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Molecular recognition of 2,4,6-trinitrotoluene by 6-aminohexanethiol and surface-enhanced Raman scattering sensor.

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Abstract

2,4,6-trinitrotoluene (TNT) is one of the most commonly used nitro aromatic explosives in landmine, military and mining industry. This article demonstrates rapid and selective identification of TNT by surface-enhanced Raman spectroscopy (SERS) using 6-aminohexanethiol (AHT) as a new recognition molecule. First, Meisenheimer complex formation between AHT and TNT is confirmed by the development of pink colour and appearance of new band around 500 nm in UV-visible spectrum. Solution Raman spectroscopy study also supported the AHT:TNT complex formation by demonstrating changes in the vibrational stretching of AHT molecule between 2800-3000 cm^{-1} . For surface enhanced Raman spectroscopy analysis, a self-assembled monolayer (SAM) of AHT is formed over the gold nanostructure (AuNS) SERS substrate in order to selectively capture TNT onto the surface. Electrochemical desorption and X-ray photoelectron studies are performed over AHT SAM modified surface to examine the presence of free amine groups with appropriate orientation for complex formation. Further, AHT and butanethiol (BT) mixed monolayer system is explored to improve the AHT:TNT complex formation efficiency. Using a 9:1 AHT:BT mixed monolayer, a very low detection limit (LOD) of 100 fM TNT was realized. The new method delivers high selectivity towards TNT over 2,4-DNT and picric acid. Finally, real sample analysis is demonstrated by the extraction and SERS detection of 302 pM of TNT from spiked.

Keywords: 2,4,6-Trinitrotoluene (TNT) sensor; 6-Aminohexanethiol (AHT) and mixed monolayer; Surface-enhanced Raman spectroscopy (SERS); Electrochemical desorption and X-ray photoelectron spectroscopy (XPS); Analysis of explosives in soil

1. Introduction

Trinitrotoluene (TNT), a highly explosive nitroaromatic compound plays very significant role in mining industry and underwater blasting [1]. Moreover, TNT is also widely used in military and terrorist activities in the form of landmines and bombs for several decades [2, 3]. Due to this widespread application, TNT pollution in environment requires ongoing monitoring [4, 5]. In particular, soil and ground water is sometimes highly contaminated beyond the human tolerant level [4, 5]. Prolonged exposures of TNT to humans result in anemia and liver abnormalities [6, 7]. Further, TNT also act as a carcinogen and leads to cancer development in living system [8]. Since TNT can easily absorb through the skin [9], external contact of humans and other organisms with TNT-contaminated water and soil may create significant damage to their biological systems.

Over the years, numerous analytical methods have been developed for the detection of TNT in soil and water [10-14]. However, the in-field and real-time determination of TNT at the ultra-trace level still face limitations in terms of sensitivity, selectivity, reproducibility, cost-effectiveness and suitability to field analysis. Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical technique known for its ultra-sensitivity and rapidity in sample analysis [15-19]. The miniaturization of Raman spectrometer into a handheld device has greatly revolutionized the in-field application of Raman spectroscopy [20]. Richard Van Duyne [21] recently demonstrated the application of handheld Raman spectrometer for the in-field forensic analysis of dyed hair by SERS. This study clearly indicates that SERS will be one of the next-generation analytical tools for the rapid in-field analysis of target molecules. To realize the rapid in-field detection by SERS, cost-effective, sensitive and selective substrates are essential [22]. In order to detect TNT in environmental samples, a cost effective disposable SERS strip that is

functionalized with a suitable binding molecule to selectively recognize TNT over other interfering analytes is highly desirable. This study focuses on finding a new recognition molecule for TNT such that the recognition molecule can be attached to the disposable SERS strips surface. This recognition molecule should selectively bind TNT onto the strip surface in a suitable orientation to enable a high SERS signal enhancement of the analyte Raman fingerprint. Further, the recognition molecule should have a higher mechanical and thermal stability which will enhance the shelf-life of the sensor [23]. The functionalized SERS strips will lead to rapid and selective in-field analysis of TNT trace amounts using handheld Raman device [24].

Several literatures have been reported over the years for sensitive and rapid SERS based detection of TNT using various nanoparticles and SERS platforms. A wide range of primary amines has been used for TNT recognition since primary amines effectively form Meisenheimer complexes with this nitro aromatic compound. For example, cysteine [25, 26], aminothiophenol [1, 27], BSA [28] and cysteamine [29, 30] have been used as selective TNT recognition molecules. In the present work, we have chosen 6-aminohexanethiol (AHT) as a potential selective TNT recognition molecule. AHT has several advantages over other primary amines that have been utilized for TNT recognition. These advantages are: i) the longer chain length of AHT compared to cysteamine provide an improved long term stable self-assembled monolayer (SAM) on SERS substrate. This is due to the enhanced van der Waals lateral interactions between adjacent AHT chains [23, 31]. This long-term stability of AHT monolayer on SERS active surface will increase the shelf-life of the sensor [23]. ii) AHT forms a vertically orientated monolayer with amine groups protruding away from the surface [31-34] and avoids the ambiguity of chelate binding of both thiol and amine groups onto the gold surface as in the case of cysteamine [35, 36]. iii) AHT delivers the flexibility in forming a mixed SAM by creating a

passive backfill layer using shorter thiols such as butanethiol (BT). This shorter backfill molecule also acts as a spacer molecule such that it will create enough space between the AHT molecules and provides easy access to TNT to bind, via the AHT bridge, in an almost perpendicular orientation to the SERS substrate [29, 36-38]. It has been demonstrated in the literature that, high SERS enhancement is realized when the molecule's Raman vibrations have large component of polarizability in the direction normal to the SERS surface [29, 39, 40]. On the other hand, the least enhanced modes are those whose Raman tensor components involve the two axes in the plane of the surface [37]. iv) AHT will not form any chelating complex with metal ions, as in the case of cysteine. This is due to the existence of only one metal ion coordinating amine group in the AHT molecule. In contrast, cysteine, with both amine and carboxylic group, has been known for its ability to form chelating complex with metal ions [41, 42]. This may hinder the amine group of the cysteine molecule to access TNT, especially in environmental samples that may contain several metal ions. v) AHT is a weak Raman scatterer and will show less interference with TNT signal when compared to other aromatic primary amines [1, 27] used for TNT recognition such as p-aminothiophenol.

