between reagents in (1) intersection where magnetic beads with antibody (MB-Ab) meet PBDE, (2) middle part of the chip where mixing process partially occurred, (3) terminal section of the channel where samples are fully mixed. Trypan-blue (10%) was used to visualize PBDE solution, pictures taken using optical microscope with 20x magnification; (b) Optimization of the flow rate in IMMU-CHIP based on colorimetric test as regards PBDE recovery and immunocomplex (HRP-PBDE-Ab-MB) signal when passing the microfluidic channel; (c) optimization of the flow rate during enzymatic reaction in electrochemical detection of HRP-PBDE (DET-CHIP). All results expressed as signal-to-noise ratio calculated as a ratio between signal represented by substrate solution without PBDE as control sample.

Regarding deposition step, similarly as in static measurements, the time of 20 sec was found as the optimal one (data not shown). The process of deposition was performed with the flow rate of 90 µL/min in order to transfer all the solution from the chip, where an enzymatic reaction occurred. We have observed that such relatively high flow rate supports the sensitivity of the assay as the background current is much lower than in static measurements. This results from the quantity of molecules adsorbed onto electrode during deposition step that is lower in higher flow rate.

3.4 Complete detection of PBDE using Lab-on-a-chip platform

Fig. 5 presents final results of all methods compared. As it can be seen in Fig. 5a, signal of SW-ASV is represented by the curves which negative current is inversely proportional to PBDE concentration. Each peak is characterized by a specific potential of about -0,35 V that shifts slightly towards -0,3 V when the concentration of HRP-PBDE increases. We have also observed that the presence of salts (PBS) influences the potential value by shifting it towards -0,4 V. However it does not affect the signal intensity (data not shown). All measurements where performed in presence of PBS (o-AP + H2O2 and PBS in a ratio 10:1) so as to keep a reproducible value of potential. In order to validate the developed technique, we performed in a same time commercial colorimetric test (Fig. 5b). The limit of detection of this technique is about 0,019 ppb PBDE what is in accordance with the results obtained in electrochemical measurements, where the limit of detection is about 0,018 ppb, both for the whole assay performed in chip as well as just detection step performed in chip. This is not surprising as the LOD of this method is limited by the immunoassay performance, rather than HRP detection as the concentration of the labelling protein is big enough, both in samples with high and low concentration of PBDE. Such limit of detection seems to be sufficient for all possible applications taking into account the level of PBDEs found in different places for instance seawater in Jiaozhou Bay in China (6,59 ppb)[40], coastal water of Aegean Sea (0,03-0,05 ppb)[41] or various indoor environments in Barcelona, Spain (7,3-13,9 ppb)[42].





(c)

PBDE spiked (ppb)	Relative signal (buffer) (%)	Relative signal (seawater) (%)	Recovery (%)	RSD (%)
1	12,01	11,30	94,09	9,60
0,5	20,20	19,10	94,55	8,10
0	100,00	103,00	103,00	5,12

Figure 5 Comparison of optical and electrochemical detection of PBDE (a) Example of SW-ASV measurements of different concentration of PBDE (0,0-1,0 ppb) using Lab-on-a-chip system; (b) Calibration curves showing detection of PBDE using colorimetric test as well as different modes of electrochemical approach; (c) electrochemical detection in seawater samples spiked with PBDE standard solutions.

Electrochemical approach comparing to optical brings higher sensitivity of the method what is clearly seen by the slope of the calibration curves (Fig. 5b). Furthermore, a major difference regarding linearity and reproducibility can be observed comparing with measurements performed just on bare electrode and those in microfluidic chip (either just detection or the whole assay). Detection based just on bare electrodes was linked with much worse coefficient of determination (R2=0,974 comparing to R2=0,994) and higher errors among all repetitions of the experiment as regards measurements in microfluidic. This is probably related to the nature of Square Wave Anodic Stripping Voltammetry, as a good performance of this technique depends on diffusion and the contact between electrode's surface and sample. Apparently this works better in microfluidics where the deposition process is performed in flow and the presence of microfluidic channel keep constant the volume of liquid being in contact with working electrode as well as prevents evaporation of the sample by limiting any influences from external environment. This confirms already reported statements on improved performance of SW-ASV in microfluidics comparing to bare screen-printed electrodes. It is especially meaningful taking into account the use of home-made electrodes fabricated using more eco-friendly materials (carbon, silver/silver-chloride) than electrodes containing for example toxic mercury [43].

