Core–shell molecularly imprinted polymer-based solid-phase microextraction fiber for ultra trace analysis of endosulfan I and II in real aqueous matrix through gas chromatography–micro electron capture detector

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**A R T I C L E   I N F O**

Article history:
Received 25 November 2013
Received in revised form 2 January 2014
Accepted 11 February 2014
Available online 20 February 2014

Keywords:
Molecular imprinting
Endosulfan I and II
Solid-phase microextraction
Plackett–Burman design
Sample clean-up

**A B S T R A C T**

In this study, core–shell molecularly imprinted polymer selective for endosulfan I and II was prepared by copolymerization of Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}-methacrylamide composites and N,N-methylene-bis-acrylamide. The synthesized polymer was thoroughly characterized by FT-IR, TGA, and SEM. The adsorption properties of the MIP and NIP were demonstrated by equilibrium rebinding experiments, pseudo-second-order kinetic model, LF-isotherm and Scatchard analysis. The competitive recognition studies were performed with endosulfan I and II and structurally similar compounds: aldrin, dieldrin and heptachlor. The imprinting factors (IF) of endosulfan I and II were found to be 10.1 and 9.1, respectively, which were much higher than the imprinting factors (IF) of other cyclodienes. The imprinted polymer was then coated on stainless steel wire to develop an easy and simple laboratory made solid phase microextraction device for selective extraction of endosulfan I and II from water samples of environmental importance. Also the main parameters influencing coating of fiber and microextraction procedure were investigated and optimized using Plackett–Burman and Central Composite designs. The developed method was thoroughly validated for its linearity, selectivity, precision and accuracy. The developed MISPE method’s linearity ranged from 7 to 5 × 10\textsuperscript{4} ng l\textsuperscript{-1} (R\textsuperscript{2} = 0.999) and from 10 to 5 × 10\textsuperscript{5} ng l\textsuperscript{-1} (R\textsuperscript{2} = 0.999) for endosulfan I and II, respectively. The limits of detection for endosulfan I and II were found to be 2 ng l\textsuperscript{-1} and 3 ng l\textsuperscript{-1}, respectively. However, the limits of quantification for endosulfan I and II were 7 ng l\textsuperscript{-1} and 10 ng l\textsuperscript{-1}, respectively.

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1. Introduction

Organochlorine pesticides are a group of chlorinated cyclodienes that are not only extremely toxic but are also highly persistent organic pollutants (POPs). Although they play a vital role in crop production and protection, their widespread use has resulted in their bioaccumulation throughout the food chain and led to the contamination of various environments. The accumulation of organochlorines can have worst effects on human health and the environment, even though many of them are banned. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9a-hexahydro-6,9-methano-2,4,3-benzo-odioxa-thiepin-3-oxide) is one of the persistent organochlorine pesticides that is not banned worldwide and is consistently used in agriculture, viticulture and horticulture [1,2]. The half-life of endosulfan in water depends on the amount of oxygen dissolved, turbidity, pH and other contaminants in the water; it varies from 3 days to 5 months. It is a mixture of two stereoisomers, namely α- and β-endosulfan in a ratio of 7:3 [3]. Endosulfan is an insecticide that acts as a contact poison in a wide variety of insects and mite such as Colorado potato beetle, cabbage worm, and pests on fruits, vegetables, tobacco and cotton. It is also used as wood preservative and home garden insecticide for tsetse fly [4]. It is an endocrine disruptor that can cause venous effects in almost all tissues of both humans and animals, including liver, lung, central nervous system, genital system, pancreas etc. [2,5–7]. It affects the biochemistry of blood and hematological values [7]. Endosulfan can be easily absorbed into an organism through its stomach, lung and even through the skin and hence is an active contact hepatotoxin [2]. India has already experienced
its devastating effects in its province named Kerala where it was sprayed constantly on the crops [8]. Due to its above-mentioned injurious effects the United Nations Environmental Protection Agency (UNEP/A) has classified endosulfan as a highly toxic chemical [9]. The European Union has set a maximum permissible limit of 1.0 μg l−1 for each pesticide in surface water and 0.1 μg l−1 in drinking water [10]. Thus monitoring endosulfan residues in water is very important for human health protection as well as for environmental control. Organochlorines are usually determined by GC–ECD, GC–MS or LC–MS preceded by sample clean-up using liquid–liquid extraction, solid phase extraction, solid-phase microextraction or single-drop microextraction [11]. Sample preparation is the bottleneck of whole analytical procedure; the enhanced selectivity during extraction or cleanup process of environmental matrix is an area of extreme importance. Although the recent techniques such as solid-phase microextraction or single-drop microextraction offer many advantages over classical techniques like liquid–liquid extraction, their non-selective nature is still a challenge for the researchers. The non-selective character of these extraction procedures offers insufficient clean-up and results in co-extracted peaks or noise in chromatography that in turn lead to the reduced column and liner life. Besides selective extraction processes improve the sensitivity and versatility of assay procedures [12].

