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Article

Detection of Multiple Nitroaromatic Explosives via Formation of a Janowsky Complex and SERS

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ABSTRACT: Military-grade explosives such as 2,4,6-trinitroluene (TNT) are still a major worldwide concern in terms of terror threat and environmental impact. The most common methods currently employed for the detection of explosives involve colorimetric tests, which are known to be rapid and portable; however, they often display false positives and lack sensitivity. Other methods used include ion mobility mass spectrometry, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS), which despite producing more reliable results often require large, expensive instrumentation and specially trained staff. Here we demonstrate an alternative approach that utilizes the formation of a colored Janowsky complex with nitroaromatic explosives through reaction of the enolate ion of 3-mercapto-2-butanone. The colored complex is formed rapidly and can then be detected sensitively using surface-enhanced Raman scattering (SERS). We demonstrate that SERS can be used as a quick, sensitive, and selective technique for the detection of 2,4,6-trinitrotoluene (TNT), hexanitrostilbene (HNS), and 2,4,6-trinitrophenylme-thylnitramine (tetryl) with a detection limit of 6.81 ng mL^{-1} achieved for TNT, 17.2 ng mL^{-1} for tetryl, and 135.1 ng mL^{-1} for HNS. This method of detection also requires minimal sample preparation, can be done in a solution-based format, and utilizes the same precursor reagents for complex formation with each of the explosives which can then be identified due to the specificity of the unique SERS response obtained. We demonstrate the ability to simultaneously identify three explosive compounds within a total analysis time of 10 min. This method of detection shows promise for the development of rapid and portable SERS-based assays which can be utilized in the field in order to achieve reliable and quantitative detection.
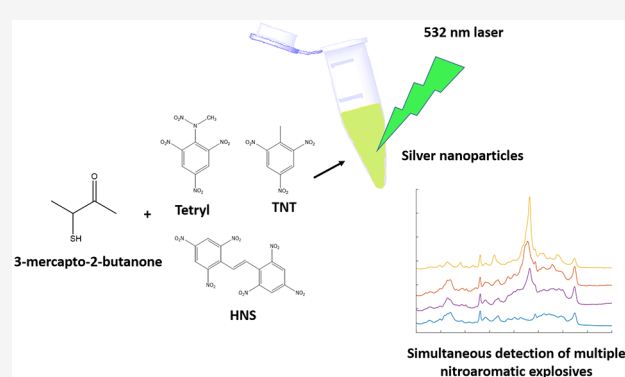
rinitrotoluene (TNT) is a highly explosive nitroaromatic compound that is still a worldwide cause for concern.[1,2] TNT is still commonly utilized as a blasting agent for controlled explosions; however, for many years it has been used for military and terrorist activities such as the development of landmines[3] and improvised explosive devices (IEDs).[4] As a result of this widespread use, TNT pollution within the environment has become a major cause for concern.[5] Prolonged human exposure to nitroaromatic compounds such as TNT can lead to many problems such as liver abnormalities[5] and anemia.[6] Since TNT is capable of crossing into the bloodstream through the skin, soil contamination has become an area of interest in monitoring TNT contamination. From a security standpoint, there is also a strive toward developing more specific, rapid, and sensitive methods for the detection of explosives such as TNT.[7] The transport of such materials poses a serious safety concern, and therefore, there is a definite need for more reliable methods of detection. Current methods of detection of nitroaromatic explosives include presumptive colorimetric tests, which have been utilized for many years in the identification of explosive compounds. Recent advances in colorimetric tests include those demon-

strated by Peters et al.[8] in 2015, which utilized a portable paper-based device for rapid detection of multiple explosive types including military-grade explosives, organic perchlorates, and inorganic explosives such as black powder. This method of detection is fast and portable, and very low detection limits of detection of 0.39 μg were achieved; however, this approach cannot distinguish individual explosives from a class of compounds. Krauss et al.[9] also demonstrated a paper-based device that was employed in the colorimetric detection of multiple illicit compounds including TNT, which were able to be stored and used over a period of 10 weeks. Long-term stability of portable devices is highly desirable as detection methods strive toward point-of-need analysis; however, quantitative results could not be obtained using this method.