The present manuscript involves the development of a new Meisenheimer complex between AHT and TNT and its subsequent characterization by UV-visible and Raman spectroscopy. SAM of AHT alone and mixed monolayer of AHT:BT are developed on gold nanostructured (AuNS) SERS substrate. The developed monolayers are examined for their performance as recognition layers that bind TNT to the SERS substrate via the formation of selective Meisenheimer complex with the analyte. Various ratios of AHT:BT are attempted for the mixed monolayer formation in order to optimize the AHT:BT monolayer for a strong SERS signal that allows for the detection of ultra-traces of TNT. To conclude, SERS substrate that is

modified with 9:1 AHT:BT mixed monolayer has been employed for the selective and sensitive detection TNT in soil through the established calibration curve.

2. Experimental Section

2.1. Chemicals and Materials

Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$), 6-aminohexanethiol hydrochloride (AHT), butanethiol (BT), cysteamine hydrochloride (CA), 2,4-dinitrotoluene (DNT), 2,4,6-trinitrophenol (picric acid) and sodium hydroxide (KOH) were purchased from Sigma Aldrich. 2,4,6-trinitrotoluene (TNT) standard (1 mg/mL in 1:1 acetonitrile:methanol) was purchased from Merck (Australia). All other chemicals and solvents were of analytical grade and were used without further purification. All dilutions were made using deionized water (18.2 M Ω .cm) from a Millipore water purification system. Polishing slurries and pads (Microcloth®) were purchased from Buehler, Germany. Polycrystalline gold discs (Au) having a geometric area of 0.502 cm² and platinum wire (A & E Metals, Australia) were respectively, used as working and counter electrode. Dry leakless electrode (DRIREF-2, World Precision Instruments, USA) was used as a reference electrode.

2.2. Instrumentation

All electrochemical experiments were carried out in Autolab PGSTAT204 potentiostat with a custom-made three-electrode cell setup. All Raman measurements were performed using the Renishaw InVia Raman microscope equipped with 785 nm laser line as excitation source. Spectra were collected using a 50 \times and 5x objective lens over a wavelength range from 500 cm⁻¹ to 2000 cm⁻¹ using a laser power of 1 mW for 10s. For each AHT-TNT spectrum, 10 spectra were randomly recorded over the entire surface and averaged. X-ray photoelectron (XPS) measurement was performed using Omicron Multiscan scanning probe microscopy. UV-vis

measurements were carried out in the wavelength range 250–800 nm using the Agilent Cary 60 UV–vis spectrometer (Agilent Technologies, USA).

2.3. SERS Substrate Preparation and mixed monolayer formation

The SERS active substrate was prepared by electrodeposition of gold nanostructure over flat gold surface (pAu/AuNS) in accordance with our previous reported method [43]. Self-assembled monolayer (SAM) of cysteamine, AHT and mixed monolayer of AHT and BT was prepared by immersing the substrate in already prepared appropriate ratio of 1 mM aqueous solution of respective thiols. After 3 h the substrates were washed with copious amount of water to remove the freely adsorbed molecules from the electrode surface.

The electrochemical desorption measurements were carried out in 0.1 M KOH at a scan rate of 0.1 Vs^{-1} . Prior to applying desorption potential, the electrochemical cell was purged with argon gas for about 10 minutes to completely remove the oxygen present in the electrolyte solution.

2.4. Preparation of Meisenheimer complex with TNT

AHT:TNT complex was prepared similar to the reported procedure for other primary amines and TNT [29, 44]. Briefly, AHT stock solution ($2 \times 10^{-4} \text{ M}$) was prepared in MilliQ water and the pH is adjusted to 8.5. Equal volumes of TNT and AHT having the desired concentration were mixed together. The mixture was then allowed to stand for 15 minutes until the pink color of the Meisenheimer complex is fully developed. To prepare the Meisenheimer complex directly on pAu/AuNS surface, few drops of TNT at the desired concentration and optimum pH (8.5) was placed for about 10 minutes on SERS substrate modified with AHT monolayer. The modified pAu/AuNS substrate was then washed with copious amount of NaOH solution (pH 8.5) to

remove any un-complexed TNT from the surface. The substrate was then dried in a gentle flow of nitrogen and used for SERS measurements.

2.5 Extraction of TNT from soil

A known concentration of TNT was spiked into soil and subsequently extracted into a water suspension by dispersing the TNT treated soil with water and subjected to centrifugation. The extracted aqueous TNT was then interacted with the AHT-modified pAu/AuNS substrate to form the Meisenheimer complex. The AHT-TNT complex was allowed to stand for 10 minutes and then washed with copious amount of NaOH solution (pH 8.5). The substrate was then dried under a stream of nitrogen and sent for SERS measurements.

3. Results and Discussion

3.1. Characterization of AHT-TNT Meisenheimer complex in solution phase

Meisenheimer complexes are σ -complexes formed by the nucleophilic addition of electron-rich species, usually amines, to the aromatic system of electron-deficient aromatic substrates [44, 45]. Due to three strong electron withdrawing nitro groups in its structure, TNT exists as an electron deficient aromatic system which readily forms Meisenheimer complex with amines [34]. AHT is an electron rich molecule with its amine group capable of contributing a lone pair of electrons to form a stable complex with TNT [44, 45]. We carried out the AHT:TNT complex formation at alkaline medium (pH 8.5). Fig. 1A shows the UV-visible spectrum of AHT, TNT and AHT:TNT mixture at pH 7 and pH 8.5. As expected, the UV-visible spectrum (pink spectrum) of AHT:TNT mixture at pH 8.5 showed a new band at 500 nm confirming the formation of Meisenheimer complex. This was adequately supported by the photograph we took at a higher concentration (Fig. 1A inset) where the pink color appeared upon the formation of

Meisenheimer complex between AHT:TNT. On the other hand, AHT:TNT at pH 7 (red spectrum) does not show any significant intense band at 500 nm which confirms that the alkaline medium (pH 8.5) is vital for the complex formation between AHT and TNT. Similarly, TNT alone at pH 8.5 (blue spectrum) does not show any bands at 500 nm. The development of AHT:TNT complex is vital for the SERS based detection of TNT using AHT as recognition molecule. In order to identify the saturation time of the complex formation, the increase of the UV-vis signal intensity with time was monitored and recorded (Fig. 1B). According to Fig. 1B, the band indicative of AHT:TNT complex formation at 500 nm saturates after 10 minutes where no further appreciable increase in absorbance can be observed.

