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PAPER

Rapid extraction and quantitative detection of the herbicide diuron in surface water by a hapten-functionalized carbon nanotubes based electrochemical analyzer†

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A solid phase extraction micro-cartridge containing a non-polar polystyrene absorbent matrix was coupled with an electrochemical immunoassay analyzer (EIA) and used for the ultra-sensitive detection of the phenyl urea herbicide diuron in real samples. The EIA was fabricated by using carboxylated carbon nanotubes (CNTs) functionalized with a hapten molecule (an amine functionalized diuron derivative). Screen printed electrodes (SPE) were modified with these haptenized CNTs and specific in-house generated anti diuron antibodies were used for bio-interface development. The immunodetection was realized in a competitive electrochemical immunoassay format using alkaline phosphatase labeled secondary anti-IgG antibody. The addition of 1-naphthyl phosphate substrate resulted in the production of an electrochemically active product, 1-naphthol, which was monitored by using differential pulse voltammetry (DPV). The assay exhibited excellent sensitivity and specificity having a dynamic response range of 0.01 pg mL^{-1} to 10 μg mL^{-1} for diuron with a limit of detection of around 0.1 pg mL^{-1} ($n = 3$) in standard water samples. The micro-cartridge coupled hapten-CNTs modified SPE provided an effective and efficient electrochemical immunoassay for the real-time monitoring of pesticides samples with a very high degree of sensitivity.

Introduction

Diuron [3-(3, 4-dichlorophenyl)-1,1-dimethylurea] is a substituted phenyl urea herbicide which is used for broad spectrum pre-emergence weed control in a wide variety of crops.[1] It functions primarily by inhibiting the Hill reaction in photosynthesis by limiting the production of high-energy compounds such as adenosine triphosphate (ATP) used for various metabolic processes.[2] Besides its use as a herbicide it is also employed as an active ingredient in antifouling boat paints and in algaecide formulations used in fountains and aquaculture.[3] This herbicide has been proved to be an endocrine disruptor by showing adverse effects on human health.[4] It is relatively persistent in soil with a half-life of 90–180 days, varying from several weeks to years in different types of environment.[5,6] Due to its extensive use, its residues are detected in ground and surface water in concentrations exceeding permissible limits.[7,8] As per the European Union Directive (1980) the maximum permissible limit for any individual pesticide in groundwater is around 0.1 ng mL^{-1}.To monitor such a low trace amount of pesticides in environmental samples, a highly sensitive and specific method of detection is needed. Several analytical approaches such as liquid chromatography, gas chromatography, mass spectrometry, electrophoresis, immunoassay techniques have been described in literature for the monitoring of diuron.[9–15]

Antibody based electrochemical sensing is a promising approach for the detection of small molecules such as pesticides as these techniques combine high specificity with low detection limits. Various nano-structured films have been used for the development of electrochemical sensors and biosensors, because of their synergistic effects on the electrochemical and catalytic activity.[16,17] Carbon nanotubes (CNTs) have recently gained interest for use in electrochemical immunoassays due to their remarkable tensile strength, surface area, flexibility and other unique structural, mechanical, electrical as well as physico-chemical properties reflecting increased signal currents.[18,19] The possibility of functionalizing these smart nano-materials directly with different biomolecules and their biocompatibility make them extremely attractive for electrochemical based sensing.[20–25] For the detection of an electroactive analyte, bioreceptors are usually immobilized on the electrode and the corresponding redox responses can be measured.[26] However biological macromolecules are found to better retain their functional activity when immobilized through extended hydrophilic spacer arms, since sorption on the surface is substantially reduced.[27] Haptens

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are usually bound to sensor or microtiter plate surfaces indirectly by immobilizing the protein–hapten conjugate, since the direct attachment of the hapten to the surface is not possible due to the lack of available functional groups.[28] In our previous study, we described a method for the direct attachment of carboxylated haptens on an amine-derivatized polystyrene support.[29] But the method is limited to polystyrene substrate. In the present study, we demonstrate a novel strategy for hapten modification of a sensor surface by grafting an amine derivatised hapten (3, 4-dichlorophenyl urea) directly onto the carboxylated CNTs using carbodiimide activation chemistry for the development of a highly sensitive EIA. The study also presents a solid phase microextraction (SPME) method using a non-polar polystyrene absorbent (styrene–divinylbenzene) matrix for the extraction of diuron from samples. Since, for the direct estimation of diuron in drinking water and soil, a pre-enrichment of the samples is essential especially in the case of low levels of pesticide contamination in samples. SPME is the most common format for the pre-concentration of pesticide contaminated samples. It utilizes a matrix which selectively retains weakly polar molecules on the stationary phase that can be eluted with a small volume of organic solvent.[30,31] A straight hydrocarbon chain consisting of 18 carbon atoms (C_{18}) is the most commonly used solid phase bed for pesticides extraction.[32,33] However, the average recovery of mildly polar diuron (solubility in water ~42 mg L^{-1}) is not efficient with C_{18} matrix and requires the use of large amounts of extraction solvent. Non-polar styrene–divinylbenzene sorbents have recently been made available by several companies for the off-line extraction of pesticides.[34,35] The study thus reports on the development of an electrochemical analyzer coupled with a micro-extraction column (Scheme 1) for the rapid extraction and real time quantitative detection of the herbicide diuron in surface water.