Quantitative methods regularly employed in the detection of explosives such as TNT include mass spectrometry (MS) and

electrochemical detection. Leppert et al.[10] recently demonstrated a hyperfast method of quantifying less thermally stable explosives such as ethylene glycol dinitrate (EGDN), nitroglycerin (NG), and pentaerythritol tetranitrate (PETN) using mass spectrometry and achieved detection limits ranging from 0.1 to 20 μg/mL. However, the instrumentation required for this sort of analysis is often very large and costly and usually requires very specialized user training which is less desirable for in-field detection. For this reason, surface-enhanced Raman scattering (SERS) has become an attractive alternative to more well-established techniques for use in this field. The enhancement in signal observed by using this technique, as opposed to conventional Raman spectroscopy, allows much lower limits of detection to be achieved, and the unique fingerprint spectrum obtained offers specificity and hence multiplex detection capabilities not offered by other techniques. Difficulties are often encountered when trying to adsorb molecules such as TNT directly onto the surface of metallic nanoparticles. Conventional methods of immobilizing molecules onto the surface of nanoparticles, such as using nanoparticles which have been conjugated with antibodies specific for a target antigen, often present size incompatibility issues as well as the instability of biological components in the harsh environments explosives are usually found in as well as increasing the cost of the sensor.[11] Stewart et al. have shown the potential of positively charged nanoparticles in such applications, achieving a detection limit of 25 μM for picric acid in 2015.[12] However, positively charged nanoparticles are not simple to prepare and often do not exhibit long-term stability.

There are many examples of SERS detection of trace levels of explosives by adsorption onto solid metallic substrates, such as Klarite, as demonstrated by Wackerbarth et al.[13] in 2010, and more recently (2018), Hakonen et al.[14] achieved detection of picric acid at a concentration of 0.02 ng/mL using commercially available Ag-capped Si nanopillar substrates. Despite significant advancements in nanotechnology and development of SERS substrates, there still exists a need for rapid and reliable detection of multiple explosives particularly in a solution-based format. A promising way of achieving this is to form colored derivatives of explosive compounds which can subsequently be detected using SERRS; for example, Jamil et al.[15] used cysteamine as a precursor to form a Meisenheimer complex and achieved a detection limit of 0.04 mg/L of TNT in wastewater samples. SERRS utilizes both a surface enhancement from the nanoparticles and a resonance contribution from the absorbance of the colored complex and has been shown to result in enhancement factors up to 10^{14} orders of magnitude.[16] There are many other examples of utilizing a Meisenheimer-type complex in order to achieve SERRS detection of TNT;[17,18] however, almost all of these methods require time-consuming preparation of functionalized Au nanoparticles and do not allow for detection of multiple explosives simultaneously.

McHugh et al.[19] also demonstrated that surface-enhanced resonance Raman scattering (SERRS) of TNT can be achieved through the formation of stilbene derivatives of TNT; however, the synthetic protocols employed were complex and often produced low yields. Another method of achieving SERRS detection of TNT was explored which involved the reduction of TNT and subsequent azo dye formation.[20] This method achieved detection limits of 1 nM; however, synthesis of the azo dyes was again time consuming.