The formation of Meisenheimer complex between AHT:TNT was further characterized by solution Raman spectroscopy. Fig. 2A and B (black) shows the Raman spectrum of clean AHT in aqueous medium at pH 8.5. AHT showed good Raman spectrum with characteristic bands in the fingerprint region between 1000 cm^{-1} and 1650 cm^{-1} . The addition of TNT to AHT at pH 8.5 (red spectrum in Fig. 2A) showed intense bands at 1349 cm^{-1} and 1618 cm^{-1} corresponding to symmetric NO_2 stretching and aromatic ring stretching modes respectively, which was not readily noticed in the AHT spectra in Figure 2A (black spectrum). To confirm the complex formation between AHT and TNT, we focused our attention to the two bands at 2800 and 3000 cm^{-1} . According to the literature the bands at 2858 cm^{-1} and 2910 cm^{-1} correspond to the symmetric and asymmetric CH_2 stretching, respectively [25, 46, 47]. Among these, the higher frequency CH_2 stretching at 2910 cm^{-1} is very sensitive to the protonation of the amine moiety and can be used as a vibrational marker to study the ionization of amine groups [46]. Significant changes in the electron density of the amine group leads to a change in the intensity of 2910 cm^{-1} band [46]. A careful examination of the CH_2 stretching region (2800 cm^{-1} - 3000 cm^{-1}

¹) in the SERS spectrum of the AHT:TNT complex (red spectrum in Fig. 2 B) indicates a change in the band shape in comparison to that of the clean AHT (black spectrum, in Fig. 2 B). The 2910 cm^{-1} band in the AHT:TNT spectrum (red spectrum) almost disappeared and only a broad band at 2858 cm^{-1} is observed. These changes in the CH_2 stretching region clearly confirms the alteration in the electron density of AHT amine group and, in effect, may indicate the development of AHT:TNT Meisenheimer complex at pH 8.5 [25, 46, 47]. On the other hand, the mixture of AHT and TNT at pH 7.0 does not show any significant change in the CH_2 stretching region of AHT and thus indicate the lack of Meisenheimer complex at this pH. Thus, both UV-visible and solution Raman studies clearly indicate the Meisenheimer complex between AHT and TNT at pH 8.5.

3.2. Electrochemical and XPS characterization of CA and AHT modified electrodes/substrates

The next part of this study is to confirm the Meisenheimer complex between TNT and AHT monolayer on pAu/AuNS surface. As we discussed in the introduction, the upright orientation of AHT with free amine groups protruding outwards is critical for the efficient complex formation between TNT and AHT. To confirm the orientation of AHT SAM and the availability of free amine groups on the substrate's surface, after functionalization with AHT, electrochemical desorption and XPS experiments were carried out. For comparison purposes, we also carried out electrochemical desorption and XPS studies of pAu/AuNS surface that was modified with cysteamine SAM. Fig. 3 shows the cathodic linear sweep voltammograms (LSVs), corresponding to desorption of cysteamine and AHT SAM from pAu/AuNS electrode surface in 0.1 M KOH. The LSV of AHT (Fig. 3, curve b) showed a sharp cathodic peak at -1.0 V corresponding to the cleavage of Au-S bond [31]. The sharp desorption peak of AHT clearly depicts that AHT has formed a well packed monolayer on the pAu/AuNS surface [31]. This

observation may provide indirect evidence that all the AHT molecules are oriented in the preferred trans conformation of alkanethiols on the surface [31, 38, 48]. To the contrary, the desorption LSV of cysteamine SAM from the pAu/AuNS surface shows two cathodic peaks at -0.91 V and -0.77 V. The presence of two desorption peaks of the cysteamine monolayer may be attributed in part to different Au-S bond environments and different orientations of cysteamine on the pAu/AuS surface [35, 36, 49]. One possible orientation is the vertical standing of cysteamine molecule where thiol is anchored to Au surface with amine group pointing vertically away from the Au surface [35]. The other possible orientation is bridge-like orientation where the amine group of cysteamine may also be bound to the Au surface in addition to the attachment of its thiol group to the same surface [35]. Further, the desorption potential of Au-S bond in the case of AHT monolayer is higher by ~ 0.1 V than that of the cysteamine monolayer. This can be attributed to the higher stability of Au-S bond in the case of AHT monolayer due to increased van der Waals lateral integration upon increasing the hydrocarbon chain length of the alkyl thiol [31, 48]. Thus, the higher stability of the AHT recognition molecule on the sensor surface is essential for the better shelf-life of the sensor.