3.5 Validation of Lab-on-a-chip in seawater samples

In order to justify the practical application of presented LOCs device, we evaluated seawater samples spiked with PBDE standard solution as described in "2 Seawater detection" in the ESM. Results are shown in Fig. 5c and there is a clear evidence of good recovery yield between 94,1% and 103,0%, so it can be assumed that the presented method is fully compatible with seawater samples. This is possible due to washing steps where all the traces are removed so there is no influence on electrochemical detection. On the other hand, as mentioned before, the presence of salts (ionic strength) does not affect the signal intensity. As the system based on magneto-immunoassay, the use of magnetic beads in chip also supports an efficient capturing of analyte from complex matrix such as seawater.

3.6 Comparison with other methods and future perspectives

Comparison between colorimetric and electrochemical method regarding the use of regents is shown in Table S4 in the ESM. As it can be seen thanks to microfluidics less reagents are spent as well as significant amount of time is saved. Just by using a syringe pump as a driving unit, an automation of the system is provided. Moreover, instead of syringe pump, programmable microfluidic flow controllers might be applied for this platform, providing full automation of the process and eliminating the need of qualified workforce. This is crucial, as PBDE, as small molecule, can be detected mainly in competitive immunoassay format. This kind of assays are generally more sophisticated than for example label-free methods thus, more time and attention is usually required to perform the analysis. However although simple and sensitive label-free methods for PBDE detection based on pattern recognition are reported, the risk of cross-reactivity and the need of data separation limits their practical application [46]. Another example of label-free detection of PBDE is Electrochemical Impedance Spectroscopy, which although specific, provides much higher LOD (1.3 ppb) comparing to our work [47].

Another possible future improvement of presented platform is its high-throughput performance. By miniaturizing all of the chips and multiplying them, it would be possible to run several assays in parallel without an extension of total size of the sensing system.

Conclusions

In this work, the need of a novel, integrated microfluidic platform for simultaneous capturing, detection and removal of polybrominated diphenyl ethers was fulfilled. The presented system consists of 3 microfluidic chips: the IMMU-CHIP where the analyte is captured and immunoreaction occurred, the DET-CHIP, where the level of HRP-PBDE is quantified using SW-ASV and the GO-CHIP, which is responsible for the removal of potentially toxic PBDEs.

The LOD achieved was 0,018 ppb, which is in accordance with currently available methods for PBDE detection. The electrochemical detection performed in the microfluidic chip showed better sensitivity compared to the commercial colorimetric method and better reproducibility and linearity as regards electrochemical measurements performed using just bare screen-printed electrode.

The practical impact of this work is twofold: firstly, the proof that electrochemical detection of PBDEs can be performed and secondly, that rGO-PDMS composite in microfluidic devices can be employed as an efficient adsorbent of various compounds. To the best of our knowledge both of the issues mentioned above are not reported yet.

Furthermore, small size, durability and suitability for operation with seawater samples suggest that the presented platform can be employed in marine environmental analysis, for example, as an integrated component of buoys. Combining this with commercially available miniaturized potentiostats (electrochemical detection) and microfluidic driving units will result in a small-sized and fully automated detection platform.

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Electronic Supplementary Material: Supplementary material (protocol of colorimetric detection of PBDE, data treatment, measurements in seawater, alternative electrochemical methods, protein adsorption to rGO-PDMS composite, comparison of electrochemical and optical methods) is available in the online version of this article at http://dx.doi.org/10.1007/s12274-***-****-* (automatically inserted by the publisher).

References

[1] Medina-Sanchez, M.; Miserere, S.; Morales-Narvaez, E.; Merkoçi, A., On-chip Magneto-immunoassay for Alzheimer's Biomarker Electrochemical Detection by Using Quantum Dots as Labels. Biosensors & Bioelectronics 2014, 54, 279-284.