In the work reported here, we investigate the ability of 3-mercapto-2-butanone (3M2B) to form a Janowsky complex[21] with multiple explosives including 2,4,6-trinitrotoluene (TNT), 2,4,6-trinitrophenylmethylnitramine (tetryl), and hexanitrostilbene (HNS). The resulting complexes have a thiol functionality allowing for covalent attachment to the surface of silver nanoparticles, resulting in a very strong SERS response. Furthermore, it has been shown that the complex formed for each of the different explosive compounds produces a distinctive fingerprint SERS spectrum, opening the potential for sensitive, simultaneous detection of multiple explosive materials at the point of need. The main advantage of this approach is the minimal sample preparation required for analysis. The same reagents can be employed as precursors for the detection of multiple explosive materials, therefore making this method an ideal approach for in-field detection. The formation of the TNT complex is shown in Figure 1.
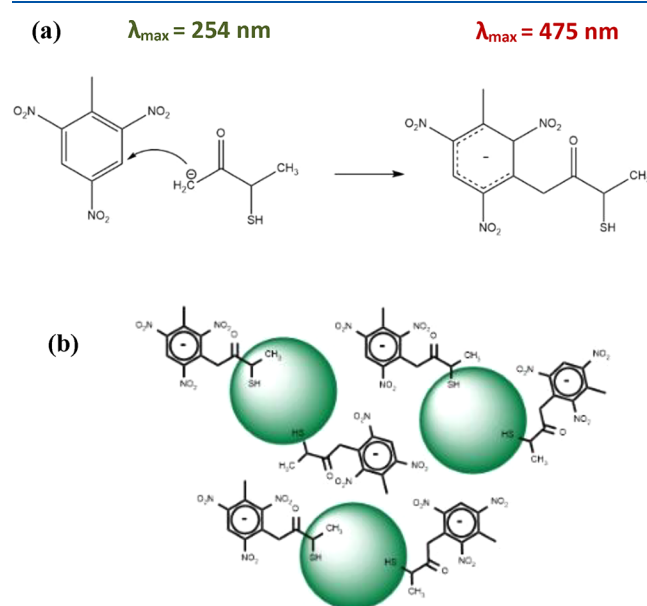

Figure 1. Formation of a Janowsky complex between 3-mercapto-2-butanone and TNT. (b) Subsequent attachment of the 3-mercapto-2-butanone/TNT Janowsky complex to the surface of silver nanoparticles via Ag−S bond formation.

■ EXPERIMENTAL SECTION

Chemicals and Materials. Silver nitrate, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 3-mercapto-2-butanone (3M2B), and sodium citrate were purchased from Sigma-Aldrich, UK. Trinitroluene (TNT), tetryl, and hexanitrostilbene (HNS) (1 mg/mL in acetonitrile) were supplied by the Defense Science Technology Laboratory[2] (DSTL), Porton Down, UK.

Instrumentation. All SERS measurements were carried out using a Renishaw Plate Reader (532 nm). All SERS measurements were obtained using 100 mW laser power and a 1 s acquisition time unless otherwise stated. Nanoparticle characterization was carried out using an Agilent Cary 60 UV−vis spectrophotometer (Agilent Technologies, USA) and Malvern Zetasizer ZS (Malvern Instruments, UK).

Colloid Synthesis. Citrate-reduced silver nanoparticles were synthesized using a modified Lee and Meisel method.[22] Silver nitrate (90 mg) was added to 490 mL of Milli-Q water

and heated to 98 °C with constant stirring. Sodium citrate (100 mg in 10 mL) was then added, and the solution was maintained at 98 °C with constant stirring for a further 90 min.

Nanoparticle Characterization. Silver nanoparticles were characterized using UV–vis extinction spectroscopy and dynamic light scattering (DLS). A 1 mL amount of nanoparticle solution was added to a 1 cm^3 quartz cuvette (diluted 20×), and the extinction was measured between 300 and 800 nm. Size and zeta potential were measured using dynamic light scattering (532 nm).

Formation of Janowsky Complex. In order to form a Janowsky complex between 3-mercapto-2-butanone and TNT, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile (ACN) (200 μL, 0.01 M) was added to 3-mercapto-2-butanone in ACN (200 μL, 0.01 M). To this solution, TNT (200 μL, 0.01 mM) in ACN was added. This addition was followed by an immediate color change from colorless to red/purple and then subsequently red/pink. The same procedure was carried out for the formation of the tetryl and HNS Janowsky complexes using solutions containing 0.01 mM in acetonitrile. The multiplex samples were prepared via the addition of each explosive (0.01 mM in acetonitrile) simultaneously to a solution containing 3M2B (0.01 M in acetonitrile) and DBU (0.01 M in acetonitrile).