Molecular imprinting is one of the most versatile and promising options to incorporate selectivity and to enhance sensitivity of extraction methods. Molecularly imprinted polymers (MIPs) are materials particularly designed to present valuable molecular recognition properties. This molecular recognition brilliance had been very attractive in many different fields, such as sensors, enantiomeric separations, biomedical and analytical applications, etc. Regarding analytical applications, MIPs have been applied successfully in many distinct ways; thus far, the main interest has focused on solid-phase microextraction (SPME), more specifically the up-and-coming technique of molecularly imprinted solid-phase microextraction (MISPME) [13]. In recent years, many different MIPs have been synthesized to selectively extract a diverse range of compounds from many different matrices [14]. The molecularly imprinted SPME was first attained by Koster et al., who obtained MIP coated silica fiber for the SPME of brombuterol from human urine [15]. However, this method showed severe difficulties in controlling polymeric film thickness, as the desired graft polymerization was always accompanied by polymerization in solution when azo-initiators were used [16]. Since then a number of procedures are explored by researchers in order to overcome the drawback of molecularly imprinted SPME methods. MIP-coated silica fiber was further improved by using surface reversible addition-fragmentation chain transfer polymerization (RAFT), which offered a better control of the thickness of MIP coating [17]. Mazzotta et al. prepared MIP coated fibers by electropolymerization of conducting polymers [18] and Djozan prepared MIP fibers inside fused-silica capillaries [19]. Apart from these, the organic–inorganic hybrid sol–gel-imprinted, coated fibers for SPME of ascorbic acid and polypropylene hollow fibers for SPME of chlorogenic acid in medicinal plants had also been developed [20,21]. All above methods offer advantages with some restrictions such as fragility, lack of porosity and thereby poor accessibility of target analytes to binding sites, etc. due to the practical limitations of immobilization of tailor-made preparation onto fiber. Thus, stability and capacity of such fibers need improvement and the quest of researchers to produce molecularly imprinted SPME fibers that are robust, cost effective, most selective and sensitive, reproducible, thermally stable, easily synthesized and having better kinetics, capacity and compatibility with environmental matrix is still continued.

In the present study, we are reporting core–shell imprinted particles selective for endosulfan that are successfully employed for enhanced and efficient pre-concentration of endosulfan I and II through solid phase microextraction technique prior to GC–μECD detection. The synthesized MIP was characterized thoroughly and examined for its adsorption capacity and selectivity. It was coated on simple and cheap laboratory made solid-phase microextraction device using very simple method. The method was devised and optimized using Plackett–Burman design of experiment and further verified with Central Composite Surface design. The developed method was validated and found robust and highly sensitive to low limits of detection for endosulfan I and II. In the present investigation, we tried to resolve the problems of low capacity, slow kinetics, poor accessibility of template molecules to binding sites, non-adherence of MIP on fiber surface, uncontrolled polymer thickness on the fiber surface and fragility of molecularly imprinted fiber.

2. Experimental

2.1. Materials

Endosulfan and tetrahydrofuran (THF) were purchased from TCI Tokyo, Japan. N,N’-methylene-bis-acrylamide (ultra-pure) was purchased from ICN Biomedicals Inc., Germany. Iron(II) chloride tetrahydrate (FeCl2 4H2O), iron(III) chloride hexahydrate (FeCl3 6H2O) and ammonium per sulphate were purchased from Merck, Germany. Toluene, ammonium hydroxide, methanol, triethylamine, mineral oil, acetone, dimethyl formamide (DMF) and dichloromethane were purchased from Fisher Scientific, UK. Methacryloyl chloride and (3-aminopropyl)triethoxysilane (APTES) were obtained from Sigma–Aldrich, Finland. Milli-Q water was obtained from ELGA Milli-Q water System (Model: Classic UVF), UK.

2.2. Preparation of endosulfan imprinted core–shell polymer (MIP)

Fe3O4 magnetite particles were used as core and prepared by a chemical co-precipitation method described previously with minor modifications [22]. Briefly, FeCl3 4H2O (1.72 g) and FeCl3 6H2O (4.72 g) were dissolved in 80 ml of de-aerated purified milli-Q water contained in a three neck round bottomed flask with vigorous stirring at 800 rpm, under nitrogen stream. The reaction mixture was maintained at 80 °C and 10 ml of ammonium hydroxide was added drop by drop into the flask till the pH of reaction mixture reached to 10. The reaction was maintained for 30 min and then the products were cooled to room temperature. The black magnetic precipitates were isolated from reaction system through a strong magnet and washed several times with distilled water until the washings became neutral. The resulting magnetic particles were dried at room temperature under vacuum.

The synthesized Fe3O4 magnetite particles were modified with APTES by the method previously reported [23]. Precisely, dried Fe3O4 particles were dispersed in methanol (100 ml) and milli-Q water (1 ml) by sonication for 15 min, followed by the addition of APTES (1.5 ml). The mixture was allowed to react for 12 h at 40 °C under continuous mechanical stirring. The resultant silanized Fe3O4 composites were collected by a strong magnet, washed 3 times with methanol and distilled water, respectively, and then dried to powder at room temperature under vacuum.

The 3-aminopropyl groups of Fe3O4@APTES composites were further modified to methacrylamide by dispersing Fe3O4@APTES composites into dried toluene, followed by addition of
methacryloyl chloride (5 ml) and triethylamine (12.7 g) to the reaction mixture. The reaction was carried out under nitrogen stream. The resulting silanized Fe₃O₄ composites with methacrylamide functionality were collected by external magnetic field, washed and then dried to powder at room temperature under vacuum.