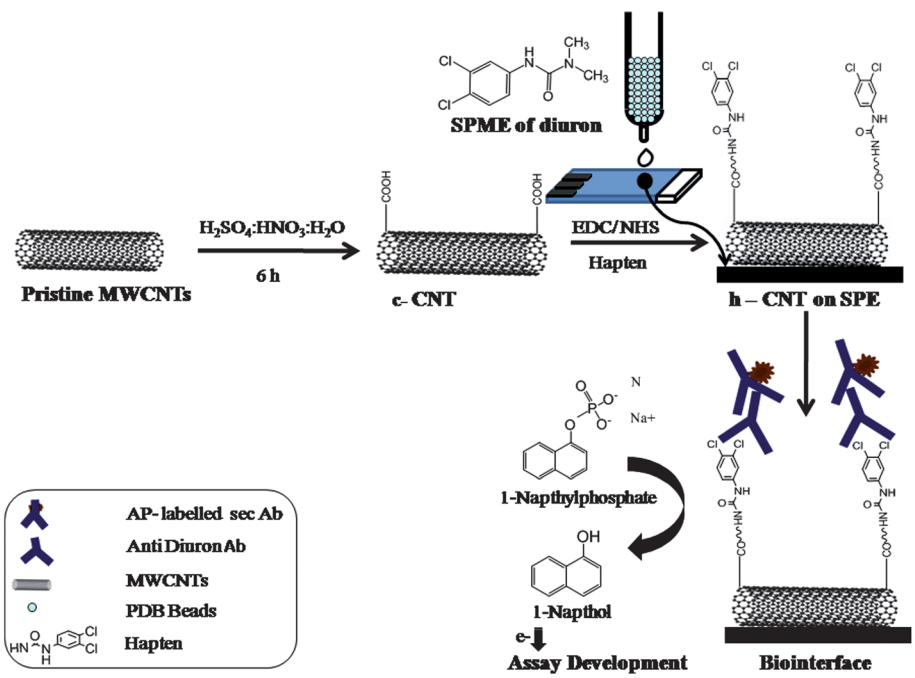
Experimental

Materials

Analytical standards of diuron, DCPU (3,4-dichlorophenylurea), bovine serum albumin (BSA), 1-napthyl phosphate, thionin acetate (THA), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxy-succinimide (NHS), poly(vinyl alcohol) (PVA): average mol. weight 30,000–70,000, polyvinyl pyrrolidone (PVP): average molecular weight 360,000, polyethylene glycol (PEG): average molecular weight 6000, casein and gelatin were purchased from Sigma Chemical Co. (USA). Alkaline phosphatase labeled anti-rabbit IgG was purchased from Genie (India). The high purity multiwalled CNTs (diameter ~30 nm) were purchased from Nanoshel (India). Styrene–divinylbenzene beads (SM-2) used for extraction of diuron were purchased from BioRad, USA. All other reagents used for preparing buffer solutions were of analytical grade. Specific anti-diuron antibodies were produced in-house by synthesizing a novel hapten 3, 4-dichlorophenylurea (DCPU), a metabolic intermediate of the diuron degradation pathway. DCPU was synthesized using a 'green route' by employing gram-positive, *Micrococcus* sp. PS-1 isolated from the diuron storage site as described in our previous study.[36]

Micro-extraction column for diuron extraction

A micro-extraction column (1 mL capacity) containing 500 mg of divinylbenzene derivatized polystyrene beads was fabricated to extract diuron from water. The column containing the bio-beads was first pre-equilibrated with 10% methanol solution followed by degassing of the slurry by placing it in a vacuum flask under mild vacuum for 5 min. The excess solvent was then decanted, and the column was equilibrated with 3 bed volumes



Scheme 1 Schematic representation of SPME micro-cartridge column coupled with an EIA using haptenized CNTS for the monitoring of phenyl urea herbicide. The pristine CNTS were carboxylated by acid treatment followed by haptenisation using carbodiimide chemistry for assay development.

of distilled water. To facilitate the dissolution of diuron in water, a stock solution of 1 mg mL^{-1} of diuron in methanol was prepared. Further dilutions were made in Milli-Q water in the range from 10 to 100 μg mL^{-1} (ppm). 100 mL of diuron solution was percolated into the column containing Bio-beads, and the samples were collected every 15 min for checking the binding of diuron on the beads. After percolation, diuron bound to the column was eluted by treating the beads with methanol solutions. The binding and elution efficiencies of diuron on the column were analyzed by a Shimadzu GC-2010 gas chromatograph equipped with an ECD detector (see ESI†).

Haptenization of CNTs

High aspect ratio (length: 15–30 μm and diameter: ~30 nm) pristine CNTS (p-CNTs) were used for the formation of hapten functionalized carbon nanotubes. The carboxylation of p-CNTs was carried out according to the previous report.[37] Briefly, (1.0 mg) CNTs were sonicated in H_2SO_4 : HNO_3 : H_2O (3 : 1 : 6, v/v) for 2 h. The resulting dispersion was washed with water and dried under vacuum overnight. Subsequently, the carboxylated CNTS (c-CNTs) were dispersed in 500 μL of DMF : water (1 : 1, v/v) solution to form an homogeneous dispersion indicating the generation of hydrophilic carboxylic groups. The degree of carboxylation on c-CNTs was quantified by using THA dye. Different concentrations of THA prepared in ethanol were used for plotting a calibration curve. The dye was incubated with c-CNTs and p-CNTs for 6 h and fluorescence measurements were recorded using a Cary eclipse spectrofluorimeter (excitation-590 nm).

The c-CNTs suspension was activated by using a solution containing 100 mM EDC and NHS respectively, followed by an incubation at room temperature for 2 h. The resulting mixture was centrifuged at 12,000 rpm for 15 min, and the supernatant was discarded. This centrifugation procedure was repeated thrice using water to remove excessive EDC and NHS. Hapten (DCPU), an analogue of diuron at a concentration of 2 mg mL^{-1}, prepared in DMF, was added to activate the c-CNTs dispersion (1 : 1, v/v) and incubated at room temperature for 2 h. The reaction mixture was again centrifuged at 12,000 rpm for 15 min, and the supernatant was removed, followed by subsequent washings to form haptenized CNTs (h-CNTs). Finally the h-CNTs were dispersed in DMF : water (1 : 1) followed by sonication for 1 h. FTIR spectra were recorded for the samples in the spectral region of 4400–400 cm^{-1} using a Thermo Scientific spectrophotometer equipped with AgCl windows. A KBr pellet with homogenized sample in 1 : 100 was made for the analysis. For thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis, a Mettler TA system (Model 6000) with STARe analysis software was used and the samples were loaded on an alumina pan. The temperature was set from 25 to 800 °C with a heating rate of 10 °C min^{-1} in an oxygen environment (20 mL min^{-1}). The weight loss percentage with decomposition temperature of CNTs and modified CNTs were determined by TGA. Simultaneously, DSC analysis was carried out under similar conditions to demonstrate the thermokinetics of the samples.