[2] Marasso, S. L.; Giuri, E.; Canavese, G.; Castagna, R.; Quaglio, M.; Ferrante, I.; Perrone, D.; Cocuzza, M., A Multilevel Lab on chip Platform for DNA Analysis. Biomedical Microdevices 2011, 13, 19-27.

[3] Lee, H. H.; Yager, P., Microfluidic lab-on-a-chip for microbial identification on a DNA microarray. Biotechnology and Bioprocess Engineering 2007, 12 (6), 634-639.

[4] Kurbanoglu, S.; Mayorga-Martinez, C. C.; Medina-Sanchez, M.; Rivas, L.; Ozkan, S. A.; Merkoçi, A., Antithyroid Drug Detection Using an Enzyme Cascade Blocking In a Nanoparticle-based lab-on-a-chip System. Biosensors & Bioelectronics 2015, 67, 670-676.

[5] Kimura, H.; Ikeda, T.; Nakayama, H.; Sakai, Y.; Fujii, T., An On-Chip Small Intestine-Liver Model for Pharmacokinetic Studies. Jala 2015, 20, 265-273.

[6] Ozhikandathil, J.; Badilescu, S.; Packirisamy, M., Detection of Bovine Growth Hormone Using Conventional and Lab-on-a-chip Technologies: a Review. International Journal of Advances in Engineering Sciences and Applied Mathematics 2015, 7 (4), 177-190.

[7] Ozhikandathil, J.; Packirisamy, M., Nano-islands Integrated Evanescence-based Lab-on-a-chip on Silica-on-silicon and Polydimethylsiloxane Hybrid Platform for Detection of Recombinant Growth Hormone. Biomicrofluidics 2012, 6, .

[8] Medina-Sanchez, M.; Cadevall, M.; Ros, J.; Merkoçi, A., Eco-friendly Electrochemical Lab-on-paper for Heavy Metal Detection. Analytical and Bioanalytical Chemistry 2015, 407, 8445-8449.

[9] da Costa, E. T.; Santos, M. F. S.; Jiao, H.; do Lago, C. L.; Gutz, I. G. R.; Garcia, C. D., Fast Production of Microfluidic Devices by CO2 Laser Engraving of Wax-coated Glass Slides. Electrophoresis 2016, 37, 1691-1695.

[10] Ambrosi, A.; Guix, M.; Merkoçi, A., Magnetic and Electrokinetic Manipulations On a Microchip Device for Bead-based Immunosensing Applications. Electrophoresis 2011, 32, 861-869.

[11] Medina-Sanchez, M.; Miserere, S.; Merkoçi, A., Nanomaterials and Lab-on-a-chip Technologies. Lab on a Chip 2012, 12, 1932-1943.

[12] Li, S. G.; Xu, Z. G.; Mazzeo, A.; Burns, D. J.; Fu, G.; Dirckx, M.; Shilpiekandula, V.; Chen, X.; Nayak, N. C.; Wong, E.; Yoon, S. F.; Fang, Z. P.; Youcef-Toumi, K.; Hardt, D.; Tor, S. B.; Yue, C. Y.; Chun, J. H. In Review of Production of Microfluidic Devices: Material, Manufacturing and Metrology, Conference on MEMS, MOEMS and Micromachining III, Strasbourg, France, Apr 09-10; Strasbourg, France, 2008.

[13] Bhise, N. S.; Manoharan, V.; Massa, S.; Tamayol, A.; Ghaderi, M.; Miscuglio, M.; Lang, Q.; Zhang, Y. S.; Shin, S. R.; Calzone, et al., A liver-on-a-chip platform with bioprinted hepatic spheroids. Biofabrication 2016, 8, 12.

[14] He, J. K.; Chen, R. M.; Lu, Y. J.; Zhan, L.; Liu, Y. X.; Li, D. C.; Jin, Z. M., Fabrication of Circular Microfluidic Network in Enzymatically-crosslinked Gelatin Hydrogel. Materials Science & Engineering C-Materials for Biological Applications 2016, 59, 53-60.

[15] Xia, Y. N.; Whitesides, G. M., Soft Lithography. Annual Review of Materials Science 1998, 28, 153-184.

[16] Mayorga-Martinez, C. C.; Hlavata, L.; Miserere, S.; Lopez-Marzo, A.; Labuda, J.; Pons, J.; Merkoçi, A., An Integrated Phenol 'Sensoremoval' Microfluidic Nanostructured Platform. Biosensors & Bioelectronics 2014, 55, 355-359.