SERS Analysis. After 5 min, the TNT Janowsky complex was added at varying concentrations (50 μL) to silver nanoparticles (100 μL). This solution was subsequently analyzed by SERS using a Renishaw Plate Reader (Renishaw, UK) with 532 nm excitation (100 mW, 1 s acquisition time). SERS analysis of the tetryl and HNS Janowsky complexes was carried out under identical conditions.

UV–vis Absorption Spectroscopy. Absorption spectroscopy was carried out using a Cary 60 UV–vis spectrophotometer. A 1 mL amount of each solution was added to a 1 cm^3 quartz cuvette, and absorption was measured between 200 and 800 nm. The Janowsky complex was analyzed at a concentration formed from the addition of TNT (200 μL, 0.01 mM) to a solution containing 3M2B (200 μL, 0.01 M) and DBU (200 μL, 0.01 M).

Data Analysis. Data analysis was carried out using WiRE 4.2 software and Matlab (version 2016, The MathWorks, Natrick MA, USA). Principal component analysis (PCA) was carried out using Matlab. All spectra were truncated and scaled prior to PCA. Analysis was carried out on an average of 5 measurements of 3 replicate samples, unless otherwise stated.

The research data associated with this paper is available at the following link: 10.15129/4f8897b7-23b6-42ae-b4c0-736fe3e994b2.

■ RESULTS AND DISCUSSION

Complex Formation. TNT forms a Janowsky complex with the enolate anion of 3-mercapto-2-butanone as shown in Figure 1a. The thiol functionality incorporated into the complex allows covalent attachment to the surface of silver nanoparticles. As 3-mercapto-2-butanone is a small molecule (104.167 g mol^{-1}), the complex comes within very close proximity to the nanoparticle surface as shown in Figure 1b and allows a strong SERS response to be achieved. Traditionally, a Janowsky complex is formed as a product of the reaction between acetone and an electron-deficient aromatic compound.[21] In this instance, acetone was replaced by 3-mercapto-2-butanone (3M2B). The Lewis basic nature of the thiol allowed for covalent attachment to the surface of silver

nanoparticles subsequent to formation of the complex between the ketone and the electron-deficient benzene ring of TNT. 1,8-Diazobicyclo (5.4.0) undec-7-ene (DBU) was employed as a base in the first step of the reaction in order to form an enolate anion via deprotonation of the acidic methyl group in the α position relative to the ketone. DBU was chosen as it is a nonaqueous base and was therefore suitable for use in acetonitrile (ACN). As the formation of a Janowsky complex is specific to nitroaromatic molecules, this method of detection is ideal for SERS detection of nitroaromatic explosives as minimal interference would be expected from other types of aromatic molecules such as PAHs commonly found in soils. In addition, nitro stretching vibrations also tend to produce a strong SERS response; this allowed for identification of three different explosives molecules due to the unique SERS response obtained for each complex. Upon addition of TNT to the 3M2B anion, a Janowsky complex was formed. This was confirmed by the formation of a red-pink-colored complex with an absorption maximum at 475 nm. Figure 2b–d shows the
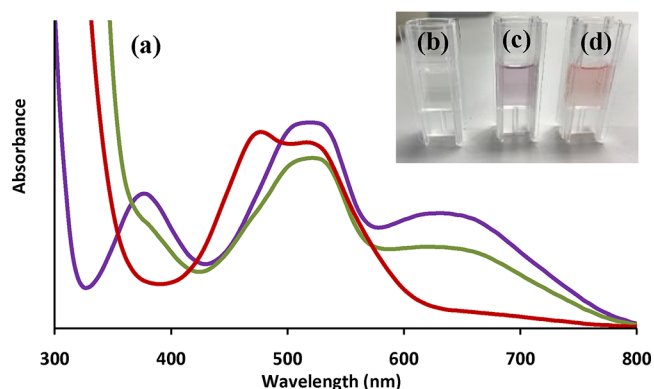