Imprinted polymer of copolymerized Fe₃O₄@SiO₂-methacrylamide composites and N,N'-methylene-bis-acrylamide was prepared by means of water in oil (W/O) suspension polymerization. The dried Fe₃O₄@SiO₂-methacrylamide composites were dispersed in an aqueous solution consisting of DMF (2 ml) and milli-Q water (8 ml), followed by the addition of endosulfan (3.0 mmol) in an ice bath. Fe₃O₄@SiO₂-methacrylamide composites and template molecules were then allowed to generate hydrogen bonding between them. The crosslinker N,N'-methylene-bis-acrylamide (5.188 mmol) and initiator ammonium persulphate (0.7 mmol) were added to the mixture and dissolved. The 20 ml mineral oil (oil phase) was taken in a flask installed with a reflux condenser and a mechanical stirrer. Oil phase was continuously stirred under nitrogen stream while the addition of aqueous phase to the flask. Polymerization was carried out for 6 h at 50 °C. MIP was separated through an external magnet and washed with methanol for 10 h by Soxhlet extraction method. The complete removal of template and unreacted substances was guaranteed by washing MIP once again with acetone and injecting acetone wash into GC–μECD. The non-imprinted polymer (NIP) was also synthesized following same procedure but in the absence of endosulfan. The MIP and NIP were dried in vacuum at room temperature and used for further studies.

**Fig. 1.** Schematic representation of imprinting of endosulfan in poly(methacrylamide functionalized magnetic composites-co-N,N'-methylene-bis-acrylamide).

### 2.3. Characterization of endosulfan imprinted (MIP) and non-imprinted (NIP) core–shell polymers

The synthesized MIP and NIP were characterized with FT-IR Nicolet 5700 Thermo Electron Corporation, USA, and thermogravimetric analyzer (TGA), Shimadzu GTG-60H system. The polymers as well as solid-phase microextraction fiber were characterized with scanning electron microscope (SEM), JSM-6490 LV, Jeol, Japan.

### 2.4. Chromatographic determination of endosulfan

In all instances aqueous solutions containing endosulfan were extracted with equal volume (single extraction) of ethyl acetate/n-hexane (1:1) and 2 μl of extract was injected onto GC–μECD. Similar procedure was adopted for constructing calibration graphs, sorption studies and samples analysis, however the non-aqueous solutions containing endosulfan were injected in GC–μECD soon after separating MIP or NIP by applying magnetic field. The chromatographic system consisted of an Agilent technologies 7890 A gas chromatograph installed with 30 m HP-5 column having 0.25 μm film thickness of 0.25 μm was used with ECD detection system. Nitrogen was used as carrier gas. The calibration curves of endosulfan I and II were obtained at column temperature starting from 140 °C to 210 °C at the rate of 30 °C min⁻¹, the temperature was held for 1 min and then raised to 260 °C at the rate of 25 °C min⁻¹, held for 2 min and finally increased to 290 °C at the rate of 40 °C min⁻¹ and held for 1 min. The temperatures of injector and detector were 250 °C and 310 °C, respectively.

### 2.5. Sorption studies

To evaluate the binding kinetics of MIP, 5 ml of 10 μg ml⁻¹ endosulfan I and II solution in methanol was added into six separate glass
flasks containing 5 mg of dried polymer, which were then shaken (100 rpm) on orbital shaker for 5, 10, 15, 20, 25 and 30 min at room temperature. To measure the adsorption capacity, 5 mg of dried MIP was added to 5 ml solutions with various concentrations of endosulfan I and II ranging from $4.8 \times 10^{-3}$ to $4.0 \times 10^{-2} \mu g/ml^{-1}$ in methanol. The mixtures were shaken for 10 min with shaking speed of 100 rpm on orbital shaker at room temperature to facilitate the adsorption of endosulfan onto the MIP sorbent. To check the adsorption capacity in aqueous solutions pH was optimized first. To find the optimum pH, 5 mg of dried MIP was added to 5 ml of 0.33 µg/ml^{-1} aqueous solutions of endosulfan I and II at pH ranging from 1 to 9. The mixtures were shaken for 10 min with shaking speed of 100 rpm on orbital shaker at room temperature to evaluate the performance of MIP in aqueous system, 5 mg of dried MIP was added to 5 ml aqueous solution maintained at pH 1.0 with various concentrations of endosulfan I and II ranging from $4.8 \times 10^{-3}$ to $3.3 \times 10^{-2} \mu g/ml^{-1}$. The mixtures were shaken for 10 min with shaking speed of 100 rpm on orbital shaker at room temperature to facilitate the adsorption of endosulfan onto the MIP sorbent. Recognition studies were performed with endosulfan I and II and other cyclodienes pesticides i.e. aldrin, dieldrin and heptachlor. 5 mg of dried MIP was placed in aqueous mixture of endosulfan I and II, aldrin, dieldrin and heptachlor with initial individual concentrations of 0.3 µg/ml^{-1} at pH 1. The mixtures were shaken for 10 min at room temperature. Subsequently MIP was separated by an external magnetic field and solutions were analyzed as given in Section 2.4. The MIP was also examined with the same procedures. The data were obtained in triplicate and reported as the mean ± standard deviation (S.D.).

2.6. Molecularly imprinted solid-phase microextraction experiments (MISPEM)

A thoroughly cleaned stainless steel wire of diameter 160 µm was sonicated in ethanol for half hour and then rinsed with milli-Q water. 1 mg of MIP was dispersed in 1 ml of THF, followed by addition of 0.4 mg of PVC (2.5:1 MIP:PVC). The stainless steel wire mounted in a laboratory made solid-phase microextraction device was dipped in the mixture contained in a micro vial till the length of 1.5 cm and sonicated till all of the solvent was evaporated. The coated wire was treated thermally at 100 °C and then used for further analyses.

2.7. Experimental designs

2.7.1. Plackett–Burman design and Central Composite design

The Plackett–Burman design (PBD) was used as a screening approach with the aim of establishing the significant factors that influence the proposed MISPE method and selecting suitable extraction conditions. The Plackett–Burman matrix is shown in Table S-2 in supplementary information, where the low (−) and high (+) levels are those specified in Table S-1 in supplementary information. After screening out the variables that did not have a significant effect on the recovery of endosulfan, the significant factors were further optimized to provide the maximum recoveries through Central Composite design. The significant variables were monitored in thirteen combinations (Table S-3), organized according to the Central Composite design. The detailed discussion of these experimental designs is given in supplementary information.