[17] Mayorga-Martinez, C. C.; Hlavata, L.; Miserere, S.; Lopez-Marzo, A.; Labuda, J.; Pons, J.; Merkoçi, A., Nanostructured CaCO3-poly(ethyleneimine) Microparticles for Phenol Sensing in Fluidic Microsystem. Electrophoresis 2013, 34, 2011-2016.

[18] Medina-Sánchez; Mariana; Mayorga-Martinez; C., C.; Watanabe, T.; A. Ivandini; Tribidasari; Honda, Y.; Pino, F.; Nakata, A.; Fujishima, A.; Einaga, Y.; Merkoçi, A., Microfluidic Platform for Environmental Contaminants Sensing and Degradation Based on Boron-doped Diamond Electrodes. Biosensors and Bioelectronics 2015, 75, 365-364.

[19] Tan, H. Y.; Loke, W. K.; Nguyen, N. T.; Tan, S. N.; Tay, N. B.; Wang, W.; Ng, S. H., Lab-on-a-chip For Rapid Electrochemical Detection of Nerve Agent Sarin. Biomedical Microdevices 2014, 16, 269-275.

[20] Ibarlucea, B.; Diez-Gil, C.; Ratera, I.; Veciana, J.; Caballero, A.; Zapata, F.; Tarraga, A.; Molina, P.; Demming, S.; Buttgenbach, et al., PDMS Based Photonic Lab-on-a-chip For The Selective Optical Detection of Heavy Metal Ions. Analyst 2013, 138, 839-844.

[21] Feng, C. Y.; Wei, J. F.; Li, Y. J.; Yang, Y. S.; Wang, Y. H.; Lu, L.; Zheng, G. X., An On-chip Pollutant Toxicity Determination Based on Marine Microalgal Swimming Inhibition. Analyst 2016, 14, 1761-1771.

[22] Zheng, G. X.; Li, Y. J.; Qi, L. L.; Liu, X. M.; Wang, H.; Yu, S. P.; Wang, Y. H., Marine Phytoplankton Motility Sensor Integrated Into A Microfluidic Chip for High-throughput Pollutant Toxicity Assessment. Marine Pollution Bulletin 2014, 84 (1-2), 147-154.

[23] Zheng, G. X.; Wang, Y. H.; Wang, Z. M.; Zhong, W. L.; Wang, H.; Li, Y. J., An Integrated Microfluidic Device In Marine Microalgae Screening Application. Marine Pollution Bulletin 2013, 72, 231-243.

[24] Hooper, K.; McDonald, T. A., The PBDEs: An Emerging Environmental Challenge and Another Reason for Breast-milk Monitoring Programs. Environmental Health Perspectives 2000, 108, 387-392.

[25] Andrade, N. A.; McConnell, L. L.; Torrents, A.; Ramirez, M., Persistence of Polybrominated Diphenyl Ethers in Agricultural Soils after Biosolids Applications. Journal of Agricultural and Food Chemistry 2010, 58, 3077-3084.

[26] Guo, W. H.; Holden, A.; Smith, S. C.; Gephart, R.; Petreas, M.; Park, J. S., PBDE Levels in Breast Milk are Decreasing in California. Chemosphere 2016, 150, 505-513.

[27] Ward, J.; Mohapatra, S. P.; Mitchell, A., An Overview of Policies for Managing Polybrominated Diphenyl Ethers (PBDEs) in The Great Lakes Basin. Environment International 2008, 34, 1148-1156.

[28] Darnerud, P. O.; Eriksen, G. S.; Johannesson, T.; Larsen, P. B.; Viluksela, M., Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology. Environmental Health Perspectives 2001, 109, 49-68.

[29] Porterfield, S. P., Vulnerability Of The Developing Brain To Thyroid Abnormalities - Environmental Insults To The Thyroid System. Environmental Health Perspectives 1994, 102, 125-130.

