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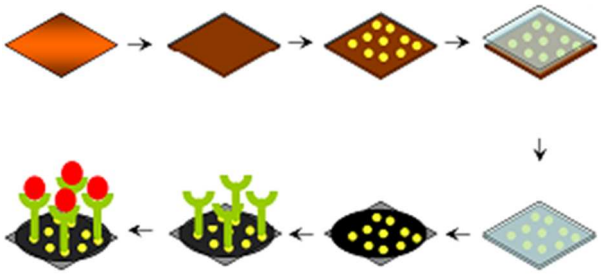

We present a graphene-based sensor for the determination of polybrominated diphenylether at the ppt level.

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ARTICLE TYPE

Impedimetric graphene – based biosensor for the detection of polybrominated diphenyl ether

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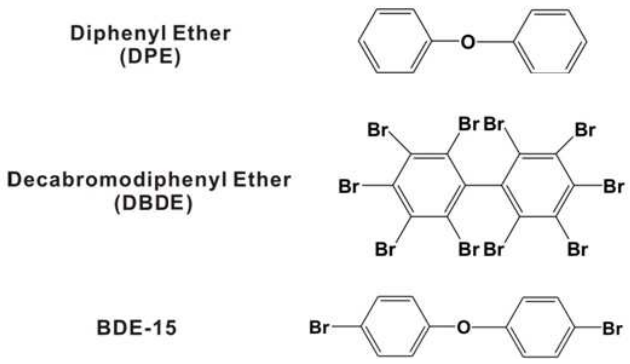
Single-layer graphene, decorated with Au nanoparticles, and a specially designed peptide are used for the first time in the detection of decabrominediphenyl ether using impedance spectroscopy. Biosensor calibration is presented, showing a good linear response from 5% to saturated dissolutions (100 ppt). Selectivity towards brominated species is demonstrated by lack of response to molecules with similar structure but without any bromines.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a 209 congener family consisting of two benzene rings attached by an ether group with different brominated positions, ranging from 1 to 10 bromines per molecule. PBDEs are used largely as flame retardants in the electronics, furniture, textile, airplanes, plastics and building materials industries.[1] Because of the persistence and bioaccumulative health hazard implications of these compounds, monitoring of PBDEs is of great interest worldwide.[2-6] One of the most commonly used PBDEs is the fully brominated congener, namely decabromodiphenyl ether (DBDE) or BDE-209. Current detection of PBDEs is based on expensive instrumentation such as gas chromatography coupled with mass spectroscopy.[7-10]

Biosensors have gained substantial interest in recent years due to their specificity and low levels of detection that are of great interest in clinical diagnosis, food quality control and national security.[11] Among the different biosensors variations, receptor-based sensors, a specific kind of biosensors that uses sequence-specific biopolymers, have received much attention in the recent years.[12-15] These sequence-specific biopolymer can be identified through rational design of anti-sense DNA or RNA sequences based genomic database or isolate novel sequences from the combinatorial high-throughput screening techniques, such as phage/bacterial/yeast display or Systematic Evolution of Ligands by Exponential Enrichment (SELEX),[16] for the desired target molecules. The resulting sequence-specific peptides or nucleic acid aptamers are shown to exhibit high binding affinity and selectivity to specific target molecules of organic, inorganic or biological origin.

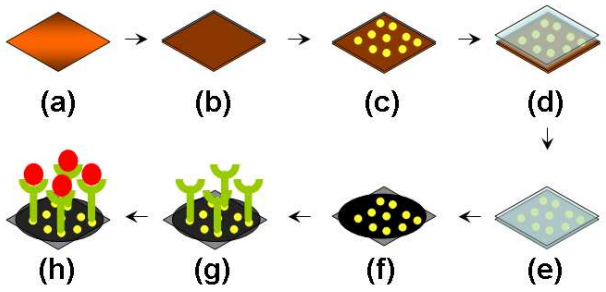
The peptide receptor-based biosensor designs provide many opportunities for gas and liquid phase biosensing detection.[17] The specific short peptide information can be easily isolated from the phage display. The resulting peptides can be chemically synthesized and used for various sensing platforms, involving mass, optical and electrical transducers. Together with high specificity, the peptide-based sensors provide long-term stability and ease of use, and are inexpensive and adaptable to diverse sensing platforms through molecular design. Previously, we developed novel specific peptides to recognize explosives (TNT and DNT).[18]