In order to validate the conclusions derived from the above electrochemical study we carried out XPS investigation of pAu/AuNS surfaces that were modified with cysteamine and AHT respectively. We examined the N 1s region of both cysteamine and AHT modified surfaces. Fig. 4 shows the raw and peak fitted spectra of the N 1s region of cysteamine and AHT modified pAu/AuNS surfaces. The N 1s region of AHT monolayer depicted a sharp peak at 400.1 eV (spectrum b in Fig. 4) confirming the existence of free amino groups on the substrate surface [31, 50]. On the other hand, the N 1s region of cysteamine monolayer showed two peaks at 400.1 eV and 398.3 eV. The peak at 400.1 eV is similar to that of the AHT monolayer and

corresponds to the free amine groups of cysteamine [31, 50]. The second peak at the lower binding energy of 398.3 eV is in agreement with the literature values for bound amine groups and, therefore, corresponds to the surface-bound amine groups of cysteamine [31, 50, 51]. Further, cysteamine monolayer also depicted higher intensity for the 398.3 eV peak in contrast to the 400.1 eV peak. This may be attributed to the higher population of the “bridged” cysteamine orientation over the vertical orientation confirming that majority of the amine groups in cysteamine monolayer are not free. Thus, based on the electrochemical and XPS study we conclude that the majority of the amine groups in AHT monolayer are freely available on the Au/pAuNS surface. Therefore AHT represents a better recognition layer for the SERS detection of TNT over cysteamine. In the forthcoming studies, we employed pAu/AuNS surface that is modified with AHT monolayer for the SERS based detection of TNT.

3.3. Characterization of AHT-TNT Meisenheimer complex on pAu/AuNS surface

Fig. 5 shows the SERS spectrum of AHT SAM (black spectrum) and subsequent AHT:TNT complex (red spectrum) formed directly on the pAu/AuNS surface. The SERS spectrum AHT SAM shows two CH₂ stretching bands at 2855 cm⁻¹ and 2919 cm⁻¹. The SERS spectrum of AHT:TNT modified pAu/AuNS showed intense signals at 1375 cm⁻¹, 1559 cm⁻¹ and 1608 cm⁻¹. The high intensity of the SERS signal at 1608 cm⁻¹ (aromatic ring stretching) may be attributed in part to the vertical orientation of the TNT:AHT complex over the substrate and the large Raman cross-section of the aromatic ring when it is in a perpendicular position to the noble metal surface [25, 52-54]. The spectrum also depicts a sharp and intense band at 1375 cm⁻¹ corresponding to NO₂ group symmetric stretching vibration of TNT [25, 52-54]. The band at 1559 cm⁻¹ corresponds to asymmetric NO₂ stretching of TNT [25, 52-54]. A closer examination of the AHT:TNT complex spectrum in the CH₂ region (Fig. 5 inset) shows two bands at 2855

cm^{-1} and 2919 cm^{-1} which are similar to those of the AHT SAM. This indicates that there may be an abundance of free amine groups on the substrate surface, which are not involved in complex formation with TNT. However, AHT forms dense monolayer on the surface and therefore TNT molecules may experience some steric hindrance to reach the amine groups of the AHT layer [55]. Therefore, optimizing the surface distribution of AHT is vital for the effective complex formation between AHT and TNT. Beforehand, it is essential to optimize the complex formation time between AHT and TNT on pAu/AuNS surface. SERS measurements were carried out for the AHT-TNT complex by varying the complex formation time (2-12 min) on the pAu/AuNS surface. Fig. 5B shows the corresponding time vs SERS intensity plot where 1608 cm^{-1} Raman band of TNT was used as a reference band for quantifying the SERS intensity. Similar to solution phase AHT-TNT complex formation study, the complex formation on surface (Fig. 5B) also gets saturated around 10 min. Therefore, in all the forthcoming studies, 10 min incubation time were permitted for the AHT-TNT complex formation on pAu/AuNS surface.

3.4. AHT and BT mixed monolayer system for efficient TNT complex formation

Mixed monolayer system is one of the best approaches to optimize the distribution of the AHT molecules on the substrate surface and minimize the potential steric hindrance of the TNT molecules attempting to bind the amine groups of the AHT layer. In this study, we have chosen butane thiol (BT) as spacer molecule for developing a mixed monolayer of BT: AHT. BT is shorter than the AHT molecule by two carbons. Therefore it can be used as a spacer between AHT molecules without causing a steric hindrance to their amine groups. Further, the monolayer of BT molecule has free methyl group at the terminal end while its thiol end is bound to the Au surface. The free methyl groups do not have the ability to form a donor-acceptor complex with TNT due to the lack of free lone pair of electrons when compared to the amine groups on AHT.

This is to say that, the methyl groups are not capable of forming complex with the electron deficient TNT molecules. We have tested the complex formation between BT and TNT at pH 8.5 using UV-visible in solution phase and also using SERS on pAu/AuNS surface. As expected, both showed negative results confirming that BT is not able to form Meisenheimer complex with TNT. Now, to arrive the best ratio of the mixed monolayer system, we carried out SERS measurements of TNT using Au/pAuNS surfaces modified with ATH/BT mixed layers at various ratios of AHT to BT. Fig. 6 shows the SERS spectrum of TNT captured by pure AHT monolayer (black), AHT:BT (1:1) (blue) and AHT:BT (9:1) (red) mixed layers. Among these three ratios, AHT:BT (9:1) mixed layer showed the best TNT signal intensity and therefore we used it in the forthcoming studies. Thus, the higher SERS intensity of TNT on AHT:BT (9:1) mixed layer system clearly confirms that the shorter BT molecule served as an effective spacer between AHT molecules at this 9:1 ratio. This provided easy access to large number of TNT to bind to the pAu/AuNS surface, via the AHT bridge, in an almost perpendicular orientation to the SERS substrate [29, 36-38] and subsequent higher SERS signal. Further, the enhanced SERS intensity of TNT on AHT:BT (9:1) mixed layer system indirectly conveys that on pure AHT layer, the recognition molecules are closely packed and hence showed hindrance to the effective complex formation with incoming TNT molecule.

For the SERS quantification of TNT by present AHT:BT (9:1) modified pAu/AuNS surface, various concentrations of TNT has been used to form Meisenheimer complex over the sensor surface. Fig. 7A shows the SERS spectra of TNT between 1 nM and 100 fM. The band at 1608 cm^{-1} was used as a reference band for the quantitative analysis of TNT. The intensity of TNT SERS signal increased monotonically with the increase in TNT concentration. Fig. 7B illustrates the linear correlation between the logarithmic SERS signal intensity at 1608 cm^{-1} and

the corresponding logarithmic solution concentration of TNT [56]. A good linear response was achieved within the given concentration range of 1 nM to 1 fM of TNT with a correlation coefficient of 0.9910. The error bars indicate the standard deviations from 10 measurements at different spots over the SERS substrate. The lower limit of quantification (LQD) for TNT by the present sensor was found to be 100 fM. The theoretical limit of detection (LOD) can be obtained from the x-axis intercept of linear regression [56, 57]. We estimated the theoretical LOD to be 6.3×10^{-14} M for the present TNT sensor [56].