[30] Ahn, K. C.; Gee, S. J.; Tsai, H. J.; Bennett, D.; Nishioka, M. G.; Blum, A.; Fishman, E.; Hammock, B. D., Immunoassay for Monitoring Environmental and Human Exposure to the Polybrominated Diphenyl Ether BDE-47. Environmental Science & Technology 2009, 43, 7784-7790.

[31] Butryn, D. M.; Gross, M. S.; Chi, L. H.; Schecter, A.; Olson, J. R.; Aga, D. S., "One-Shot" Analysis Of Polybrominated Diphenyl Ethers and Their Hydroxylated and Methoxylated Analogs In Human Breast Milk and Serum Using Gas Chromatography-Tandem Mass Spectrometry. Analytica Chimica Acta 2015, 892, 140-147.

[32] Li, Z.; Li, C.; Lin, D.; Kang, W. J.; Pan, D. Y.; Wu, M. H., GC/MS Analysis of Polybrominated Diphenyl Ethers in Vegetables Collected from Shanghai, China. Proceedings of the 2013 International Conference on Material Science and Environmental Engineering (Msee 2013) 2013, 292-296.

[33] Liu, Q.; Shi, J. B.; Sun, J. T.; Wang, T.; Zeng, L. X.; Zhu, N. L.; Jiang, G. B., Graphene-Assisted Matrix Solid-Phase Dispersion for Extraction of Polybrominated Diphenyl Ethers and Their Methoxylated and Hydroxylated Analogs From Environmental Samples. Analytica Chimica Acta 2011, 708, 61-68.

[34] Zhang, H.; Lee, H. K., Plunger-In-Needle Solid-Phase Microextraction with Graphene-Based Sol-Gel Coating as Sorbent for Determination of Polybrominated Diphenyl Ethers. Journal of Chromatography A 2011, 1218, 4509-4516.

[35] Orozco, J.; Mercante, L. A.; Pol, R.; Merkoçi, A., Graphene-based Janus Micromotors for the Dynamic Removal of Pollutants. Journal of Materials Chemistry A 2016, 4, 3371-3378.

[36] Chen, M. T.; Tao, T.; Zhang, L.; Gao, W.; Li, C. Z., Highly Conductive and Stretchable Polymer Composites Based on Graphene/MWCNT network. Chemical Communications 2013, 49, 1612-1614.

[37] Shahzad, M. I.; Giorcelli, M.; Shahzad, N.; Guastella, S.; Castellino, M.; Jagdale, P.; Tagliaferro, A. In Study of carbon nanotubes based Polydimethylsiloxane composite films, 6th Vacuum and Surface Sciences Conference of Asia and Australia (VASSCAA), Islamabad, Pakistan, Oct 09-13; Islamabad, Pakistan, 2012.

[38] Xu, R. Q.; Lu, Y. Q.; Jiang, C. H.; Chen, J.; Mao, P.; Gao, G. H.; Zhang, L. B.; Wu, S., Facile Fabrication of Three-Dimensional Graphene Foam/Poly(dimethylsiloxane) Composites and Their Potential Application as Strain Sensor. Acs Applied Materials & Interfaces 2014, 6, 13455-13460.

[39] Lee, A. C.; Liu, G. D.; Heng, C. K.; Tan, S. N.; Lim, T. M.; Lin, Y. H., Sensitive Electrochemical Detection of Horseradish Peroxidase at Disposable Screen-printed Carbon Electrode. Electroanalysis 2008, 20 (18), 2040-2046.

[40] Ju, T.; Ge, W.; Jiang, T.; Chai, C., Polybrominated Diphenyl Ethers in Dissolved and Suspended Phases of Seawater and in Surface Sediment from Jiaozhou Bay, North China. Science of the Total Environment 2016, 557, 571-578.

[41] Lammel, G.; Audy, O.; Besis, A.; Efstathiou, C.; Eleftheriadis, K.; Kohoutek, J.; Kukucka, P.; Mulder, M. D.; Pribylova, P.; Prokes, et al., Air and Seawater Pollution and Air-Sea Gas Exchange Of Persistent Toxic Substances in The Aegean Sea: Spatial Trends of PAHs, PCBs, OCPs and PBDEs. Environmental Science and Pollution Research 2015, 22, 11301-11313.

