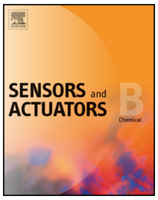
Sensors and Actuators B xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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



A dual-template biomimetic molecularly imprinted dendrimer-based piezoelectric sensor for ultratrace analysis of organochlorine pesticides

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A R T I C L E I N F O

Article history:
Received 27 June 2014
Received in revised form 21 October 2014
Accepted 25 October 2014
Available online xxx

Keywords:
Molecularly imprinted dendrimer
Quartz crystal microbalance
Dichlorodiphenyltrichloroethane
Hexacholrobenzene
Ultra trace analysis

A B S T R A C T

A double-template imprinted biomimetic dendritic nano-fibers based piezoelectric sensor was developed for the analysis of dichlorodiphenyltrichloroethane and hexachorobenzene prevalent together as organo-chlorinated pesticide residues in real samples, without any cross reactivity and false positives. For this, the immobilization of 2,5-thiophene dicarbonyl dichloride molecules was initially carried out on the surface of gold quartz crystal via Au–S links. Latter, these molecules were covalently attached to dendron molecules (monomer) followed by the free-radical polymerization at 65 °C, in the presence of both target analytes, cross-linker and initiator, leading to the development of self-assembled molecularly imprinted dendrimer nano-fibers on the gold surface. The method of analysis necessarily involved a typical 'saturation approach' in this work. Accordingly, one of the molecular cavities was completely saturated with an authentic amount of the respective analyte, whereas the cavity specific for other analyte was exposed to rebinding resulting in the frequency change in accordance with the mass of the analyte in question. Detection limits of dichlorodiphenyltrichloroethane and hexachorobenzene were realized as low as 0.75 and 0.69 ng mL^{-1} (S/N = 3), and linearity observed in the concentration ranges 5.0–150.0 and 5.0–75.0 ng mL^{-1}, respectively.

1. Introduction

Although many public benefits have been realized by the use of pesticides, their sustained impact on the environment and human health is substantial. Organochlorine pesticides (OCPs) are known to raise much global concern primarily due to their toxicity, resistance to degradation, potential for long-range transport, and their tendency to accumulate in fatty tissues (lipophilicity). Among the OCPs, dichlorodiphenyltrichoroethane (DDT) and hexachlorobenzene (HCB) are the most widely used product for pest and fungus control. These OCPs have demonstrated estrogenic activity in in vitro assays and adverse reproductive effects in fish and wildlife [1], along with carcinogenic and endocrine disrupting effects in humans [2,3] Transported into the human body by consumption of ordinary foods, these tend to biomagnify up to 70,000 times in the food chain [4,5]. Although OCPs production, usage and disposal to the environment have been regulated or prohibited in most countries [6,7], DDT is still being used for hygiene purposes, such as malaria control, in many low latitude developing countries, including India [8,9]. On the other hand, HCB, a by-product of organic chemical production processes, is still produced outside the United States [10]. Even after such restrictions, large quantities of residual fractions still remain in the natural environment due to their persistence and cause adverse effects on organisms and human beings. The precise analysis of OCPs in ecoaquatic systems and biological samples is thus warranted for the accurate information on the distribution of persistent organic pollutants and background data useful in bioremediation and ecological risk management.

Till date many methods have been developed for the simultaneous analysis of DDT and HCB in real samples; the most common methods include thin-layer chromatography and high-performance thin-layer chromatography [11], and gas chromatography (GC) [12]. Because of lipo-solubility and low volatility of these pesticides, GC–mass spectrometry and electron capture detector have been the preferred approach due to their high sensitivity and selectivity [13]. However, these techniques are expensive and tedious involving time-consuming separation steps [14,15].