2.8. Validation study

The developed MISPE method was validated by evaluation of following validation parameters: selectivity, linearity, sensitivity, precision, accuracy and detection and quantification limits. This study was performed on synthetic wastewater samples spiked with endosulfan I and II to provide samples containing concentration range of 3 x 10^{-4}–5 x 10^{-3} ng/l. The linearity and sensitivity were established through the calibration graph obtained by triplicate analysis of endosulfan spiked synthetic wastewater samples. Linearity was demonstrated calculating the regression line by the least squares method and expressed by coefficient of determination (R²) where sensitivity is the slope of the calibration curve. Selectivity of the method was evaluated by the comparison of chromatograms obtained from spiked synthetic wastewater sample extracted through polydimethylsiloxane (PDMS) fiber and MIPs-based fiber. Its purpose was to verify possible presence of interference that could compromise the determination of endosulfan. The PDMS fiber of 100 µm film thickness was conditioned at 250 °C for 30 min prior to use. Synthetic wastewater sample was prepared by the method as reported earlier [24].

Intra-assay and inter-assay precision data were determined using low, medium, and high concentrations (Table 3). Intra-assay precision was assessed using five replicates of each concentration in the same day. Inter-assay precision was evaluated for three replicates analyzed on three separate days (n = 3). The results were expressed as percent relative standard deviation. The accuracy of the method was determined as percent recovery, by spiking river water and wastewater samples at three different concentrations: 10, 5 x 10^{2} and 5 x 10^{3} ng/l^{-1}, in triplicate analyses. The limit of detection and limit of quantification were determined from signal-to-noise ratios of 3 and 10, respectively, measured at the approximate retention time of the corresponding analytes peaks.

3. Results and discussion

3.1. Synthesis

As shown in Fig. 1, the synthesis of the MIP was a multistep process, which involved synthesis of Fe_{3}O_{4} magnetite particles as core, silica–shell deposition (Fe_{3}O_{4}@APTES), modification of Fe_{3}O_{4}@APTES with MIP and removal of endosulfan template. At first, Fe_{3}O_{4} magnetite particles were synthesized by the coprecipitation method. The motivation for the use of magnetite particles as core was their exceptional compatibility with variety of polymer shells as well as their unique physicochemical properties due to surface effect and finite size effect [25]. Secondly, the surface of Fe_{3}O_{4} particles was coated with silica by APTES in a sol–gel process. SiO_{2} shell provided a biocompatible and hydrophilic surface, and prevented oxidation of Fe_{3}O_{4}. Furthermore, 3-aminopropyl groups present in APTES were beneficial to chemical modification on the surface of Fe_{3}O_{4}@APTES. Thus, methacrylamide-group was introduced onto Fe_{3}O_{4}@APTES using methacryloyl chloride to ensure tight growth of imprinted polymer layer. The major purpose of using APTES as silane was to introduce methacrylamide modification on the surface of core particles. Finally, a hydrophilic molecularly imprinted layer on Fe_{3}O_{4}@SiO_{2}-methacrylamide was produced via water in oil (W/O) suspension polymerization. Endosulfan imprinted polymer reported so far contains methacrylate-based polymer backbone [26]. Although some MIPS synthesized by conventional imprinting protocol based on poly (methacrylic acid-co-ethylene glycol dimethacrylate) [27,28] exhibit recognition properties under aqueous conditions, but this technology often fails to generate MIPS for use in pure aqueous environments. This is often due to nonspecific hydrophobically driven binding [28,29], the extent of which depends on the hydrophobicity of the template and the exposed surface of the materials. Suppressing the nonspecific binding may result in the formation of MIPS which can be implemented in separations or chemical sensors in aqueous medium such as biological fluids
and environmental waters [30]. Thus, in the current study, such type of hydrophilic imprinted material was synthesized using core–shell magnetite particles having modified hydrophilic surface and cross-linker; N,N-methylene-bis-acrylamide, as their surface shows low non-specific adsorption in aqueous medium. In the pre-polymerization mixture, the template molecules interacted with the functional monomer via hydrogen bonding, and during the polymerization process, the template molecules were well-implemented in the polymer matrix (Fig. 1). The MIP was synthesized by extremely simple method; also washing of polymer through soxhlet extraction method confirmed the complete removal of template as well as unreacted monomer and crosslinker. However conventional methods for the preparation of MIPs require 20–24 h where polymerization time and removal of template are extremely exhausting tasks [31]. The resulting polymer was also magnetically susceptible and therefore easily separated by external magnetic field after batch adsorption process. The surface of magnetic core–shell molecularly imprinted polymers (MIP) is small in dimension but extremely large in surface to volume ratio and they are easy to prepare and chemically stable. Furthermore, a mass of recognition sites is situated at the surface of the core–shell materials that allows faster rebinding and easy removal of template due to easy accessibility and low mass transfer resistance to target molecules. Besides, the MIPs also display excellent adsorption ability and selectivity [32]. Due to above significant properties of magnetic core–shell molecularly imprinted polymers we chose to use them as SPME coating. The synthesized polymer was first critically characterized and tested for selectivity, kinetics, robustness and capacity and then applied as coating for SPME fiber.