Scheme 1 Chemical structures of the tested molecules

Electrochemical impedance spectroscopy (EIS) is currently one of the most sensitive techniques in the detection of interfacial phenomena on the surface of electrochemical sensors.[19] EIS is based in the response of the electrochemical cell to an applied oscillating voltage of varied frequencies. Scanning the frequency allows one to obtain information related to diffusion and charge transfer phenomena on the surface, as well as resistivity values for the system. In order to fully exploit an aptamer's selectivity and sensitivity, an excellent transducer material is necessary.

Graphene, a single carbon layer with sp^2 hybridization, presents many desired properties for the next generation impedimetric biosensors because of its remarkable



Scheme 2. Biosensor construction. From a copper foil (a) graphene is grown (b) and decorated with Au NP by a galvanic displacement process (c). PMMA spin coating (d) allows the etching of the copper foil (e) and transfer on the glassy carbon electrode (f) prior to peptide immobilitzation by incubation (g) and PBDE binding (h).

mechanical, thermal and electronic properties.[20] These include high electron conductivity, high surface area, high signal to noise ratio due to its fast heterogeneous electron transfer, and decreasing production cost due to technological breakthroughs.[21]

To exploit these desired properties, we have fabricated an impedimetric biosensor based on graphene modified with gold nanoparticles and a synthetic peptide for the detection of DBDE. To the best of our knowledge, it is the first time that this type of biosensor is reported. The response of the here presented biosensor has been studied in the range of 5% to saturated solutions of DBDE.

Experimental

Materials

The decabromo diphenyl ether (DBDE) binding peptide (DBDE-bp; WHWNAWNWSSQQ) was previously discovered by our laboratory using phage display. The DBDE-bp franked with tri-glycines and cysteine at the c-terminus was synthesized using FMOC-solid phase peptide synthesis approaches and purified using high-performance liquid chromatography (HPLC).[22].

Sodium phosphate dibasic heptahydrate (99.4% purity) for the preparation of phosphate buffer solution (PBS) at pH 7.0, potassium chloride (ACS purity) and concentrated hydrochloric acid (ACS plus purity) to adjust the PBS buffer pH were purchased from Fluka. Potassium hexacyanoferrate (III) (99% purity) was purchased from Alfa, acetonitrile (99.8% purity) was purchased from J. T. Baker. DBDEs (98% purity), diphenyl ether (DPE 99%) and dibromo phenylether (BDE-15 99%) were purchased from Aldrich. Scheme 1 presents the chemical structure of the main target molecule, DBDE, as well as two similar chemical compounds also tested with the developed aptamer.

Apparatus

Electrochemical impedance spectroscopy was performed using a CH660D potentiostat-galvanostat (CH Instruments, USA). A standard three-electrode configuration was used with a AgCl-covered Ag wire acting as reference electrode, a Pt wire as the auxiliary electrode and the peptide-AuNP-graphene on a glassy carbon electrode (CH Instruments, USA) as the working electrode.

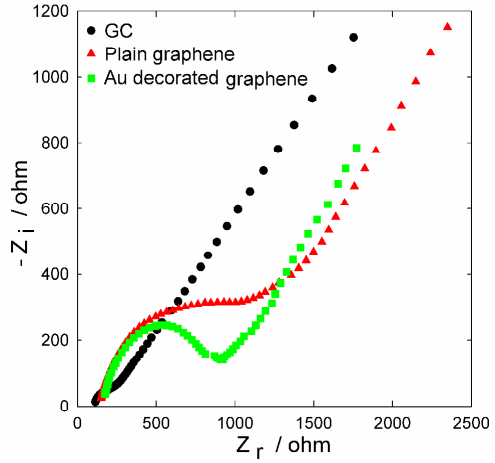


Fig 1. Nyquist plot of the pristine glassy carbon electrode (black dots), undecorated graphene (red triangles) and Au-decorated graphene (green squares).