3.5. Interference Study

2,4-DNT and PA are two major contaminants usually co-exist with TNT [29]. Further, both compounds have Raman fingerprints similar to TNT. Therefore, it is very important to selectively identify TNT in presence of DNT and PA in order to demonstrate the method for practical in-field applications. We reacted both DNT and PA individually with AHT at pH 8.5 and subsequently monitored the UV-visible spectrum. The UV-vis spectra of the 2,4-DNT/AHT and PA/AHT mixtures did not show any change to the absorption spectra of 2,4-DNT or PA (Fig. 8). Also, the visual inspection did not indicate any colour change unlike the AHT-TNT reaction. This clearly indicates that AHT selectively forms a Meisenheimer complex with TNT while completely disregarding 2,4-DNT and PA even though both molecules are also electron deficient. The reason for this is the lack of nitro group in 2,4-DNT prevents distribution of anionic charge throughout the benzene ring and therefore 2,4-DNT may not form Meisenheimer complex with AHT [25]. Although PA has a similar structure to TNT, the -OH group can easily lose a proton which would reduce the charge transfer between AHT and PA and thus less likely to form a complex [52]. In order to further confirm the selectivity of the AHT towards TNT, SERS investigation of DNT and PA was carried out on AHT modified surface. Both AHT-DNT

and AHT-PA modified surfaces were prepared similar to that of AHT-TNT modified surface as presented in the experimental section. As expected, the SERS spectra of AHT-DNT and AHT-PA surface do not show Raman bands corresponding to aromatic NO₂ stretching except the SERS spectral features similar to AHT monolayer (Fig. 2A black spectrum). This clearly confirms that AHT does not form Meisenheimer complex with DNT and PA on surface and hence no interference.

3.6. Detection of TNT in soil

In order to demonstrate the practical application of the present sensor, we carried out the detection of TNT spiked into soil. Before spiking the TNT into soil, the soil sample was tested for any pre-existing TNT. The SERS spectrum of the soil extract, before spiking with TNT, confirmed the absence of pre-existing TNT. 2 nM TNT was then spiked in soil and subsequently extracted into the aqueous medium. The TNT extract was then screened by pAu/AuNS SERS substrate modified with 9:1 AHT:BT mixed monolayer (Fig. 9). The SERS spectrum of extracted TNT showed similar fingerprint pattern to that of TNT standard. To demonstrate the repeatability of TNT detection from soil, SERS measurements were repeated on two separate dates with 10 SERS spectra measured on each date (total n=20). The reproducibility of the SERS sensor was determined based on the SERS signal obtained from random spots across the entire substrate under the same experimental conditions. The relative standard deviation of the characteristic SERS peaks of TNT for the 20 measurements was found to be 5.23% confirming the reproducibility of the sensor. The concentration of TNT extracted by water medium was calculated from the calibration curve (Fig. 7B) and was found to be 302 pM only. This is due to the poor extraction of TNT that is caused by its poor solubility in water (0.13 g/L at 20 °C) [58]. Despite the low recovery, these results clearly demonstrate that the present methodology can be employed to identify TNT in-field for environmental and forensic applications.

4. Conclusion

In this article, we have demonstrated a simple, rapid and selective approach for the detection of trace amounts of TNT in aqueous media via SERS. The method utilized AHT as the new TNT recognition molecule. The Meisenheimer complex formed between AHT and TNT was supported by UV-visible and Raman spectroscopy. The complex formation was rapid and the reaction completed in almost 10 min, which laid platform for the rapidity of the present method. Prior to the formation of AHT:TNT complex on the surface, AHT SAM modified surface was characterized by electrochemical desorption and XPS studies. Both the studies provided supportive evidence for the availability of free amine groups on the surface with suitable orientation. AHT SAM and AHT:BT mixed SAM system was explored to enhance the TNT SERS signal. AHT:BT mixed SAM in 9:1 ratio delivered higher TNT SERS signal with a LOD of 100 fM. Further, the new sensor is selective towards TNT over 2,4-DNT and picric acid. Thus, the present method is rapid and selective which satisfies the requirements for the detection of TNT in real life samples. Finally, this new sensor successfully demonstrated the detection of TNT in soil with a calibration curve developed between 1 nM and 100 fM of TNT concentration. Thus, the present TNT sensor can be used for the rapid screening of TNT residues in soil and water.

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Figures

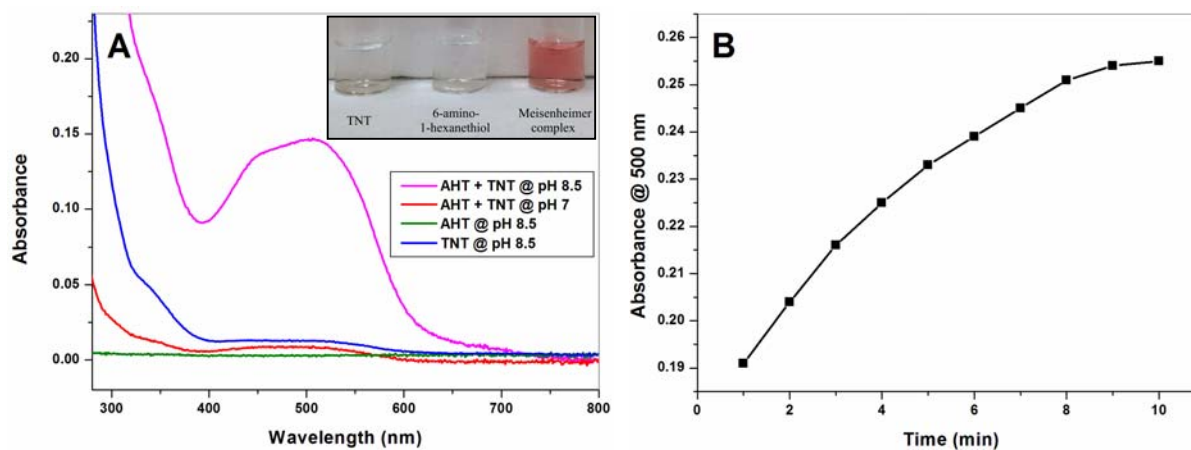


Fig. 1. (A) UV-vis absorption spectra of aqueous 1:1 Meisenheimer complex between AHT and TNT at neutral and alkaline pH and (B) Absorbance vs time plot for the formation of Meisenheimer complex between AHT and TNT at pH 8.5.