[42] Cristale, J.; Hurtado, A.; Gomez-Canela, C.; Lacorte, S., Occurrence and Sources of Brominated and Organophosphorus Flame Retardants in Dust From Different Indoor Environments in Barcelona, Spain. Environmental Research 2016, 149, 66-76.

[43] Medina-Sanchez, M.; Miserere, S.; Marin, S.; Aragay, G.; Merkoçi, A., On-Chip Electrochemical Detection of Cds Quantum Dots Using Normal and Multiple Recycling Flow Through Modes. Lab on a Chip 2012, 12, 2000-2005.

[44] Zhang, J. L.; Yang, H. J.; Shen, G. X.; Cheng, P.; Zhang, J. Y.; Guo, S. W., Reduction of Graphene Oxide via L-ascorbic acid. Chemical Communications 2010, 46, 1112-1114.

[45] Nourani, S.; Ghourchian, H.; Boutorabi, S. M., Magnetic Nanoparticle-based Immunosensor for Electrochemical Detection of Hepatitis B Surface Antigen. Analytical Biochemistry 2013, 441, 1-7.

[46] Xu D.; Zhu W.; Wang C.; Tian T.; Li J.; Lan Y.; Zhang G.; Zhang D.; Li G., Label-free detection and discrimination of poly-brominated diphenylethers using molecularly imprinted photonic cross-reactive sensor arrays. Chemical Communications 2014, 50, 14133-14136

[47] Radhakrishnan, R.; Suni, I. I.; Bever, C. S.; & Hammock, B. D., Impedance Biosensors: Applications to Sustainability and Remaining Technical Challenges. ACS Sustainable Chemistry & Engineering 2014, 2, 1649–1655.

Electronic Supplementary Material

Colorimetric detection of PBDE

Colorimetric test as a well-established and reproducible method was used as a reference technique regarding electrochemical measurements and optimization of flow operation in microfluidic chips. All steps were performed as follows, according to manufacturer´s manual. Magnetic beads, antibodies, HRP-PBDE and PBDE standard were used for development of on-chip electrochemical assay.

Procedure of colorimetric test

1. Add 250 µL of the appropriate standard, control, or sample.
2. Mix the PBDE Antibody Coupled Paramagnetic Particles thoroughly and add 500 µL to each tube.
3. Vortex for 1 to 2 seconds minimizing foaming.
4. Incubate for 20 minutes at room temperature.

5. Add 250 µL of PBDE Enzyme Conjugate to each tube.

6. Vortex for 1 to 2 seconds minimizing foaming.

7. Incubate for 20 minutes at room temperature

8. Separate in the Magnetic Separation Rack for two (2) minutes.

9. Decant and gently blot all tubes briefly in a consistent

manner.

10. Add 1 mL of Washing Solution T to each tube and vortex tubes for 1-2 seconds. Return tubes and allow to remain in the magnetic separation unit for two (2) minutes.

11. Decant and gently blot all tubes briefly in a consistent manner.

12. Repeat Steps 10 and 11 an additional time.

13. Remove the rack from the separator and add 500 µL of Color Solution to each tube.

14.Vortex for 1 to 2 seconds minimizing foaming.

15. Incubate for 20 minutes at room temperature.

16. Add 500 µL of Stopping Solution to each tube.

17. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 17.

18. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

Data treatment

The mean absorbance of each sample (or standard solution) is normalized towards control sample containing 0.0 ppb of PBDE as follow:

A = Ax/A0 (%)

Where Ax is the mean absorbance of a given sample and A0 is the mean absorbance of standard without PBDE standard. Calibration curve is obtained by plotting the ln(A) on Y axis versus ln(C) (concentration) of X axis.

Limit of detection

Limit of detection of the colorimetric method is expressed as a concentration that represents 90% of signal of control sample 0,0 ppb PBDE. It´s estimated by manufacturer at around 0,017 ppb, however in our case the limit of 0,019 ppb was obtained. Same approach was applied to determine the LOD of electrochemical measurements.

Seawater samples

Preparation of seawater sample was in accordance with the protocol for PBDE detection provided by Abraxis. Briefly, seawater sample (Barcelona, Spain) was collected in sterile glass vessel and diluted immediately (1:1) with methanol. In order to get rid of macroscopic impurities, seawater sample was filtrated through 20 µm syringe filter. Such prepared seawater samples were tested for the presence of PBDE following the same procedures as for commercial standard solutions both in optical and electrochemical experiments.