Surface modification of SPE with h-CNTs

The well characterized h-CNTs (1 mg) were first dispersed in 1 mL solution of (1 : 1 v/v DMF and water). 5 μL of the dispersion was drop casted on the working electrode surface of screen printed electrodes (SPE) with a 3 mm diameter carbon paste working electrode, a counter electrode and Ag/AgCl reference electrode (Model TE 100; CH instruments USA). The electrodes were incubated at 60 °C in a vaccum oven for 2 h to ensure the absolute evaporation of solvent. The h-CNTs modified electrodes were further characterized by FE-SEM (Hitachi S-4300) by directly mounting the electrodes on an aluminum stub using a conductive double adhesive carbon tape. In control experiments, c-CNTs were dropcasted on the electrode surface and then coated with hapten–protein conjugate (DCPU–BSA) by activating the carboxyl groups of CNTs using the same carbodiimide activation chemistry followed by an overnight incubation at 4 °C. The electrode surface after various surface modifications was characterized electrochemically by using cyclic voltammetry. The electrochemical stability of h-CNTs on SPE was tested by repeated CV scanning (25 cycles). All the electrochemical measurements were performed using a 660D electrochemical workstation (CH Instruments, Austin, TX).

Electrochemical immunoassay development

SPEs modified with h-CNTs were used for the development of an electrochemical immunoassay for diuron detection in a competitive immunoassay format. Anti-diuron antibodies (25 μg mL^{-1}) were first mixed with varying concentrations of standard diuron samples (10 pg mL^{-1} to 10 μg ml^{-1}; 50 μL each) and kept for incubation for 20 min at room temperature. The respective solutions were placed on the working area of the functionalized SPE and incubated for 1 h at 37 °C followed by washing with phosphate buffer (PB). To check for blocking efficiency the electrodes were incubated with different blocking agents (2%) followed by incubation for 1 h at 37 °C. After washing, 50 μL of anti-rabbit IgG-alkaline phosphatase (1 : 20 K diluted in PB) was incubated for 30 min at 37 °C. The electrodes were finally washed 4x with PB and 2x with PB/T. 50 μL of substrate solution (1-napthyl phosphate) at concentration of 2 mg mL^{-1} prepared in 0.1 M Tris-HCl, pH 10 containing 10 mM $MgCl_2$ was added to the electrodes and incubated for 15 min at 37 °C. The electrodes were subjected to differential pulse voltammetric (DPV) scanning and the height of the resulting 1-naphthol oxidation peak waveform was recorded. For comparison, a parallel competitive assay was developed for the protein–hapten conjugate by tagging it to c-CNTs modified SPE. The development of a bio-interface on the SPE was characterized by using RITC labeled anti diuron antibodies and the electrodes were viewed under a confocal microscope (Zeiss LSM 510).

Results and discussion

Solid phase micro-extraction of diuron from water samples

An indigenously developed micro-extraction column (1 mL) containing a divinylbenzene derivatized polystyrene adsorbent matrix was used for the extraction of diuron from water samples where the analyte was selectively retained on the stationary

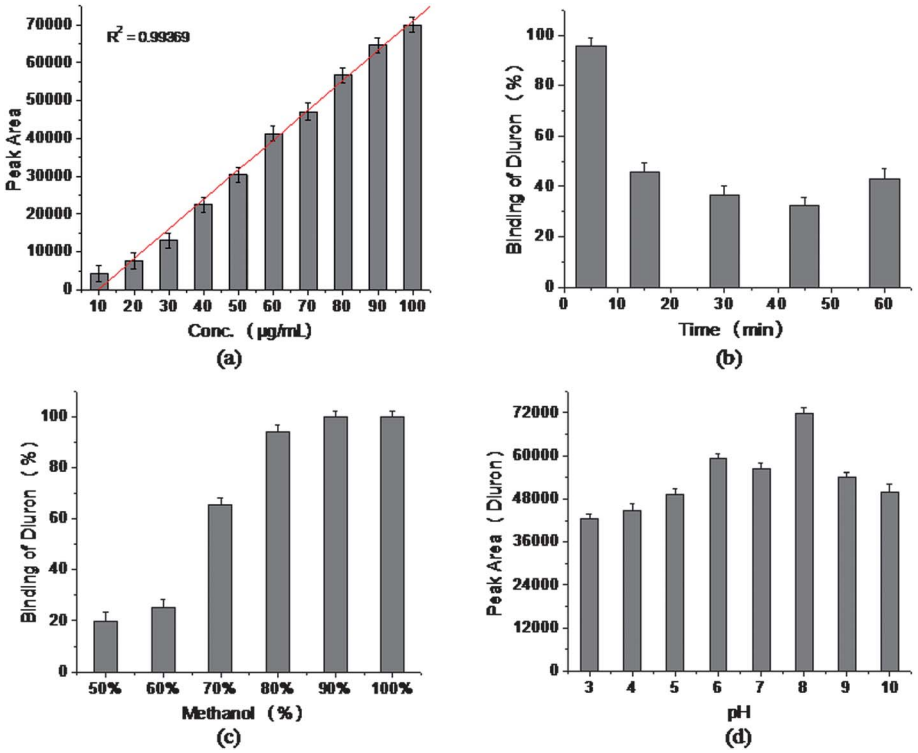


Fig. 1 Binding (a) and elution (b) kinetics of diuron on SM-2 beads. 100 mL of diuron solution (100 μg mL^{-1}) was percolated into the column containing the beads. Diuron was eluted from the column using (50–100%) methanol solutions. The effect of pH (3–10) of the spiked water samples on the binding ability of diuron on the beads is shown (c). Calibration plot for different concentration (1–100 μg mL^{-1}) of diuron standard solutions was prepared using standard GC analysis (d). For all sets of experiments, each point represents the average of three experiments.