Impedance spectra were recorded between 0.1-10^4 Hz with sinusoidal voltage amplitude of 50 mV centered at 0.0 V potential. All measurements were carried out at room temperature in a 5mM potassium ferrocyanate (III), used as the redox probe plus 0.1M KCl to set a stable reference potential for the AgCl-covered Ag wire throughout the experiments. Deionized (DI) water (Barnsted Nanopure, USA) was used throughout.Data were represented as Nyquist plots with final adjustments to the conventional Randles circuit. The Nyquist plots were then analyzed following circuit modeling to obtain the charge transfer resistance, R_t, across the electric double layer.[19]

Procedures

Scheme 2 describes the complete biosensor construction process. Graphene was grown by chemical vapor deposition (CVD) on 10x2 cm^2 copper foils (Alfa, 99.8%) of 0.001 in. thickness as described in previous works.[23-24] After graphene growth, the decoration of graphene with Au nanoparticles (AuNP) was achieved via a spontaneous galvanic displacement by dipping the graphene-copper foil in a 1 mM $KAuCl_4$ (98% purity, Sigma) dissolution for 30 seconds. The sample was then triple rinsed with DI water and dried under N_2 flow. In order to transfer the decorated graphene, 8% w/w polymethylmetacrylate (PMMA, Sigma) in toluene (analytical grade, EMD) was spin coated at 2000 rpm for 60 seconds on one side of the Cu foil only. For the final electrode transfer, 3x3 mm^2 pieces were cut, floated in a 1 M $FeCl_3$ dissolution (98% purity Strem Chemicals), until copper was completely etched. Triple rinse in DI water was performed prior to final transfer onto the glassy carbon electrodes. After drying, PMMA was dissolved by careful soaking of the surface in toluene followed by acetone and isopropanol spraying.

Peptide immobilization was performed by incubation of the AuNP-functionalized graphene electrode in 0.1 mg/ml of the synthesized peptide, dissolved in a 1:1 acetonitrile / PBS buffer (pH=7.0) dissolution for 24 hours. After incubation, the electrode was rinsed with DI water. The electrodes modified with the peptide were then incubated in a DI water dissolution containing the desired amount of DBDE.

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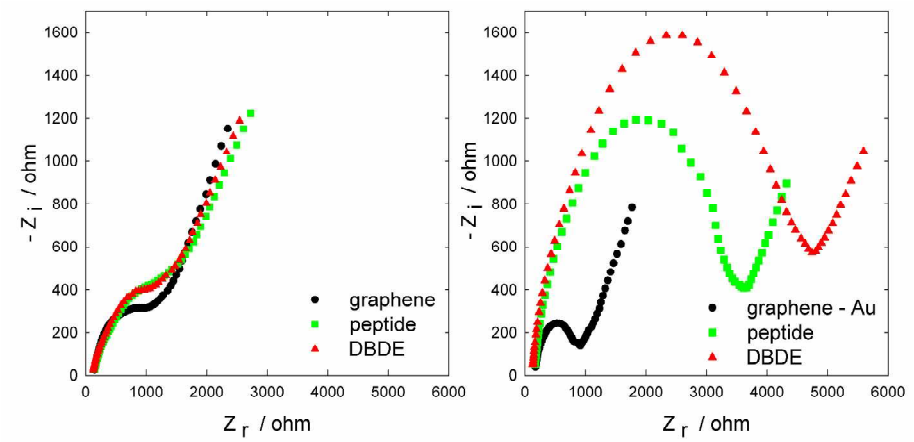