Figure 2. (a) UV–vis absorption measurements of 3-mercapto-2-butanone and DBU immediately after addition of TNT (gray), after 5 min (red), and TNT and DBU (purple). (Inset) Color change observed upon addition of TNT to a solution containing 3-mercapto-2-butanone and DBU (b), immediately after addition of TNT (c), and after 5 min (d).

change in color observed upon addition of TNT (0.1 mM) to a solution containing 3M2B (0.01 M) and DBU (0.01 M) (Figure 2b), where Figure 2c is the purple-colored stabilized benzyl carbanion of TNT formed immediately after the addition of TNT to a solution containing 3M2B and DBU due to deprotonation of the methyl group of TNT and Figure 2d shows the change in color observed after 5 min due to formation of a Janowsky complex between 3M2B and TNT. The resulting UV–vis absorption spectrum displayed two absorption maxima at 475 and 520 nm as shown in Figure 2a.

The final complex shows multiple absorbance bands (Figure 2a, red spectrum) with a maximum at 475 nm as would be expected for a Janowsky complex. It appears that the solution containing only TNT and DBU (Figure 2a, purple spectrum) also forms a purple/red-colored complex with absorption maxima at 520 and 650 nm. This is thought to be a result of both stabilized benzyl carbanion formation and pseudo-Janowsky[3] complex formation due to the presence of excess base in the reaction mixture.[23] In such circumstances, TNT ions are capable of forming unstable charge transfer complexes with other TNT molecules; however, these complexes are unstable and usually short lived. However, it appears that this

type of complex may also form a minor product in the reaction with 3M2B as the peak at 520 nm remains when 3M2B is introduced into the reaction mixture (red spectrum).

The reaction between the 3M2B anion and TNT was monitored over a period of 30 min (Figure 3). These data
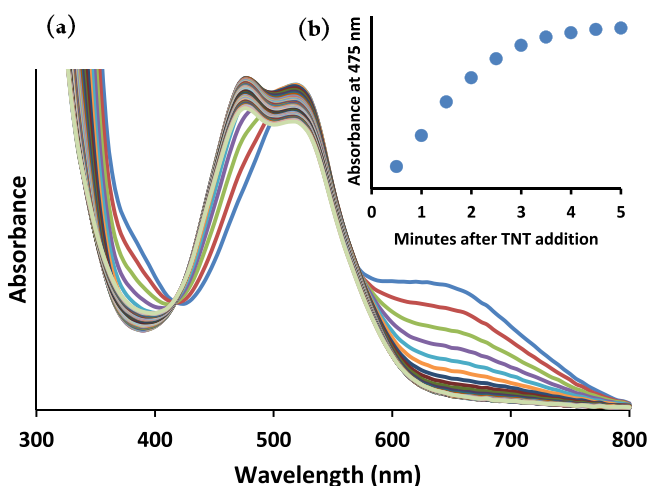

Figure 3. (a) UV−vis absorption spectra of a solution containing 3-mercapto-2-butanone (0.01 M), DBU (0.01 M), and TNT (0.01 mM) over a period of 30 min with a measurement taken every minute. (b) Increase in the measured absorbance at 475 nm each minute after TNT addition for a period of 5 min.

suggest that a pseudo-Janowsky complex forms immediately after addition of TNT to a solution containing 3M2B and DBU followed by formation of the stable 3M2B/TNT Janowsky complex. Figure 3b shows the increase in absorbance at 475 nm over a period of 5 min.

It can be observed that over a period of 5 min the absorbance at 650 nm decreased to a negligible intensity while the absorbance peak at 520 nm also decreased and a new absorbance peak appeared at 475 nm. It can therefore be deduced that the absorbance peak present at 520 nm is likely due to the pseudo-Janowsky complex which is formed as a product of TNT forming CT complexes with other TNT molecules. The peak at 650 nm can be attributed to the presence of a stabilized benzyl carbanion as a result of the deprotonation of the methyl group of TNT. However, in the presence of 3M2B, this product is disfavored and therefore short lived, since after 3.5 min the peak at 475 nm attributed to the complex formed between the ketone 3M2B and TNT had reached a constant absorbance value. The peak at 520 nm continued to decrease in intensity after this time point; however, it is still present in the absorbance spectrum after 30 min, suggesting two species are present in the final solution

SERS Measurements. In order to determine whether differences in the SERS spectra between 3-mercapto-2-butanone and the 3-mercapto-2-butanone/TNT Janowsky complex could be obtained, both samples were added to a colloidal solution of silver nanoparticles (OD = 1) and SERS analysis was carried out using a 532 nm laser excitation (100 mW). The resulting spectra are shown in Figure 4.