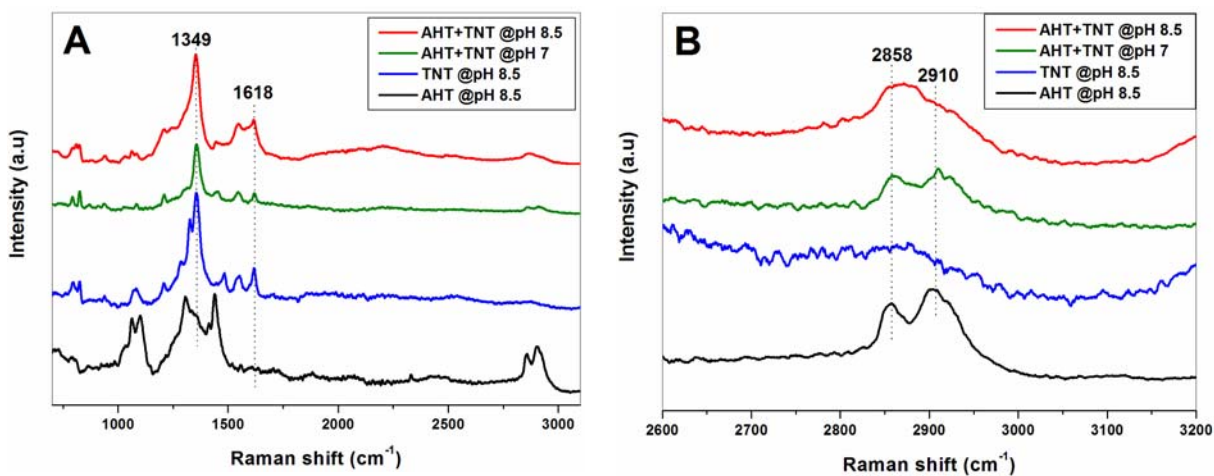


Fig. 2. (A) Solution Raman spectra of 1 mM AHT at pH 8.5 (black), TNT at pH 8.5 (blue), AHT:TNT mixture at pH 7 (green) and pH 8.5 (red). (B) The same spectra as shown in A focused at 2800-3000 cm^{-1} region.

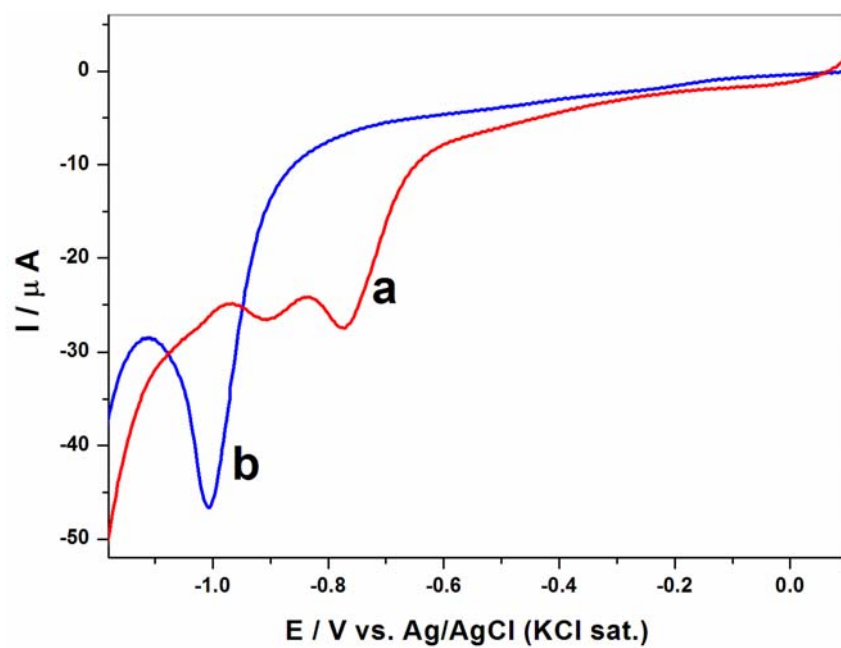


Fig. 3. Cyclic voltammograms obtained for the self-assembled monolayer of (a) cysteamine (b) AHT in 0.1 M KOH at a scan rate of 0.1 Vs^{-1} .

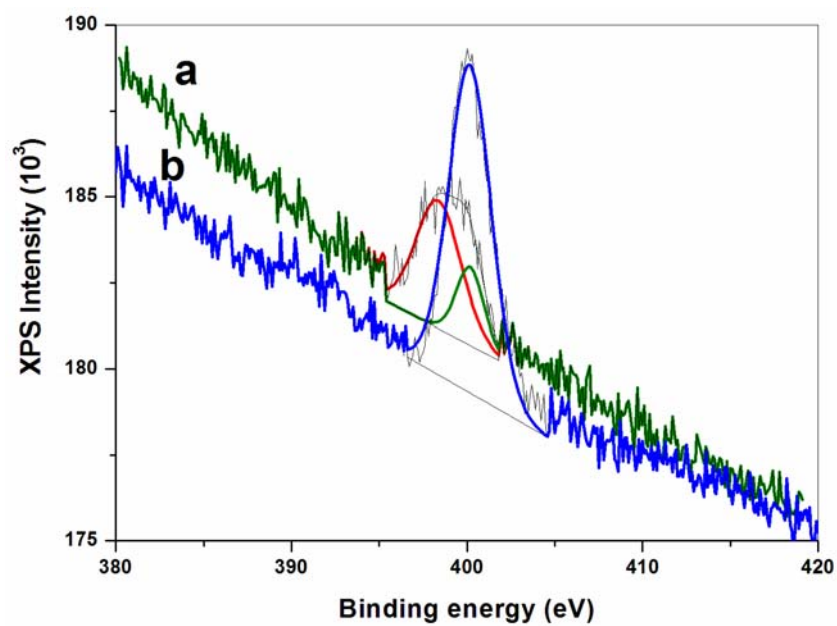


Fig. 4. XPS spectra corresponding to N 1s region of self-assembled monolayer of (a) cysteamine (b) AHT.