3.2. Characterization

FT-IR spectroscopy was used to study the chemical structure of MIP. A peak indicating stretching vibration of Fe–O was observed at ~576 cm⁻¹ for all surface modified samples, indicating that the main structure was not changed by the modification [33] (Fig. 2). The absorption bands at ~2854 cm⁻¹ and ~2928 cm⁻¹ (C–H stretching) in Fig. 2b indicated the presence of –CH₃ groups of APTES coated on Fe₃O₄ composites [34] and bands at ~1621 cm⁻¹, ~1464 cm⁻¹ and ~1350 cm⁻¹ corresponded to N–H bending vibration of primary amine, C–N aliphatic vibration and C–H bending vibration of aminopropyl group, respectively. Further the bands at ~970 cm⁻¹ and ~753 cm⁻¹ that were due to the Si–O–H and Si–O stretching vibrations confirmed the silanization of Fe₃O₄ particles [35]. In Fig. 2c sharp bands at ~1676 cm⁻¹ and ~1541 cm⁻¹ corresponded to non conjugated C=C stretching and N–H bending of secondary amide, respectively, that confirmed the modification of 3-aminopropyl group of Fe₃O₄-APTES to acrylamide. A broad band at ~3400 cm⁻¹ in FT-IR spectra of MIP (Fig. 2d) due to N–H stretching of secondary amides confirmed the completion of polymerization process, also sharp bands at ~1600 cm⁻¹ and ~1460 cm⁻¹ corresponded to carbonyl (C=O) stretching of secondary amide and C–H bending of methyl groups, respectively. These bands confirmed the presence of monomer and crosslinker in polymer structure.

Fig. 3a shows the SEM image of MIP that reveals that most of the particles are homogeneous in size; however the MIP particles were agglomerated. Fig. 3b is the SEM image of bare stainless steel wire that can be easily differentiated from Fig. 3c that is SEM image of MIP coated stainless steel wire. The SEM image revealed that the polymer had been coated on wire homogeneously resulting in porous fiber with appropriate thickness of MIP. Approximately 20 μm thick layer of MIP contributed to fine efficiency of MISPME.

TGA curve of MIP and its explanation is given in supplementary information (Fig. S-1).

Fig. 2. FT-IR spectra of (a) Fe₃O₄ (b) Fe₃O₄@SiO₂ (c) Fe₃O₄@SiO₂-methacrylamide (d) endosulfan imprinted poly(methacrylamide functionalized magnetic composites-co-N,N-methylene-bis-acrylamide).

Fig. 3. (a) SEM image of MIP; (b) SEM image of bare stainless steel wire; (c) SEM image of MIP coated MISPME fiber.
3.3. Sorption studies

Fig. 4a presents the adsorption kinetics of endosulfan I and II solutions onto MIP and NIP. The kinetic studies were performed by adding 5 mg of polymer to 5 ml of endosulfan I and II solution (10 μg ml⁻¹). The results showed that the MIP had fast uptake kinetic for both endosulfan I and II and the binding equilibrium was almost reached within 10 min. The property of rapid adsorption kinetics of the MIP is an advantage for using the MIP as an adsorbent for the SPME.

The pseudo-second-order kinetic model was used to describe the adsorption process:

\[ \frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{t}{q_e}, \]

where \( k \) is the rate constant of second-order sorption (mg g⁻¹ min⁻¹) and \( q_e \) is the adsorption capacity at any time (mg g⁻¹). From the equilibrium, \( t/q_t \) versus \( t \) is plotted in Fig. 4a the coefficients of determination (\( R^2 \)) were 0.9975 and 0.9969 for endosulfan I and endosulfan II and the \( q_e \) values (9.99 mg g⁻¹ for endosulfan I and 9.98 mg g⁻¹ for endosulfan II) obtained from the pseudo-second-order kinetic model were very close to the \( q_e \) values (9.97 mg g⁻¹ for endosulfan I and 9.86 mg g⁻¹ for endosulfan II) obtained from experiment, which indicated that the adsorption of the MIP toward endosulfan I and II follows the pseudo-second-order kinetic model very well.

To investigate the affinity of endosulfan imprinted MIP and NIP, a steady-state binding method and subsequent Scatchard and LF analysis were carried out. The binding isotherms of endosulfan to MIP and NIP were determined in the concentration range of \( 4.8 \times 10^{-3} - 4 \times 10^2 \) μg ml⁻¹ and the results were shown in Fig. 4b. The calculations of static adsorption capacities of the polymer were based on the following formula:

\[ Q = \frac{(C_i - C_f) \times V}{m} \]

where \( Q \) (mg g⁻¹) is the mass of endosulfan adsorbed per gram of polymer, \( C_i \) (mg l⁻¹) is the initial concentration of endosulfan, \( C_f \) (mg l⁻¹) is its final concentration after adsorption, \( V \) (l) is the total volume of the adsorption mixture, and \( m \) (g) is the mass of polymer. The data (Fig. 4b) indicated the amount of endosulfan I and II bound to the MIP was increased along with increased initial concentration till the concentration reached to 50 μg ml⁻¹; the adsorption capacity curve became relatively flat and reached saturation at high endosulfan I and II concentrations. However, the amount of endosulfan isomers bound to NIP at equilibrium experiment was only increased to 16.89 μg ml⁻¹ and reached saturation. These results indicated that the amount of endosulfan isomers bound to MIP was dramatically higher than NIP at higher concentrations because of the fact that more specific binding sites of MIP were generated at higher concentrations and that was obvious due to imprinting. It seems that during the polymerization process, large population of endosulfan specific binding sites had been produced which were more activated even at higher concentrations of endosulfan. In order to find adsorption behavior of MIP in aqueous environment, first the effect of pH was optimized. Adsorption was carried out in the aqueous solutions of concentration 0.33 μg ml⁻¹ at the pH ranging from 1 to 9. Fig. 4c reveals that the adsorption was most favorable at acidic pH values and maximum adsorption was observed at pH 1; this may be due to the fact that endosulfan is stable at acidic pH values however it degrades in alkaline solution [36]. The binding isomers of endosulfan isomers to MIP were also determined in aqueous solutions of endosulfan at various concentrations ranging from \( 4.8 \times 10^{-3} - 33 \times 10^{-2} \) μg ml⁻¹. The concentration window was narrow due to limited solubility of endosulfan in aqueous solution. Fig. S-2 in supplementary
The saturation binding data were further processed to generate a Scatchard equation to estimate the binding properties of MIP and NIP. The Scatchard equation was as follows:

$$\frac{Q}{[\text{Endosulfan}]} = \frac{Q_{\text{max}} - Q}{K_D} \tag{3}$$

where \(Q\) is the amount of endosulfan bound to polymer at equilibrium; \(Q_{\text{max}}\) is the apparent maximum adsorption capacity; [Endosulfan] is the free analytical concentration at equilibrium and \(K_D\) is the dissociation constant. The values of \(K_D\) and \(Q_{\text{max}}\) could be calculated from the slope and intercept of the linear curve plotted at \(Q/[\text{Endosulfan}]\) versus \(Q\).

The Scatchard analysis of MIP and NIP was performed. It was observed that biphasic curves with two straight lines were obtained in the case of MIP in the plot region (Fig. 4d), which indicated that there existed two kinds of binding sites of high and low affinity.

For NIP biphasic curve was not observed which revealed that NIP only had low affinity binding sites. The adsorption capacity values of NIP for endosulfan I and II were 15.85 mg g\(^{-1}\) and 18.46 mg g\(^{-1}\), respectively, where as for MIP they were 56.29 mg g\(^{-1}\) and 54.46 mg g\(^{-1}\), respectively, from right slope and 2.95 mg g\(^{-1}\) and 1.28 mg g\(^{-1}\), respectively, from left slope of biphasic curves. These results reveal that the higher adsorption capacity of MIP and presence of both high and low affinity binding sites is due to imprinting, also the values of dissociation constant \(K_D\) of NIP were much higher than MIP that exposes the low binding strength of NIP. It also hinted that NIP did not have the specific adsorption. However, the specific adsorption of MIP was achieved through imprinting.

The MIP and NIP were also characterized through LF-isotherm (Fig. 4e).

The LF–isotherm equation is as follows:

$$B = \frac{N_i a F_m}{1 + a F_m} \tag{4}$$

where \(B\) and \(F\) are the equilibrium concentrations of bound and free guest in heterogeneous system, respectively. Whereas \(N_i\), \(a\) and \(m\) are the fitting coefficients and have physical meaning. \(N_i\) is the total number of binding sites. The variable ‘\(a\)’ is related to the median binding affinity \((K_a)\) via \(K_a = a^{1/m}\) and \(m\) is the heterogeneity index. The LF Fitting parameters were calculated from experimental data using solver function in MS Excel using \(R^2\) value to 1 and changing fitting coefficients i.e. \(N_i\), \(a\) and \(m\). One of the primary advantages of applying the LF binding model to MIP was that binding properties could be readily measured. These parameters enabled direct comparison of the binding parameters of MIP even with the polymers that have very different distribution of binding sites. For example comparison of binding parameters of MIP and NIP (Table 1) reveals that MIP has higher concentration of binding sites per gram \((N_i = 89.3 \pm 1.6 \text{ mg g}^{-1}\) for endosulfan I and 84.4 \pm 1.6 \text{ mg g}^{-1}\) for endosulfan II). Also MIP is more heterogeneous \((m = 0.204\) for endosulfan I and 0.200 for endosulfan II) as compared to NIP \((m = 0.59\) for endosulfan I and 0.64 for endosulfan II), which is due to imprinting. The accuracy of these values is evaluated with respect to the concentration window in which they were measured. This can be assessed by determining whether \(K_a\) falls between the limits 1/\(F_{\text{min}}\) and 1/\(F_{\text{max}}\) and by confirming that the standard errors in the fitting coefficients are not excessively large. These requirements are met in this study (Table 1).

In order to verify the selectivity of the MIP and NIP to endosulfan I and II, three different cyclodienes (heptachlor, aldrin and dieldrin) were selected as analogs. The adsorption capacity of MIP and NIP for the mixture of endosulfan I, endosulfan II, heptachlor, aldrin and dieldrin having individual concentration of 0.3 \(\mu\)g ml\(^{-1}\) in 5 ml aqueous solution was listed in Table 2.