phase, and then eluted with a small volume of solvent. The use of SM-2 beads for herbicide extraction was primarily studied to overcome the difficulties associated with the extraction of polar compounds from aqueous sample using C_{18} matrix[38] where the extraction of pesticides is basically due to partitioning of analytes based on their hydrophobic character and the non-polar analytes are partitioned preferentially onto the stationary phase as water is being pulled out.[39] However, the average recovery of mildly polar diuron (solubility in water ~42 mg L^{-1}) is not efficient with C_{18} matrix and a high amount of extraction solvent is needed. To overcome these problems, the developed SM-2 micro-extraction column was used and the standard diuron samples spiked in water were loaded on to it directly. Gas chromatographic analysis quantified the amount of diuron bound to the matrix before and after loading. A non-polar solvent such as methanol was selected for stripping off the bound diuron as it may facilitate the partitioning of diuron, resulting in its desorption from the beads. Kinetic studies of diuron immobilized beads kept in methanol for different time intervals (Fig. 1a) suggested that 5 min time was sufficient for stripping off the bound diuron on beads. Further study using different percentage of methanol prepared in water (50–100%) demonstrates that methanol solution (~80%) was quite effective in stripping off the bound diuron from the column in 5 min (Fig. 1b). To confirm the effect of pH of the spiked water samples on the performance of the packed column, water samples adjusted at different pH between 3 to10 showed that the maximum recovery of diuron from the column was obtained at an optimum of pH 8 (~97%) as depicted in Fig. 1c. A calibration graph for diuron standard solutions was prepared at different concentrations of diuron (10–100 μg mL^{-1}) using GC analysis. The respective peak area recorded at retention time (R_t) ~5.3 corresponds to the diuron standard sample. The amount of bound and eluted diuron from beads was correlated with the standard curve as depicted in Fig. 1d.

Haptenization of CNTs and their characterization

Pristine CNTS (p-CNTs) were first acid-oxidized to generate carboxylic groups and subjected to FTIR analysis to confirm the presence of carboxyl groups on CNTs. FTIR spectra showed a characteristic peak at 1716 cm^{-1} corresponding to >C═O (See ESI S1†). The extent of carboxylation (—COOH groups density) was quantified by using a positively charged fluorescent dye, THA.[40] Different concentrations of the dye were prepared and a standard curve was plotted by measuring the relative fluorescence intensity (Fig. 2 inset). The optimum concentration of dye (1 μM) in ethanolic solution was used and incubated with p-CNTs and c-CNTs for 6 h and 12 h. It was observed that by increasing the concentration of the dye above 1 mM causes a decrease in fluorescence intensity due to self quenching. A decrease in fluorescence intensity was observed in c-CNTs followed by a color change from deep blue to purple (Fig. 2). The fluorescence decrease was attributed to the cationic nature of the dye (T+) that forms ion pairs with the carboxylic groups of c-CNTs. The estimated values of acidic moieties (–COOH groups) on c-CNTs was found to be approximately 0.125 mmol of COOH mg^{-1} of oxidized CNTs. The thus formed c-CNTs were activated with EDC/NHS to form an active ester intermediate