Optimization of the on-chip washing step

In order to check the optimum conditions for washing step, MB-Ab-PBDE-HRP complex was mixed with 10% (v/v) Trypan Blue solution and IMMU-CHIP was primed with it. Using optical microscope the removal of ink was observed under the proper washing. It was found that that the flow of 150 µL/min provides the optimum washing performance taking into account the time of this step as well as buffer usage.

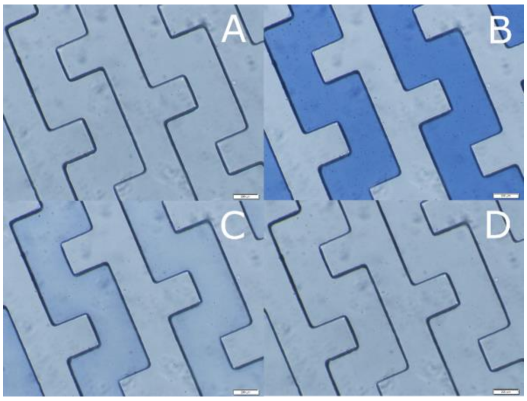

Figure S1 Visualization of the washing step using optical microscope and MB-Ab-PBDE-HRP samples mixed with 10% Trypan Blue. (A) microfluidic channel before priming the solution (B) microfluidic channel after priming the solution, (C) microfluidic channel after 2 minutes of washing step with the flow rate 150 µL/min, (D) microfluidic channel after 5 minutes of washing step with the flow rate 150 µL/min, no more ink is visible.

Cyclic Voltammetry and Differential Pulse Voltammetry for PBDE detection

Besides Square Wave Anodic Stripping Voltammetry, other electrochemical techniques such as CV or DPV might be used. However, comparing to SW-ASV they show lower sensitivity. Fig. S2. shows examples of curved for positive control (1.0 ppb PBDE), negative control (0.0 ppb PBDE) and substrate solution (o-AP + H2O2).

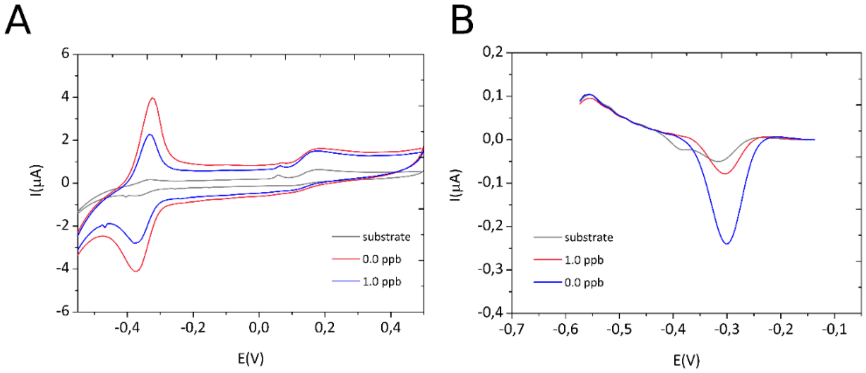

Figure S2 Alternative techniques for PBDE detection: (A) Cyclic Voltammetry, (B) Differential Pulse Voltammetry. All curves obtained in static measurements according to the same protocol as in 3.3.

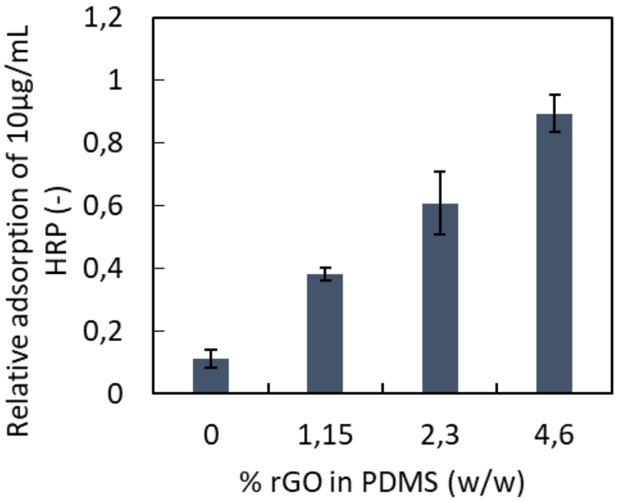

Figure S3 Adsorption of protein (HRP) to rGO-PDMS surface.