The SERS spectrum of the TNT Janowsky complex (Figure 4, black) displays a strong peak at 1300 cm^{-1}, which can be assigned to a symmetrical nitro stretching mode due to the presence of TNT. The presence of this band is highly indicative of the formation of a Janowsky complex and the
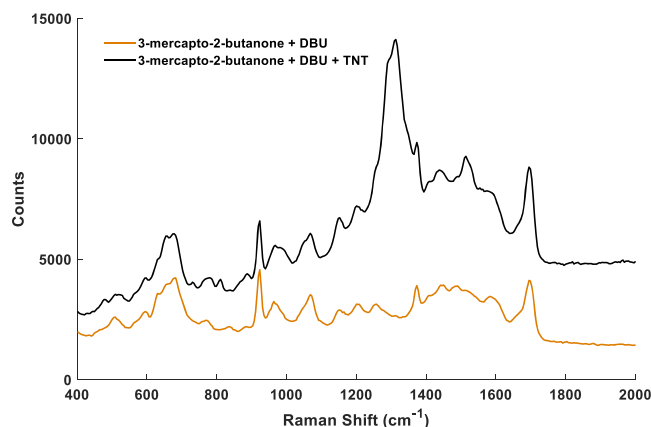

Figure 4. SERS spectra obtained from addition of 3-mercapto-2-butanone to hydroxylamine-capped silver nanoparticles (orange) and resultant Janowsky complex (black). All spectra were collected using a Renishaw Plate Reader with a 532 nm excitation wavelength (100 mW) and a 0.8 s acquisition time.

successful attachment of this complex to the silver nanoparticles. The peak present at 1516 cm^{-1} could also be due to the presence of asymmetrical nitro stretches and aromatic ring-breathing C−H modes. To ensure the formation of the complex was responsible for the SERS spectrum observed, control samples were analyzed containing TNT added directly to silver nanoparticles from which only solvent peaks were observed and a solution containing 1,3,5-trinitro-1,3,5-triazinane (RDX) in place of TNT. As RDX is a nonaromatic explosive and does not possess the electron-deficient arene ring required to form a Janowsky complex, it was expected that no complex would be formed from the addition of RDX to 3M2B and DBU and as a result no SERS response obtained from the RDX. The resulting spectra are shown in Figure S1. As expected, only solvent peaks and peaks associated with 3M2B were observed, and no TNT-specific peaks were present in any of the control samples. Similarly, the colored complex resulting from the addition of TNT to DBU was also analyzed, and again, only solvent peaks and bands associated with DBU were observed. This result is congruent with the assumption that TNT forms a pseudo-Janowsky complex under these conditions; however, as this type of complex would have little affinity for the surface of the silver nanoparticles, no SERS response was obtained. To determine whether the SERS response obtained was correlated to the concentration of TNT present in the sample, various concentrations of TNT (ranging from 0.1 to 2.2 μM) were added to a solution of 3M2B (0.01 M) and DBU (0.01 M). Figure 5a shows the spectra obtained. It was observed that the symmetrical nitro stretch at 1300 cm^{-1} displays a change in intensity relative to the concentration of TNT present in the sample. The intensity of the symmetrical nitro stretch at 1300 cm^{-1} decreased as the concentration of TNT in the solution was lowered, as would be expected. The intensity of this peak was plotted against the concentration of TNT in the solution, which produced a linear relationship over the concentration range 0.1−2.2 μM with a correlation coefficient of 0.99 as shown in Figure 5b.