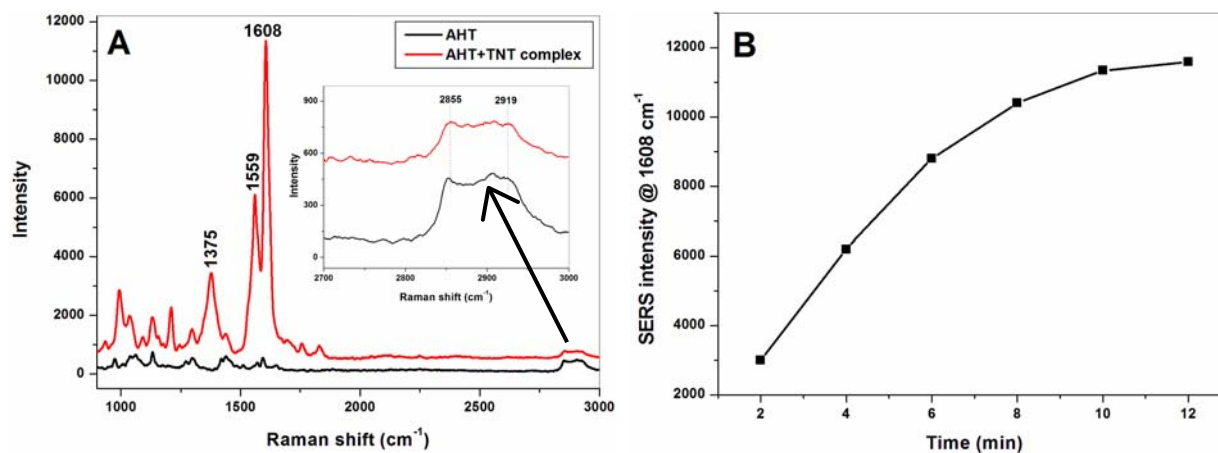


Fig. 5 (A) SERS spectra of AHT monolayer (black) and AHT+TNT surface bound complex on pAu/AuNS surface at pH 8.5. 1 nM TNT concentration was used for complex formation. Inset shows the zoomed in amine stretching region. (B) SERS intensity vs time plot for the formation of Meisenheimer complex between AHT and TNT at pH 8.5.

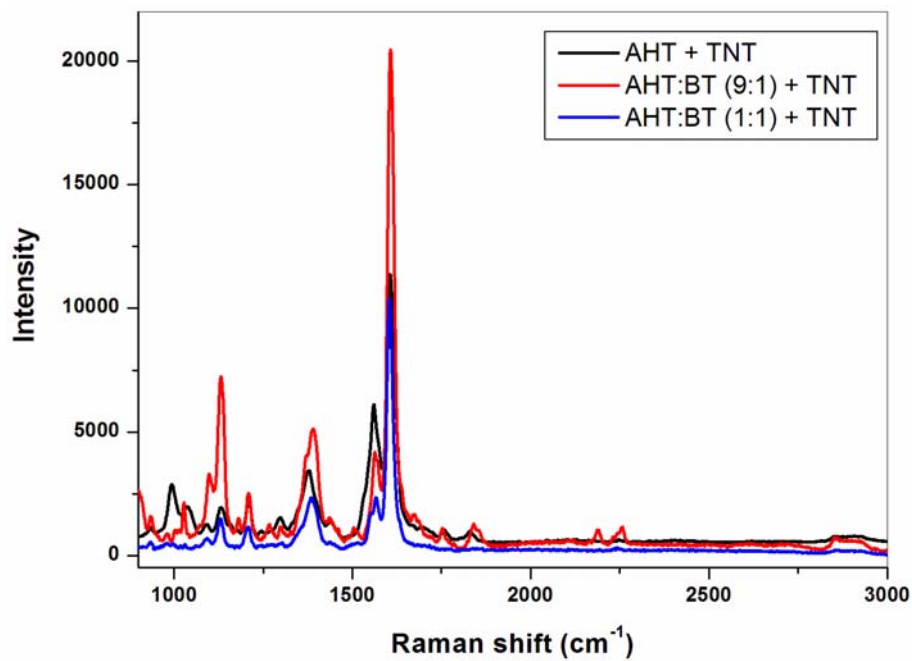


Fig. 6. SERS spectra of TNT complex at pH 8.5 on pure AHT monolayer (black), AHT and BT mixed monolayer in the ratio of 1:1 (blue) and 9:1 (red).