Table S4 Comparison of reagents usage (in µL) in different systems

	Colorimetric	Electrochemical (SPE)	Electrochemical (LOC)
MB-Ab	500	250	
PBDE/sample	250	125	
PBDE-HRP	250	125	
Washing buffer	1000	400	
Colour Solution	500	-	-
Stopping Solution	500	-	-
o-AP+H2O2	-	100	27
PBS	10	3	

Adsorption capability of GO-CHIP

The aim of this experiment was to measure the maximum concentration of PBDE that can be adsorbed by GO-CHIP using constant flow rate. Samples of PBDE (500 µL) dissolved in buffer (10% methanol in water) or seawater (pre-treatment as mentioned in a "Seawater samples" in the ESM) were injected to GO-CHIP with the flow rate of 90 µL/min. Samples were collected at the outlet of the chip and diluted accordingly prior to colorimetric test. The level of adsorbed PBDE was determined following the same procedure as in 2.7.

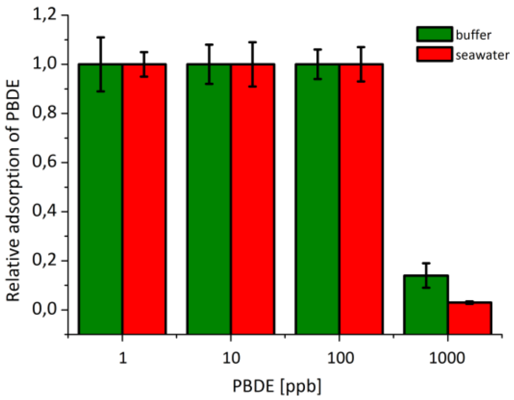

Figure S4 Adsorption of PBDE (1, 10, 100, 1000 ppb) to GO-CHIP in buffer and seawater. Results obtained from 3 repetitions using a new GO-CHIP in each case.

Reusability of GO-CHIP

10 ppb PBDE (500 µL) standard solution was injected to GO-CHIP similarly as in other experiments. Samples were collected at the outlet of the chip and diluted accordingly prior to colorimetric test, in order to proof that all PBDE is adsorbed by GO-CHIP. Afterwards, GO-CHIP was dried by injecting nitrogen. Solvent (500 µL) was injected to GO-CHIP with the flow rate of 40 µL/min and samples were treated as in previous step (collection, dilution, colorimetric test). Results were normalized towards the signal from non-treated 10 ppb PBDE standard solution. Each GO-CHIP was evaluated 4 times by repeating the same procedure. After each cycle, the channel of GO-CHIP was rinsed with miliQ water.

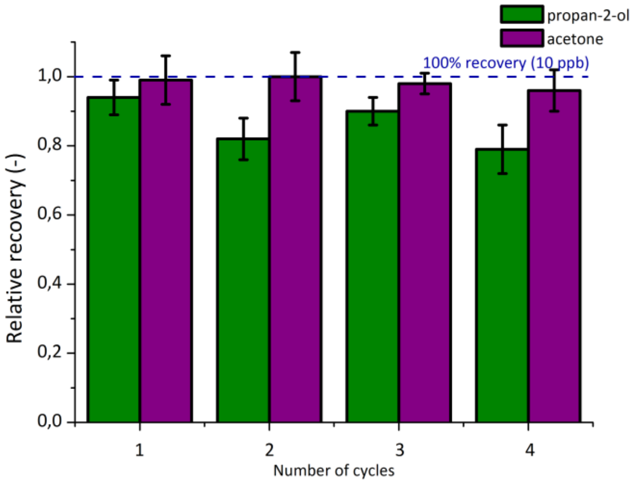

Figure S5 Recovery of PBDE (10 ppb) adsorbed by GO-CHIP using propan-2-ol and acetone as solvents for extraction. Results obtained from 3 repetitions using a new GO-CHIP in each case.