The specificity of MIP and NIP was estimated by the partition coefficients of selected cyclodienes between polymers and the

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>MIP</th>
<th>NIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_i)</td>
<td>89 ± 16 (\mu)mol g(^{-1})</td>
<td>25 ± 3.6 (\mu)mol g(^{-1})</td>
</tr>
<tr>
<td>(a)</td>
<td>1.2 ± 0.36 mM(^{-1})</td>
<td>3.2 ± 0.36 mM(^{-1})</td>
</tr>
<tr>
<td>(m)</td>
<td>0.2 ± 0.04</td>
<td>0.6 ± 0.02</td>
</tr>
<tr>
<td>(K_a)</td>
<td>2.5 mM(^{-1})</td>
<td>7.3 mM(^{-1})</td>
</tr>
<tr>
<td>Limits of affinity distribution(\text{I})</td>
<td>1.19–279.492.6 mM(^{-1})</td>
<td>1.05–97.845.1 mM(^{-1})</td>
</tr>
<tr>
<td>(N_i)</td>
<td>84 ± 15.8 (\mu)mol g(^{-1})</td>
<td>21.9 ± 4 (\mu)mol g(^{-1})</td>
</tr>
<tr>
<td>(a)</td>
<td>1.2 ± 0.36 mM(^{-1})</td>
<td>3.3 ± 0.33 mM(^{-1})</td>
</tr>
<tr>
<td>(m)</td>
<td>0.2 ± 0.04</td>
<td>0.64 ± 0.03</td>
</tr>
<tr>
<td>(K_a)</td>
<td>2.64 mM(^{-1})</td>
<td>6.5 mM(^{-1})</td>
</tr>
<tr>
<td>Limits of affinity distribution(\text{I})</td>
<td>1.17–282.633.5 mM(^{-1})</td>
<td>1.05–96.212.1 mM(^{-1})</td>
</tr>
</tbody>
</table>

\(\text{I}\) These limits were calculated from the maximum and minimum values of free guest concentration \((F_{\text{max}}\) and \(F_{\text{min}}\)) by the relationships \(F_{\text{min}} = 1/F_{\text{max}}\) and \(F_{\text{max}} = 1/F_{\text{min}}\).

### Table 2

<table>
<thead>
<tr>
<th>Cyclodiene</th>
<th>(Q_{\text{av}}) (mg g(^{-1}))</th>
<th>(Q_{\text{av}}) (mg g(^{-1}))</th>
<th>(K_{\text{av}}) (ml g(^{-1}))</th>
<th>(K_{\text{av}}) (ml g(^{-1}))</th>
<th>(I)F</th>
<th>SC Endosulfan I</th>
<th>SC Endosulfan II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptachlor</td>
<td>0.281</td>
<td>0.252</td>
<td>18.7</td>
<td>5.7</td>
<td>3.3</td>
<td>4.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.281</td>
<td>0.262</td>
<td>14.8</td>
<td>6.9</td>
<td>2.14</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.280</td>
<td>0.266</td>
<td>14.8</td>
<td>6.9</td>
<td>2.15</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>0.283</td>
<td>0.197</td>
<td>17.45</td>
<td>1.9</td>
<td>9.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>0.284</td>
<td>0.189</td>
<td>17.2</td>
<td>1.7</td>
<td>10.1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The adsorption capacity, partition coefficients, imprinting factors and selectivity coefficients of endosulfan I, endosulfan II, heptachlor, aldrin and dieldrin on MIP and NIP.
solution. The partition coefficient $K$ was determined according to the following formula:

$$K = \frac{C_P}{C_S}$$ (5)

where $C_P$ was the amount of test analyte bound by MIP and NIP, and $C_S$ was the concentration of test analyte remaining in the solution.

Additionally, the imprinting factor (IF) and selectivity coefficient (SC) were used to evaluate the selectivity properties of MIP and NIP toward endosulfan I and II and structurally related cyclodienes heptachlor, aldrin and dieldrin. The IF and SC was calculated by the following formula:

Imprinting factor (IF) = \frac{K_i}{K_c} \quad (6)

Selectivity coefficient (SC) = \frac{IF_{\text{endosulfan}}}{IF_i} \quad (7)

where $K_i$ and $K_c$ (Eq. (6)) represent the partition coefficients of analyte for MIP and NIP, respectively. $IF_{\text{endosulfan}}$ is the imprinting factor of endosulfan I or II and $IF_i$ is the imprinting factor of other three cyclodienes (Eq. (7)).

As shown in Table 2, the bound amount of endosulfan I and II for MIP was higher than that of the other three cyclodienes, suggesting that template molecule had a relatively higher affinity for the imprinted polymer than its analogs. Moreover, the IF of endosulfan I and II was also much higher than those of other three cyclodienes. As shown in Fig. 5 endosulfan has sulphate group in its structure whereas other three cyclodienes do not contain any sulphate group in their structure; presence of sulphate group differentiates it from other cyclodienes and hence makes MIP more specific for endosulfan.

3.4. Molecularly imprinted solid-phase microextraction experiments (MISPME)

The synthesized MIP was used to devise solid-phase microextraction fiber and resulting MISPME was evaluated for preconcentration of endosulfan isomers from water samples aiming at the direct determination of endosulfan by GC–μECD. Considering the SPME protocols and MIP, it was recognized that some factors such as amount of MIP for coating, ratio of PVC to MIP amount in order to get homogenous coating, pH of extraction medium, temperature of extraction medium, extraction time, stirring speed, desorption time and ionic strength of extraction medium were main effective factors. The low and high levels in screening the effectiveness of factors were shown in Table S-1 in supplementary information; however we did not include the factor pH of extraction medium because it was already optimized at the time of executing adsorption studies in aqueous medium, so these studies were also carried out at pH 1. The detailed discussion of results obtained from Plackett–Burman design and Central Composite design is provided in supplementary information.

3.5. Validation of MISPME method for endosulfan

MISPME fiber did not require time consuming conditioning steps before use. The optimized conditions for the extraction and detection of endosulfan isomers through MISPME were straightforward and easily achievable. The method for the determination of endosulfan I and II in water samples was validated using the MISPME procedure on spiked synthetic wastewater followed by GC–μECD quantitation.