A particularly useful molecular sensing technology is based on quartz crystal microbalances (QCMs), a family of very sensitive and

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http://dx.doi.org/10.1016/j.snb.2014.10.115
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inexpensive sensors that produce a frequency shift in quartz crystal vibration when molecules interact with the biomimetic sensor surface. As an advanced approach, QCM (based on micro-gravimetric measurements) and molecularly imprinted polymers (MIPs) have been combined together to design sensors for the selective and sensitive sensing of various molecules [16–20].

Molecular imprinting involves arranging suitable functional monomers around a template followed by their polymerization and cross linking to obtain biomimetic materials. After the template removal, the MIP can recognize and bind specific chemical/biochemical species. MIP sensors could be considered as best alternatives to biosensors, primarily in terms of higher shelf lives and biomimetic characteristics. With the recent advent of multi/double-imprinting in a single polymer format, multi-components often present in a sample can simultaneously be analyzed in the cost-effective manner saving both time and labor [21–31]. However, such analysis was rather challenging in many cases owing to the overlapped analyte signals and matrix effect. Unlike the traditional concept of trapping a single template in a linear polymer possessing the random coil structure or trapping a dual-template in a single linear polymer motif, the three dimensional motif of dendrimers could be quite capable of template inclusion in the empty spaces (dendritic boxes) available either in the interior or on the surface of molecularly imprinted dendritic model so produced [32]. Furthermore, despite the fact that multi-step synthesis is required to obtain higher generation dendrimers, such systems with large number of end-groups allow a sufficient degree of cross-linking to form a stable and ‘perforated’ dendritic infrastructure [33] suitable for the facile ingress-egress of test analyte(s).

Although various attempts have been made in order to fabricate MIPs for detection of DDT and HCB, individually [34–38], no MIP has been, hitherto, reported for their determination in binary mixture. To the best of our knowledge this is the first attempt toward the fabrication of a dual-template molecularly imprinted dendrimer (MID)-QCM sensor for the determination of DDT and HCB prevalent together, in real samples. Although selectivity with an individual target using MID-QCM sensor is not a problem, the difficulty arises in the measurement when both templates are allowed to be trapped simultaneously in a double-templated single format. To address this problem, we have resorted to a typical novel approach that exclusively involves the saturation of one type of pockets (dendritic boxes) with an authentic amount of respective analyte, allowing the remaining pockets to be accessible for the specific exposure to other analyte, and vice versa. Furthermore, in order to improve the sensitivity, the surface area of sensing material (MID) was substantially enhanced simply by adopting the ‘grafting from’ approach [39] to obtain a homogeneous growth of the nano-fibers on the QCM surface.

2. Experimental

2.1. Materials and reagents

Demineralized triple distilled water (conducting range $0.06–0.07 \times 10^{-6}\,\mathrm{S\,cm^{-1}}$) was used throughout the experiment. Acrylamide, 2,2-azoisobutyronitrile (AIBN), melamine (mel), and thionyl chloride, were purchased from Loba chemie (Mumbai, India). Solvents, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), N,N-dimethyl acetamide (DAC), chloroform ($CHCl_3$), and ethanol were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Ethylene glycol dimethacrylate (EGDMA), Hexachlorobenzene (HCB), and terepthalic acid were provided by Fluka (Steinheim, Germany). 2,5-Thiophene dicarbonyl dichloride (TDD), 1,1,1-Trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), and all interferents were purchased from Aldrich (Steinheimer, Germany) and Supelco (Pennsylvania, USA). All chemicals were of analytical grade and used without further purification. The pH values of test solutions were adjusted by the addition of a few drops of either 0.1 M HCl or 0.1 M NH_4OH. Standard stock solutions of DDT and HCB ($500.0\,\mu\mathrm{g\,mL^{-1}}$) were prepared in ethanol. The working solutions were prepared by diluting stock solution with ethanol.

Human blood serum was obtained from the Institute of Medical Science, Banaras Hindu University (Varanasi, India). Serum samples were brought in ice packs and stored in a refrigerator at ∼4 °C, till use. Water samples were obtained from the bank of a local river (Ganges) with the help of a water-sampler unit. Any pre-treatment of real sample (ultra filtration, ultra centrifugation, de-proteinizations etc.) was deliberately avoided since it may lead to inaccuracies in ultra trace analysis.