Fig 2. Nyquist plot of the undecorated graphene (left) and the Au-decorated graphene (right) after transfer (black dots), after peptide incubation (green squares) and after saturated DBDE incubation

Results and discussion

Graphene decorated with AuNPs was employed as an electrochemical platform for the construction of a DBDE biosensor as described above. Impedimetric measurements after the decorated graphene transfer, after peptide incubation and after DBDE incubation were performed to fully characterize the biosensor response. In all cases, the response is based on the accessibility of the surface by the redox probe molecule ($Fe(CN)_6^{3-/4-}$) and the monitoring of the blocking capacity of the Au-graphene-peptide-DBDE structure. Negative controls along the process were also performed.

Figure 1 shows the impedimetric response, shown as a Nyquist plot, of the bare glassy carbon (black dots), undecorated graphene (red triangles) and the Au-decorated graphene (green squares). A clear decrease in the charge transfer resistance of the Au-decorated graphene is observed. Further biosensor robustness studies were made with the incubation of undecorated (plain) and Au-decorated graphene platforms. Figure 2 shows the response of the undecorated (left) and Au-decorated (right) graphene platforms after graphene transfer (black dots), after peptide incubation (red dots) and after subsequent incubation in a saturated PBDE dissolution in DI water (green dots). It is clearly observed that the undecorated graphene substrate did not present any change in response after peptide or DBDE incubations, confirming the need of the anchoring Au nanoparticles for peptide immobilization. On the other hand, the response of the Au-decorated graphene presents a clear increase in the charge transfer resistance after the two incubation processes, confirming first the immobilization of the peptide and also the peptide-DBDE interaction. Increase in the charge transfer resistance is attributed to the higher blocking capacity of the peptide-DBDE structure. The exact solubility value of DBDE is uncertain and different values can be found in the literature. Because of this reason we assumed the saturation value as 0.1 ppb as the highest value reported by the Environmental Policy Agency (EPA).[25] Dilutions of the saturated dissolution were performed in order to fully characterize and calibrate the biosensor response. For each dilution, a new Au-graphene was transferred onto a polished glassy carbon electrode followed by a 24-hour incubation in the peptide dissolution.

Figure 3 shows the obtained response (after graphene transfer and after DBDE incubation) for the 10% dilution (10 ppt), the 50% dilution (50 ppt) and the saturated dissolution (100 ppt). The clear increase in the impedimetric signal after incubation reveals the excellent response of the developed biosensor. In order to complete the calibration, three more dilutions were prepared (5% or 5 ppt, 25% or 25 ppt, and 75% or 75 ppt) and measured. Figure 4 shows the obtained calibration plot, using the ratio of impedimetric signal, R_t, before and after incubation as response in the concentration range of 5 to 100 ppt of DBDE.

The response of the graphene-based biosensor to chemical species similar to DBDE was also characterized using the same experimental procedure described above, but replacing the saturated solution of DBDE with saturated solutions of diphenyl ether (DPE) and 3,3'- dibromodiphenyl ether (BDE-15). Figure 5 shows the response when DPE (left) and BDE-15 (right) were used as target molecules. It is clearly observed that the non-brominated species did not present significant change in the impedimetric signal, because of the specificity of the synthetic peptide towards brominated species. On the other hand, for BDE-15, a decrease in the charge transfer resistance was obtained from the Randles circuit analysis which may be explained as either the partial removal of the peptide from the surface due to the more stable structure of peptide bond to BDE-15 in solution or the change in conformation after the binding that allows the redox probe molecule to reach the surface more easily as found and explained in a recent paper.[26]