The MISPME fiber was immersed in samples of synthetic wastewater spiked with different concentrations of endosulfan isomers maintained at pH 1 and temperature of 35 °C with stirring speed of 50 rpm for 2 min and directly injected to GC–μECD. Desorption was carried out for 2 min and fiber was removed by pulling the plunger of laboratory made MISPME device.

The results of MISPME method are summarized in Table 3. The linear range was between 7 and 5 × 10^3 ng l^{-1} with a linearity of 0.999 for endosulfan I and II, respectively. The limit of detection and quantitation were 2.09 ng l^{-1} and 7 ng l^{-1} for endosulfan I and 3.04 ng l^{-1} and 10 ng l^{-1} for endosulfan II, respectively, established by signal-to-noise ratios of 3 and 10.

The tests of intra- and inter-day precisions produced acceptable relative standard deviation (Table 3). The LODs obtained in this work were appropriate for the chromatographic technique, GC–μECD. The Gonzalez et al. produced LOD and LOQ 23 ng l^{-1} and 69 ng l^{-1} for endosulfan I and 18 ng l^{-1} and 54 ng l^{-1} for endosulfan II, respectively, using commercially available polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber. Also the SPME was carried out by 45 min of extraction time [37], however the extraction and desorption time of MISPME was just 2 min that reveals the quick kinetics of MISPME and is due to the specific binding sites produced via imprinting process using core shell technique. The core–shell imprinted polymer produced an efficient MISPME device due to
Table 3

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Endosulfan I</th>
<th>Endosulfan II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (ng L⁻¹)</td>
<td>7–5 × 10⁵</td>
<td>10–5 × 10⁶</td>
</tr>
<tr>
<td>Linearity (R²)</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Slope (a)</td>
<td>2029 (±0.286)</td>
<td>1014 (±0.143)</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>1464 (±344)</td>
<td>1058 (±201)</td>
</tr>
<tr>
<td>LOD (ng L⁻¹)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>LOQ (ng L⁻¹)</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

Intra-assay precision (% RSD)

| 10 ng L⁻¹ (n=5)     | 0.023 | 0.09 |
| 5 × 10² ng L⁻¹ (n=5)| 6 × 10⁻¹ | 0.026 |
| 5 × 10⁴ ng L⁻¹ (n=5)| 7 × 10⁻³ | 0.05  |

Interassay precision (% RSD)

| 10 ng L⁻¹ (n=3, 3 days) | 1.25 | 0.48 |
| 5 × 10⁻¹ ng L⁻¹ (n=3, 3 days) | 0.45 | 0.74 |
| 5 × 10⁻¹ ng L⁻¹ (n=3, 3 days) | 0.021 | 0.076 |

Accuracy (% recovery) river water

| 10 ng L⁻¹ (n=3)     | 108 | 105 |
| 5 × 10⁻⁵ ng L⁻¹ (n=3)| 120 | 112 |
| 5 × 10⁻⁵ ng L⁻¹ (n=3)| 124 | 117 |

Accuracy (% recovery) waste water

| 10 ng L⁻¹ (n=3)     | 97.7 | 95 |
| 5 × 10⁻⁵ ng L⁻¹ (n=3)| 99 | 96 |
| 5 × 10⁻⁵ ng L⁻¹ (n=3)| 104 | 101 |

the presence of superficial binding sites that lead to the quicker adsorption/desorption process.

To test the selectivity of MISPME method synthetic wastewater spiked with 0.3 μg·mL⁻¹ of endosulfan isomers was extracted through MISPME fiber as well as 100 μm polydimethylsiloxane (PDMS) fiber and their chromatograms were compared (Fig. 6a and b). This test was performed to investigate possible interfering effects that could affect the selectivity or efficiency of MISPME fiber. The chromatogram obtained after extraction through PDMS fiber showed decreased intensity of endosulfan I and II peaks as PDMS is a non-specific fiber and its adsorption capacity for endosulfan will be influenced by the presence of other analytes in the sample. However the chromatogram obtained after MISPME shows prominent peaks of endosulfan I and II with four times greater intensity as compared to chromatogram obtained through PDMS fiber extraction that reveals highly specific adsorption of endosulfan isomers via MISPME. Also it explains that the presence of interfering species could not affect the efficiency and specificity of binding sites.

The validated method was applied to the analysis of river samples collected from river Indus, Sind, Pakistan, as well as to the municipal wastewater. The samples were spiked with three different concentrations (10, 5 × 10² and 5 × 10⁴ ng L⁻¹). The percent recoveries (Table 3) showed that the river water is more contaminated with endosulfan I and II as compared to municipal wastewater. This may be due to the heavy runoff of rain water directly from the agricultural farms to the river as well as due to the cultivation of crops at the river banks.

The reported MISPME method overcomes most of the limitations reported earlier; the magnetite particles used as core allowed controlled formation of polymer shell that in turn produced MISPME fiber with surface implanted binding sites having excellent kinetics and adsorption capacity.

4. Conclusions

The MIP was prepared as selective extraction sorbent for the analysis of endosulfan I and II in complex aqueous samples. The obtained core–shell MIP and NIP were characterized through FT-IR, TGA, and SEM. Adsorption studies revealed that MIP is incredibly selective for endosulfan I and II with exceptional adsorption capacity. A sensitive analytical method comprising of MISPME coupled with GC–μECD has been developed to quantify trace levels of endosulfan I and II in real water matrices. The MISPME method has been optimized by experimental designs, which significantly reduced the resources used. Sample preparation time as well as consumption of toxic organic solvents was reduced without decreasing the sensitivity of method. This easy-to-handle and cost-effective method represents an attractive alternative to both conventional and more recently proposed sample preparation methods, as it offers better results.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.02.035.