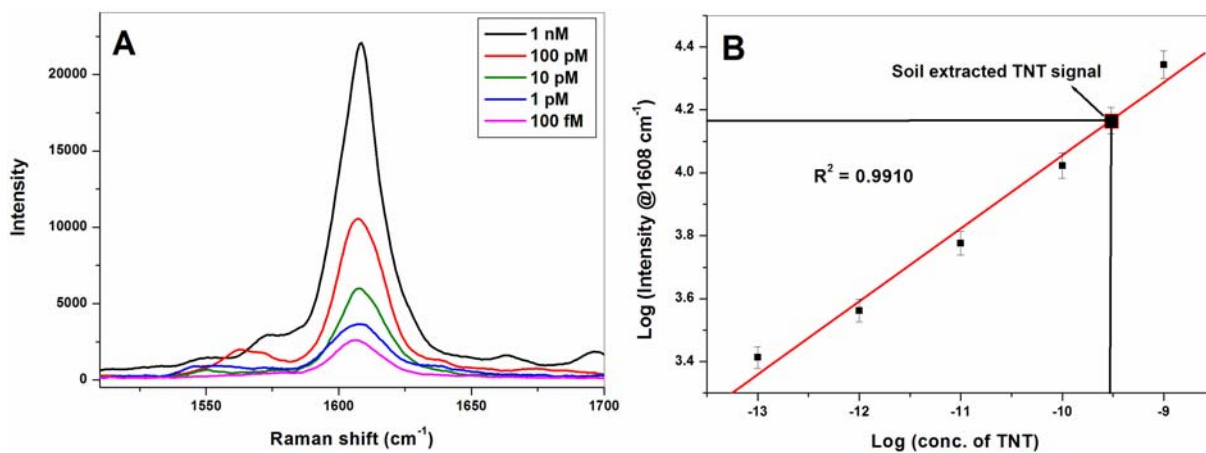


Fig. 7. (A) SERS spectra @1608 cm⁻¹ obtained for various concentrations of TNT on AHT:BHT (9:1) modified pAu/AuNS surface at pH 8.5. (B) Plot demonstrating the linear correlation between logarithmic solution concentration of TNT and logarithmic SERS intensity of the AHT-TNT complex.

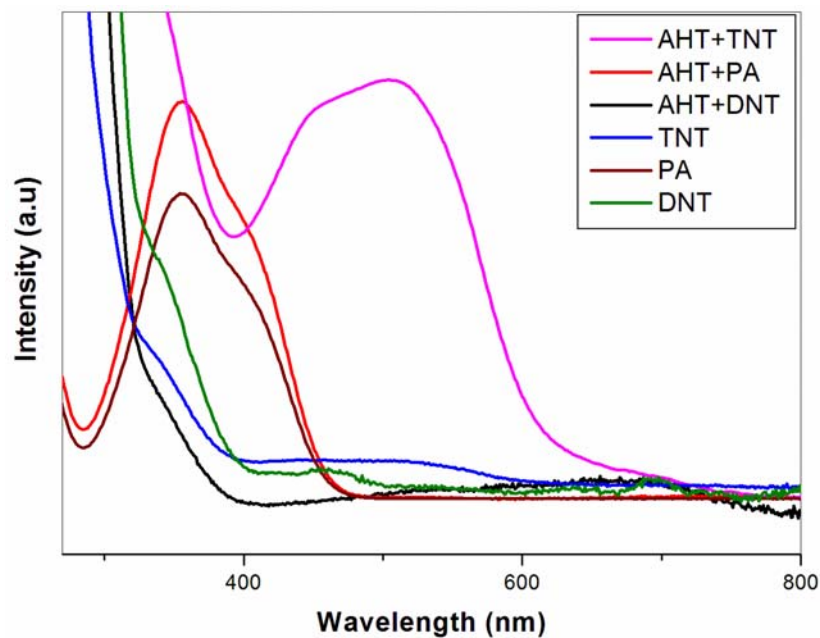


Fig. 8. UV-vis absorption spectra of DNT (green), PA (brown), TNT (blue), AHT+DNT (black), AHT+PA (red) and AHT+TNT (pink) at pH 8.5.

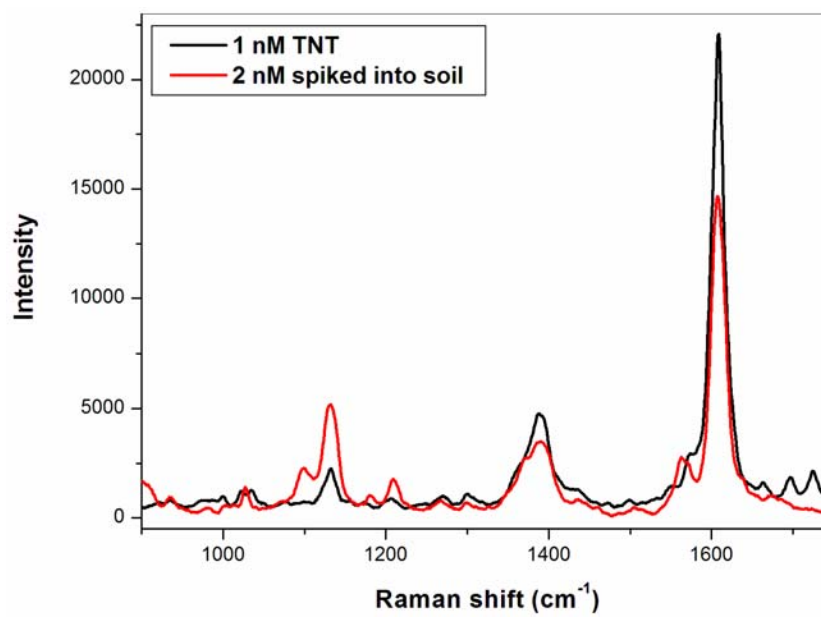


Fig. 9. SERS spectra of 1 nM (black) and 2 nM soil extracted (red) TNT on AHT:BHT (9:1) modified pAu/AuNS surface at pH 8.5.

Highlights

- 6-Aminohexanethiol (AHT) was used as new recognition molecule for TNT via selective Meisenheimer Complex
- Self-assembled monolayer (SAM) of AHT was formed over gold nanostructured (AuNS) SERS active surface to capture TNT
- The availability of the free amine groups and vertical orientation of AHT SAM was supported by electrochemical desorption and XPS studies
- AHT:Butanethiol (9:1) mixed monolayer system showed enhanced SERS signal for TNT
- Cysteamine selectively forms Meisenheimer complex with trinitrotoluene in presence of dinitrotoluene and picric acid
- Detection of 302 pM TNT was achieved over AuNS SERS substrate
- Selective detection of TNT in soil was demonstrated

Graphical abstract