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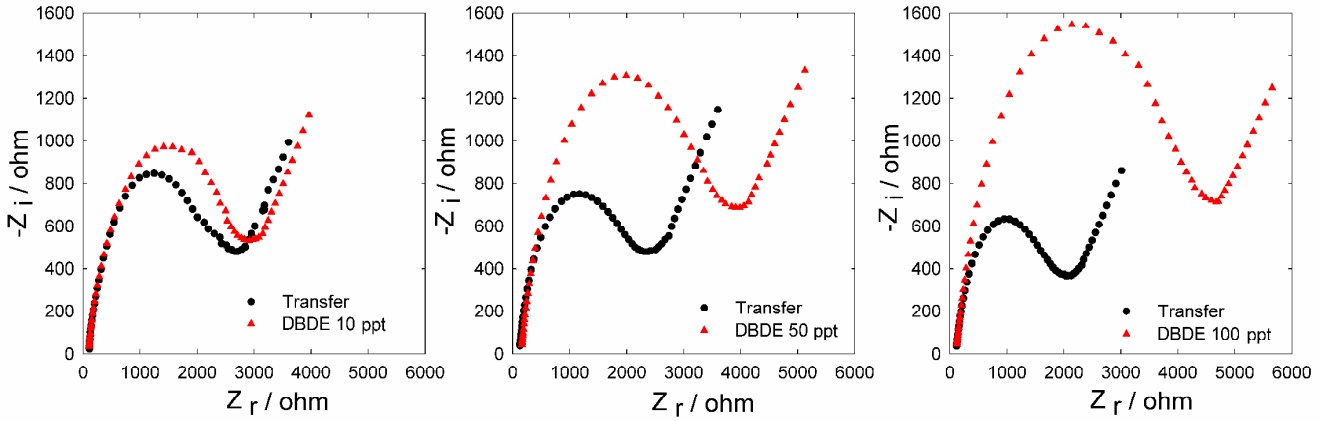

Fig. 3 Biosensor response at different DBDE concentrations (red triangles): 10 ppt (left), 50 ppt (center) and 100 ppt (right). Response after transfer is also plotted for each electrode represented in black dots

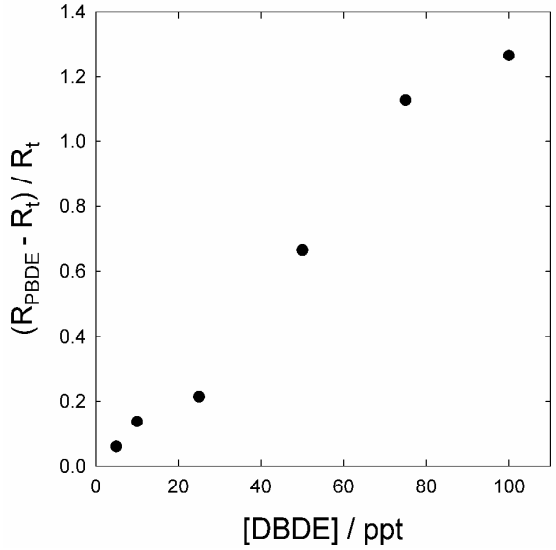

Fig. 4 Calibration plot for the biosensor in the 5 to 100 ppt range.

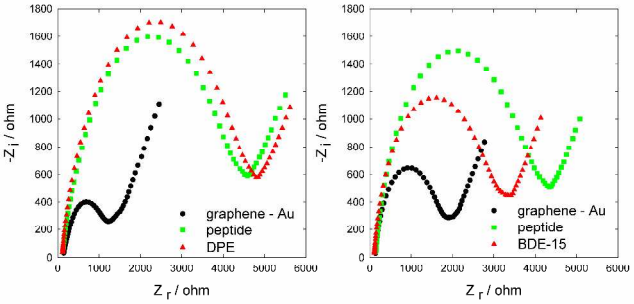

Fig. 5 Biosensor response after incubation in a saturated dissolution of DPE (left) and BDE-15 (right).

Conclusions

In conclusion, a gold decorated graphene impedimetric biosensor for the detection of DBDE is presented for the first time. Response towards DBDE in the range between 5% to saturation limit (~5 to 100 ppt range) presents a good linearity while response to molecules with similar structure but without any bromines (DPE) is insignificant.