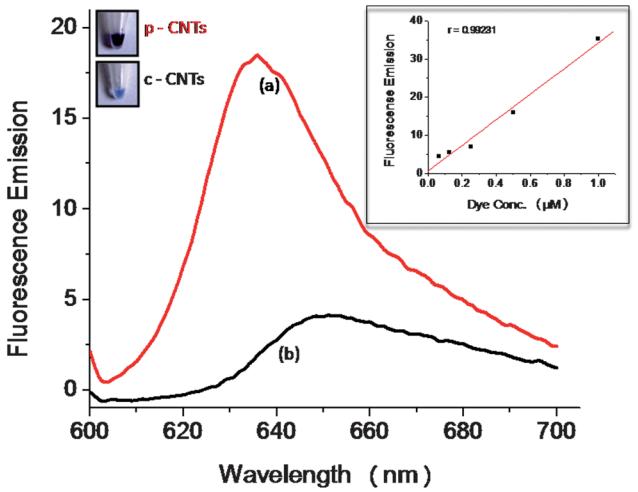
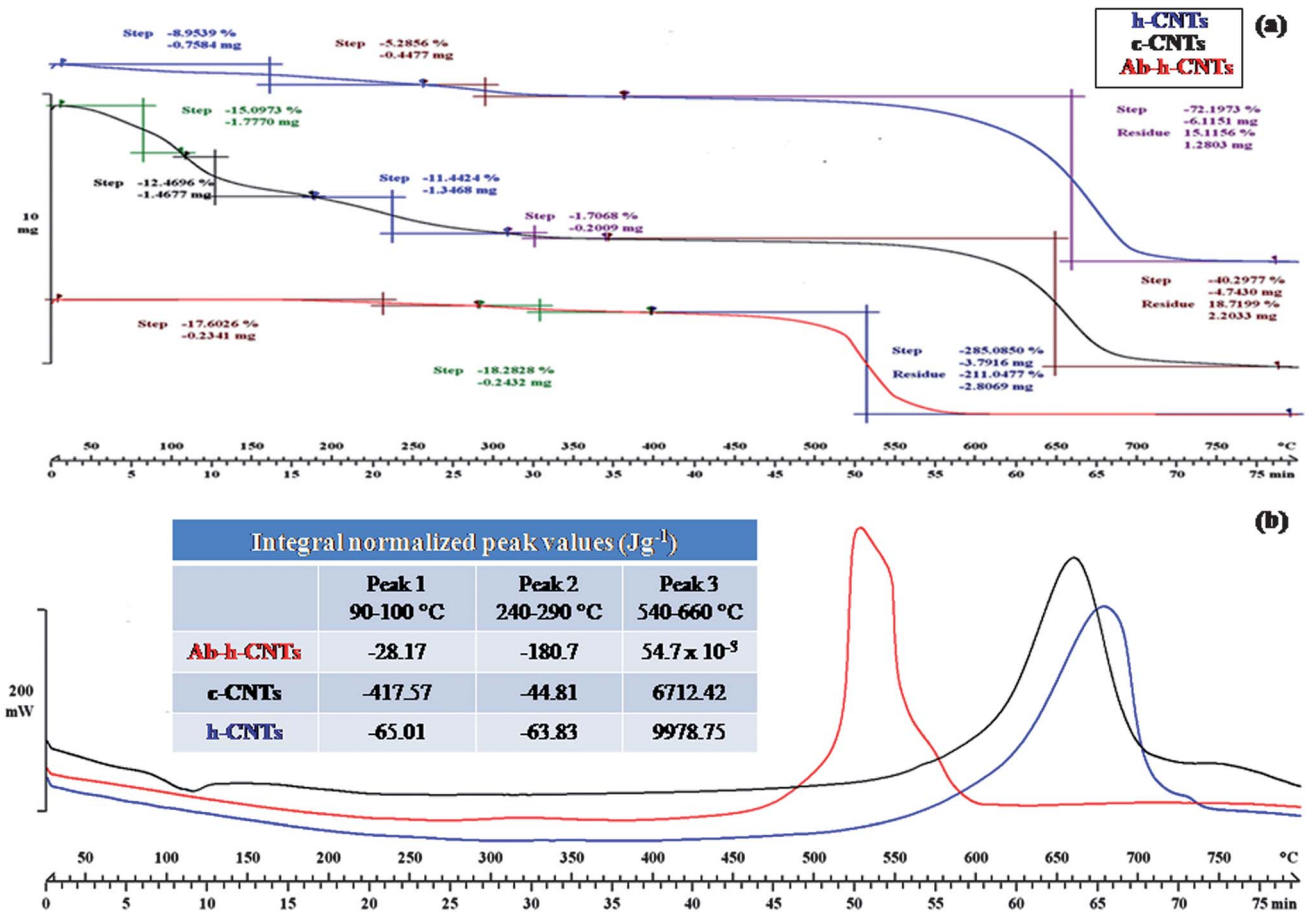


Fig. 2 Fluoresence spectra of (a) carboxylated and (b) pristine CNTs respectively. A standard curve of THA dye was prepared by taking different concentration (0.125–1 μM) of the dye. Inset shows the change in color from deep blue to purple for carboxylated CNTs depicting the extent of carboxylation on CNTs in presence of dye.

compound which reacts with the amine groups of DCPU to form a stable amide bond between the CNTs and DCPU to form h-CNTs.

The h-CNTs were further characterized for their stability with hapten and subsequently with antibody binding using TGA analysis by measuring the weight loss percentage at respective decomposition temperatures. Fig. 3 demonstrates the shift in the decomposition temperatures at 650 °C, 660 °C and 530 °C for c-CNTs, h-CNTs and antibody bound h-CNTs (Ab-h-CNTs) respectively. Relatively higher decomposition temperature was observed in c-CNTs because of the presence of carboxyl groups at their surface forming hydrogen bonds.[41] The h-CNTs further imparted stability by showing increased relative decomposition temperature because of the hydrophobic nature of the hapten which tends to form the aggregates of disordered ensemble of high aspect ratio h-CNTs. The thermokinetics of the samples were further demonstrated by DSC in order to understand the sample's stability and exothermic decomposition reactions. The respective onset temperatures (T_0) 526 °C, 612 °C, 616 °C and peak temperatures (Tp) 542 °C, 669 °C, 688 °C for Ab-h-CNTs, c-CNTs and h-CNTs respectively observed by DSC in Fig. 3 suggested that the h-CNTs were less triggered for exothermic reaction due to the increased thermal delay effect calculated by the relationship (T_p–T_0).[42] The high diffusivity and thermal



Integral normalized peak values (Jg^{-1})	Peak 1 90-100 °C	Peak 2 240-290 °C	Peak 3 540-660 °C
Ab-h-CNTs	-28.17	-180.7	54.7×10^{-3}
c-CNTs	-417.57	-44.81	6712.42
h-CNTs	-65.01	-63.83	9978.75

Fig. 3 (a) Percentage weight loss *vs.* decomposition temperature by TGA analysis for c-CNTs, h-CNTs and Ab-h-CNTs respectively and (b) shows the DSC analysis of c-CNTs, h-CNTs and Ab-h-CNTs respectively. Inset (table) shows the normalized integrated values calculated from the respective peak area. Heat rate was selected at 10 °C min^{-1}.

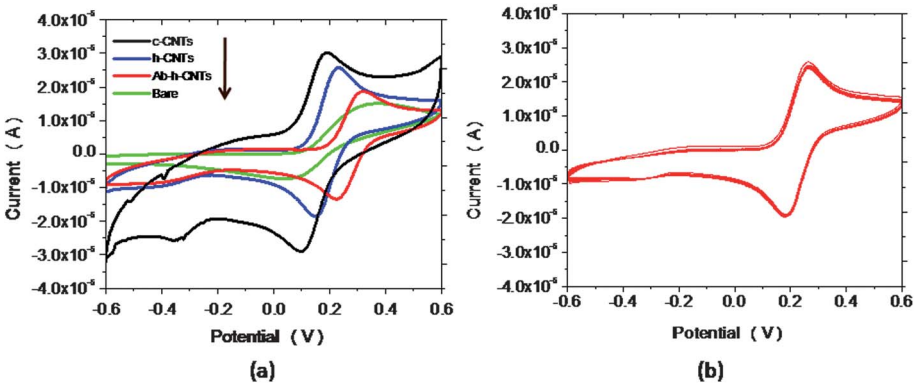


Fig. 4 (a) CV scans of CNTs functionalized SPE (c-CNTs, h-CNTs, Ab-h-CNTs and bare electrodes respectively) and (b) presents the no. of scan cycles (25 cycles) of h-CNTs on SPE. The scans were obtained in 2.5 mM ferrocyanide prepared in PBS (pH 7.4) at a scan rate 50 mV s^{-1}.