2.2. Apparatus

Micro-gravimetric measurements were performed using 5 MHz (AT-cut quartz crystals in a Teflon holder and a quartz crystal analyzer) model. The following equation [40] has been established for an AT-cut shear mode QCM:

$$\Delta f = -2f_0^2(\mu_\mathrm{q}\rho_\mathrm{q})^{-1/2}\frac{\Delta m}{A} \qquad (1)$$

where Δf is the measured frequency shift in hertz due to added mass, f_0 is the fundamental resonant frequency of the dry quartz crystal (5 MHz), Δm the surface mass loading in grams, ρ_q the density of quartz ($2.65\,\mathrm{g\,cm^{-3}}$), μ_q the shear modulus ($2.95 \times 10^{11}\,\mathrm{dyn\,cm^{-2}}$), and A is piezo-electrically active area ($1.37\,\mathrm{cm^2}$). For the 5 MHz quartz crystals used in this work, Eq. (1) predicted $k_1 = -2f_0^2(\mu_\mathrm{q}\rho_\mathrm{q})^{-1/2} = -5.66 \times 10^7$ and the frequency change of 1 Hz corresponds to a mass increase (Δm) of 24.2 ng. FT-IR (KBr) spectra of scrapped coated layers were recorded on Varian FT/IR (USA) spectrometer. Thermo-gravimetric analyses (TGA) of MID-templates adduct and MID were carried out with a PerkinElmer-STA 6000 (USA). Exeter Analytical Inc., model CE-400 Elemental analyzer, Mexico, was used for elemental analysis. Surface morphologies of modified gold crystal surfaces were studied with scanning electron microscope (SEM) [JEOL, JSM model-840 A (Netherland)] and atomic force microscope (AFM) (using a NT-MDT microscope, NT-MDT Co., Russia) in the semi contact mode. All experiments were carried out at 25 ± 1° C.

2.3. Synthesis of dendron monomer

The following steps were carried out for the synthesis of dendron monomer (Scheme 1):

1. *Synthesis of terepthaloyl dichloride (**1**)*: Terepthalic acid was converted as terepthaloyl dichloride (TPD) using the reagent thionyl chloride (for details, vide Supplementary data Section S.1].
2. *Synthesis of building block (**2**)*: AB_2 type building block (**2**) as mentioned in Scheme 1, was obtained by combining mel and TPD in 1:2 molar ratio (for details, vide Supplementary data Section S.1).
3. *Synthesis of G1 dendron (**3**)*: DMF suspensions of acrylamide (0.284 g, 4 mmol) and **2** (0.918 g, 2 mmol) were mixed together in 2:1 molar ratio, and stirred for an hour. The reaction mixture was refluxed overnight at 80 °C. The product obtained (∼56% yield) was filtered, washed with hot (35 °C) DMF–water (10 mL, 1:1, v/v) to remove residual precursors, if any. This was characterized by the elemental and FT-IR analyses [CHN analysis: Calculated for $C_{25}H_{20}N_8O_6$: C, 56.8%; H, 3.8%; N, 21.2%. Found: C, 56.6%; H, 3.5%; N, 21.3%; FT-IR (KBr) $\nu_\mathrm{max}/\mathrm{cm^{-1}}$: 3429 ($NH_2$ stretch), 3135 (Ar C–H stertch), 1659 (amide I), 1639 (amide II), 1601 (double

Please cite this article in press as: B.B. Prasad, D. Jauhari, A dual-template biomimetic molecularly imprinted dendrimer-based piezoelectric sensor for ultratrace analysis of organochlorine pesticides, Sens. Actuators B: Chem. (2014), http://dx.doi.org/10.1016/j.snb.2014.10.115