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Notes and references

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1 C. A. de Wit, *Chemosphere,* 2002, **46**, 583.
2 M.L. Hardy, *Chemosphere*, 2002, **46**, 717.
3 M.L. Hardy, *Chemosphere,* 2002, **46**, 757.
4 E. Van den Steen, A. Covaci, V.L.B. Jaspers, T. Dauwe, S. Voorspoels, M. Eens, and R. Pinxten, *Environ. Pollut.*, 2007, **148**, 648.
5 L. Liu, W. Zhu, L. Xiao, and L. Yang, *J. Hazard. Mater.*, 2011, **186**, 883.
6 F.M. Jaward, N.J. Farrar, T. Harner, A.J. Sweetman and K.C. Jones, *Environ. Sci. Technol.*, 2004, **38**, 34.
7 W. Zhang, Y. Sun, C. Wu, J. Xing, and J. Li, *Anal. Chem.*, 2009, **81**, 2912.
8 *M.J. La Guardia, R.C. Hale and E. Harvey, Environ. Sci. Technol.*, 2006, ***40***, 6247.
9 S. A. Mackintosh, A. Pérez-Fuentetaja, L. R. Zimmerman and G. Pacepavicius, M. Clapsadl, M. Alaee, D. S. Aga, *Anal. Chim. Acta*, 2012, **747**, 67.
10 I. Fulara and M. Czaplicka, *J. Sep. Sci.*, 2012, **35**, 2075.

11 D. W. Kimmel, G. LeBlanc, M. E. Meschievitz, and David E. Cliffel, *Anal Chem*., 2012, **84**,685.
12 C. Ocaña, M. Pacios and M. del Valle, *Sensors*, 2012, **12**, 3037.
13 I. Szymanska, H. Radecka, J. Radecki and R. Kaliszan, *Biosens. Bioelectron*., 2007, **22**, 1955.
14 S. Liu, X. Xing, J. Yu, W. Lian, J. Li, M. Cui and J. Huang, *Biosens. Bioelectron*., 2012, **36**, 186.
15 A. H. Loo, A. Bonanni and Martin Pumera, *Nanoscale*, 2012, **4**. 143.
16 A.D. Ellington and J.W. Szostak, *Nature*, 1990, **346**, 818.
17 A. Sett, S. Das, P. Sharma and U. Bora, Open *J. Appl. Biosens*., 2012, **1**, 9.
18 T.H. Kim, B.Y. Lee, J. Jaworski, K. Yokoyama, W.J. Chung, E. Wang, S.H. Hong, A. Majumdar and S.W. Lee, 2011, *ACS Nano*. **5**, 2824
19 E. Katz and I. Willner, *Electroanal*., 2003, **15**, 913.
20 A. Bonanni, A.H. Loo and M. Pumera, *TRAC-Trends Anal. Chem*., 2012, **37**, 12.
21 T. Kobayashi, M. Bando, N. Kimura, K. Shimizu, K. Kadono, N. Umezu, K. Miyahara, S. Hayazaki, S. Nagai, Y. Mizuguchi, Y. Murakami and Daisuke Hobara, *Appl. Phys. Lett*., 2013, **102**, 023112.
22 J.W. Jaworski, D. Raorane, J.H. Huh, A. Majumdar, and S.W. Lee, *Langmuir*., 2008, **24**, 4938.
23 A. Gutés, B. Hsia, A. Sussman, W. Mickelson, A. Zettl, C. Carraro and R. Maboudian, *Nanoscale*, 2012, **4**, 438.
24 A. Gutés, C. Carraro and R. Maboudian, *Biosens. Bioelectron*., 2012, **33**, 56.
25 www.epa.gov/iris/toxreviews/0035tr.pdf. Consulted on Feb 14th 2013.
26 A. Bonanni and M. Pumera, *ACS Nano*, 2011, **5**, 2356.