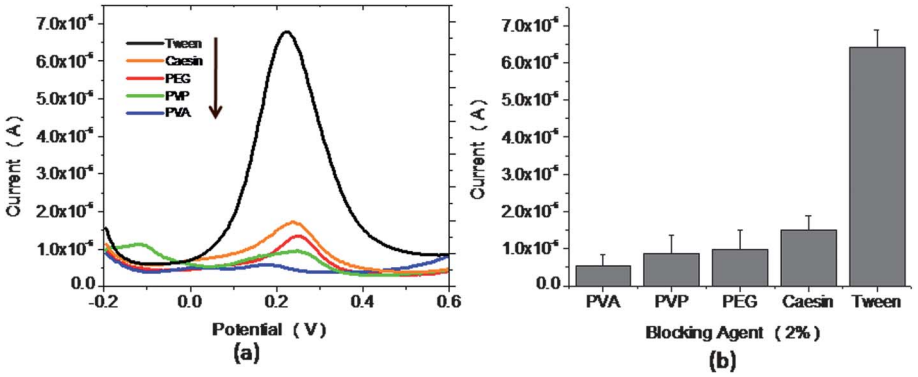


Fig. 5 Blocking efficiency of various blocking agents in terms of (a) DPV Scans and (b) the corresponding current signal response observed in the electrochemical assay ($n = 3$).

conductivity as observed in h-CNTs is mainly due to the enhanced thermal delay effect. The corresponding integrated peak areas were also deduced to calculate enthalpy of fusion (ΔH) from the thermal curves. The data presented as an inset in Fig. 3 suggests that ΔH of the modified CNTs were augmented which further supports the highest stability of h-CNTs. Thus, h-CNTs modification of the sensor has the advantage of longer shelf life besides providing high sensitivity to the developed electrochemical assay.

Morphological characterization of h-CNTs modified SPE and the biointerface development

A homogeneous dispersion of well characterized h-CNTs was prepared in a mixture of DMF : water (1 : 1).[43] An antibody functionalized biointerface was developed on h-CNTs modified SPE. Confocal microscopic study with anti-diuron antibodies labeled with RITC fluorophore suggested uniform coverage of antibody on CNTs functionalized SPE surface (See S3, ESI†). The control experiments without h-CNTs surface modification showed negligible fluorescence spots confirming low non-specific binding. The pixel data further demonstrated that high fluorescence intensity (~200) over the whole scan area was observed on h-CNTs functionalized SPE clearly indicating the full coverage of bio-receptors on the electrode.

Development of electrochemical immunoassay

The CV characteristics for the redox of ferrocyanide for the variously modified electrode surfaces namely c-CNTs, h-CNTs and Ab-h-CNTs showed that the modified electrodes exhibited 2–3 fold increase in anodic and cathodic peak current as compared to the bare SPE surface (Fig. 4a). The c-CNTs modification of the electrodes showed higher peak current indicating a larger electrode active area in comparison to bare SPE. On the other hand the h-CNTs modification of the surface leads to a decrease in the peak current with respect to c-CNTs modification. This may be explained because of the hydrophobic nature of the hapten molecule causing a decrease in peak current. A slight shift in peak potential, as observed in the voltammograms, could be ascribed due to the positively charged nature of the hapten molecules on the negatively charged c-CNTs. Furthermore binding of anti-diuron antibodies on a h-CNTs modified sensor surface leads to a decrease in peak current because of the resistance to charge transfer (R_{ct}) posed by the robust biomolecule. The electrochemical stability of h-CNTs on SPE was tested by repeated CV scanning (25 cycles) in buffer solution, which showed insignificant change in the signal intensity as reflected by negligible change in the peak currents (Fig. 4b). Various blocking agents (PVA, PVP, PEG, casein and gelatin)[44] prepared in PBS were investigated for their efficacy to avoid nonspecific binding onto the h-CNTs modified SPE

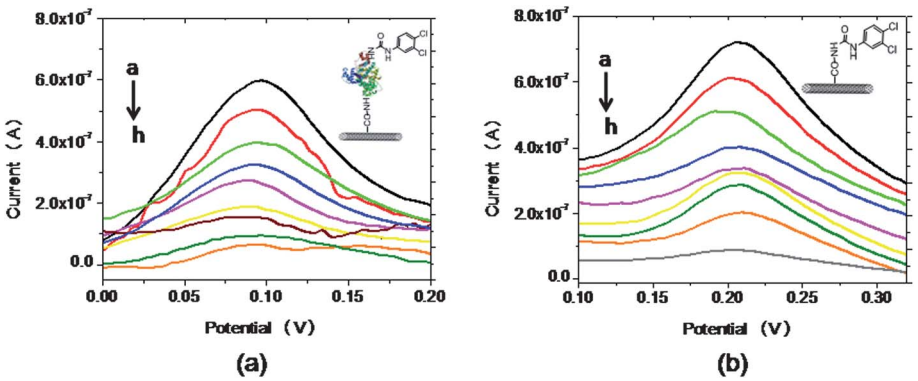


Fig. 6 Response curves (left) DCPU-conjugate coated c-CNTs modified SPE and (right) h-CNTs on SPE at different concentrations of diuron samples (a–i) 0 to 10^6 pg mL^{-1} free diuron respectively. The signal was measured as oxidation of 1-napthol after 10 min incubation in Tris pH 8, $MgCl_2$, differential pulse voltammetry technique at amplitude 50 mV, pulse width 0.2 s, pulse period 0.5 s.

surface (Fig. 5 a, b). We observed that the 2% PVA proved to be the most efficient blocking agent as it showed negligible current signal attributable to the non-specific binding of AP labeled antibodies on the sensor surface. In a control experiment, we also checked the non-specificity by taking diuron samples on the sensor surface followed by incubation with a secondary antibody. No significant response was observed in this case confirming the efficiency of PVA as an effective blocking agent.

For the development of a competitive electrochemical immunoassay a series of diuron standards 0.1 pg mL^{-1} to 10 μg mL^{-1} were mixed with a fixed pre-calibrated concentration of anti-diuron antibodies. After incubation secondary AP labeled antibody was added and DPV scans were recorded after 10 min. Fig. 6 shows the waveforms presenting an oxidation peak for 1-naphthol for hapten protein conjugate (a) and h-CNTs modified electrode surface (b). The observed reduction current due to oxidation of naphthyl phosphate in the standard competitive inhibition approach in the presence of AP decreased with the increase in diuron concentration in standard water samples (0.01 pg mL^{-1} to 10 μg mL^{-1}). The data was normalized by % B/B_0 transformation[36] and the percent amplitude of the signal decreased with an increase in analyte concentration. We compared the performance of the electrochemical immunoassay analyzer for a conjugate modified surface (BSA:h-CNTs) *vs.* a h-CNTs modified surface. Fig. 7 depicts that the hapten modified CNTs SPE electrodes show higher sensitivity of detection with IC_{50} value (~5 pg mL^{-1}) as compared to BSA:h-CNTs modified (~100 pg mL^{-1}). This could be mainly due to the large number of hapten molecules available on the h-CNTs modified surface than the CNTs surface modified with protein–hapten molecules. The sensitivity of the assay was higher than the existing chromatographic techniques such as (GC and HPLC) with prevailing diuron detection limits of 0.25 and 2.0 mg mL^{-1}.[45]

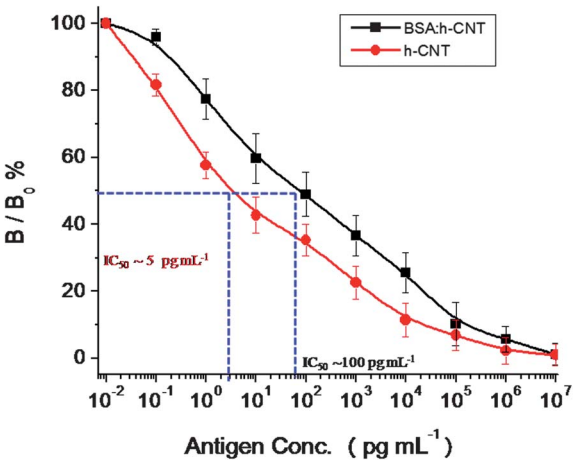


Fig. 7 Competitive electrochemical inhibition assay ($n = 3$) of diuron using (●) direct hapten coated CNTS functionalized SPE and (■) BSA–DCPU conjugate coated CNTS functionalized SPE. The concentration of diuron was selected between 10^{-2} to 10^7 pg mL^{-1}.

Conclusions

The present study deals with the development of hapten CNT-modified screen printed electrodes. The electrochemical analysis was coupled to a micro-cartridge based solid phase extraction for the enrichment of diuron sample. The solid phase micro-extraction column that consists of 250 mg of non-polar polystyrene absorbent (styrene–divinylbenzene) matrix was more successful for the extraction of diuron from real water with demonstrated recovery of diuron ~97% which is 25% higher than that obtained using a cartridge consisting of 500 mg C_{18}. The h-CNTs modification of SPE surface together with highly specific diuron antibody exhibited an excellent limit of detection of 0.1 pg mL^{-1} for diuron spiked water samples. The h-CNTs modification of the sensor surface imparted good stability due to the hydrophobic nature of the hapten which tends to form aggregates of disordered ensemble of high aspect ratio h-CNTs. The hapten modified sensors did not show any loss of signal response even after 30 days storage at room temperature, exhibiting longer shelf life. The proposed solid phase extraction based micro-cartridge coupled with electrochemical immunoassay analyzer (EIA) has significant advantages over other reported methods of pesticides detection, particularly the short analysis time, and does not need sophisticated equipment. The EI analyzer provided an efficient platform for the real-time monitoring of pesticides samples with a detection limit of 0.1 pg mL^{-1}.

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References

1 C. Tomlin, 11th ed., *British Crop Protection Council EU*, 1997.
2 S. Bazot, P. Bois, C. Joyeux and T. Lebeau, *Biotechnol. Lett.*, 2007, **29**, 749.
3 S. R. Sorensen, C. N. Albers and J. Aamand, *Appl. Environ. Microbiol.*, 2008, **74**, 2332.
4 C. Tixier, M. Sancelme, F. Bonnmoy, A. Cuer and H. Veschambre, *Environ. Toxicol. Chem.*, 2001, **20**, 1381.
5 S. Giacomazzi and N. Cochet, *Chemosphere*, 2004, **56**, 1021.
6 X. Louchart and M. Voltz, *Environ. Sci. Technol.*, 2007, **41**, 1137.
7 D. J. Lapworth and D. C. Gooddy, *Environ. Pollut.*, 2006, **144**, 1031.
8 E. Eriksson, A. Baun, P. S. Mikkelsen and A. Ledin, *Desalination*, 2007, **215**, 187.
9 A. Kotrikla, G. Gatidou and T. D. Lekkas, *J. Environ. Sci. Health, Part B*, 2006, **41**, 135.
10 G. Ozhan, S. Ozden and B. Alpertung, *J. Environ. Sci. Health, Part B*, 2005, **40**, 827.
11 Y. Li, J. E. George, C. L. McCarty and S. C. Wendelken, *J. Chromatogr., A*, 2006, **1134**, 170.
12 C. L. da Silva, E. C. de Lima and M. F. Tavares, *J. Chromatogr., A*, 2003, **1014**, 109.
13 I. M. Ciumasu, P. M. Kramer, C. M. Weber, G. Kolb, D. Tiemann, S. Windisch, I. Frese and A. A. Kettrup, *Biosens. Bioelectron.*, 2005, **21**, 354.
14 M. Rohde, J. A. Schenk, S. Heymann, O. Behrsing, G. Scharte, G. Kempter, J. Woller, W. E. Hohne, A. Warsinke and B. Micheel, *Appl. Biochem. Biotechnol.*, 1998, **75**, 129.
15 N. Lee, J. H. Skerritt, M. Thomas, W. Korth, K. H. Bowmer, K. A. Larkin and B. S. Ferguson, *Bull. EnV iron. Contam. Toxicol.*, 1995, **55**, 479.
16 H. V. Tran, R. Yougnia, S. Reisberg, B. Pir, N. Serradji, T. D. Nguyen, L. D. Tran, C. Z. Dong and M. C. Pham, *Biosens. Bioelectron.*, 2012, **31**, 62, DOI: 10.1016/j.bios.2011.09.035.
17 S. Qiu, S. Gao, L. Xie, H. Chen, Q. Liu, Z. Lin, B. Qiu and G. Chen, *Analyst*, 2011, **136**, 3962.
18 M. Hannion, *J. Chromatogr. A.*, 1999, **856**, 54.
19 I. Vassilakija, D. Tsipib and M. Scoullosa, *J. Chromatogr., A*, 1998, **823**, 49.
20 G. A. Junk and J. J. Richard, *Anal. Chem.*, 1988, **60**, 451.
21 V. Camel, *TrAC, Trends Anal. Chem.*, 2000, **19**, 229.
22 J. Hodgeson, J. Collins and W. Bashe, *J. Chromatogr., A*, 1994, **659**, 395.
23 C. Crespo, R. M. Marcé and F. Borrull, *J. Chromatogr., A*, 1994, **670**, 135.
24 V. Pichon, C. Cau Dit Coumes, L. Chen, S. Guenu and M. C. Hennion, *J. Chromatogr., A*, 1996, **737**, 25.
25 Y. Yun, A. Bange, W. R. Heinemanb, H. B. Halsall, V. N. Shanov, Z. Dong, S. Pixley, M. Behbehani, A. Jazieh, Y. Tue, D. K. Y. Wong, A. Bhattacharya and M. J. Schulz, *Sens. Actuators, B*, 2007, **123**, 177.
26 S. Hu, J. Xie, Q. Xu, K. Rong, G. Shen and R. Yu, *Talanta*, 2003, **61**, 769.
27 J. Buijs, J. W. T. Linchtenbelt and W. J. N. Lyklema, *Colloids Surf., B*, 1995, **5**, 11.
28 J. Kaur, K. V. Singh, M. Raje, G. C. Varshney and C. R. Suri, *Anal. Chim. Acta*, 2004, **506**, 133.
29 K. Fujiwara and T. Kitagawa, *J. Biochem.*, 2003, **114**, 708.
30 S. Piermarini, L. Micheli, N. H. S. Ammida, G. Palleschi and D. Moscone, *Biosens. Bioelectron.*, 2007, **22**, 1434.
31 V. N. Khabashesku, J. L. Margrave and E. V. Barrera, *Diamond Relat. Mater.*, 2005, **14**, 859.
32 J. Hu, J. Shi, S. Li, Y. Quin, Z. Guo, Y. Song and D. Zhu, *Chem. Phys. Lett.*, 2005, **401**, 352.
33 S. Daniel, T. Rao, K. Rao, S. Rani, G. Naidu, H. Lee and T. Kawai, *Sens. Actuators, B*, 2007, **122**, 672.
34 D. Vairavapandian, P. Vichchulada and M. D. Lay, *Anal. Chim. Acta*, 2008, **626**, 119.
35 J. Okuno, K. Maehashi, K. Kerman, Y. Takamura, K. Matsumoto and E. Tamiya, *Biosens. Bioelectron.*, 2007, **22**, 2377.
36 P. Sharma and C. R. Suri, *Bioresour. Technol.*, 2011, **102**, 3119.
37 I. Putu, M. Wijaya, T. J. Nie, S. Gandhi, R. Boro, A. Palaniappan, G. W. Hau, I. Rodriguez, C. R. Suri and S. G. Mhaisalkar, *Lab Chip*, 2010, **10**, 634–638.
38 G. A. Junk and J. J. Richard, *Anal. Chem.*, 1988, **60**, 451.
39 L. Wu, F. Yan and H. Ju, *J. Immunol. Methods*, 2007, **322**, 12.
40 Y. Chen, Y. Chuan, C. Lin, T. Feng, Hsieh and C. Shu, *Aerosol and Air Quality Research.*, 2010, **10**, 212.
41 S. Vinsentin, N. Barbero, S. Musso, V. Mussi, C. Biale, R. Ploeger and G. A. Viscardi, *Chem. Commun.*, 2010, **46**, 1443.
42 J. Braga, M. Rezende and M. Costa, *J. Aerosp. Technol. Manage.*, 2011, **3**, 179.
43 F. Bolado, P. Queipo, P. Lamas-Ardisana and P. J. Costa Garcia, *Talanta*, 2007, **74**, 427.
44 P. Sharma, K. Sablok, V. Bhalla and C. R. Suri, *Biosens. Bioelectron.*, 2011, **26**, 4209.
45 J. W. C. Wong, M. Fang, Z. Zhao and B. Xing, *J. Environ. Qual.*, 2004, **33**, 2015.