The limit of detection established for TNT using 3 times the standard deviation of the blank divided by the gradient of the straight line was found to be 6.81 ng/mL, which is comparable to the detection limits of already established techniques such as mass spectrometry[24] and colorimetry.[25] At trace-level concen-

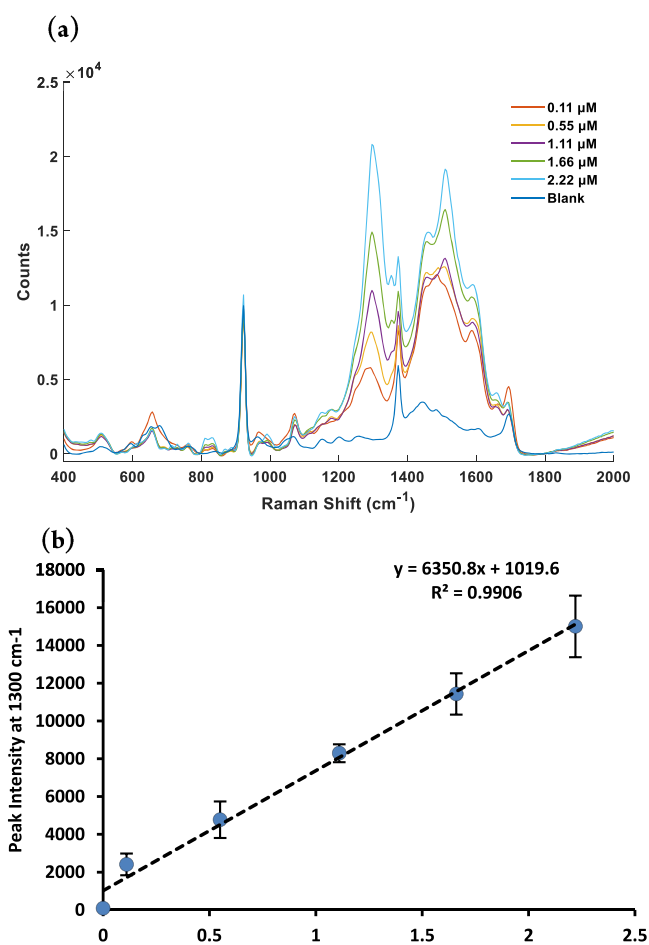**(a)**

(b)

y = 6350.8x + 1019.6
R² = 0.9906

Figure 5. (a) SERS spectra obtained upon addition of various concentrations of TNT to 3-mercapto-2-butanone anion and subsequent addition to silver nanoparticles. All spectra were collected using a Renishaw Plate Reader with a 532 nm excitation wavelength (100 mW) and a 0.8 s acquisition time. (b) Plot of the intensity of the 1300 cm^{-1} peak against TNT concentration (μM) showing a linear relationship in the range 0.1−2.2 μM (coefficient of determination R = 0.99). Error bars indicate the standard deviation on 3 replicate samples with 5 measurements taken of each.

trations, such as those shown in Figure 5, the complex displays a very strong band at 1516 cm^{-1} thought to be due to aromatic ring breathing C−H modes from the TNT molecule. The reason these bands appear more intense at lower concentrations of TNT could be due to a change in the orientation of the complex on the nanoparticle surface. Less TNT could allow the complex to change the orientation when attaching to the surface of the nanoparticles as there would likely be less steric hindrance. The doublet peaks present at 660 and 675 cm^{-1}, which can be attributed to C−S stretching of the ketone, appears less intense when TNT is present in the sample, which is also indicative of a change in the surface orientation in the presence of TNT. In addition to a sensitive limit of detection, another major benefit of SERS is the availability of portable Raman instrumentation, the low time taken for analysis, 5 min, and minimal sample preparation providing a potential detection method for in-field analysis.

Another significant advantage of this method over other methods that are currently employed for explosive detection is the capability to apply the same reaction to multiple explosives of interest. Nitroaromatic explosives such as tetryl (2,4,6-
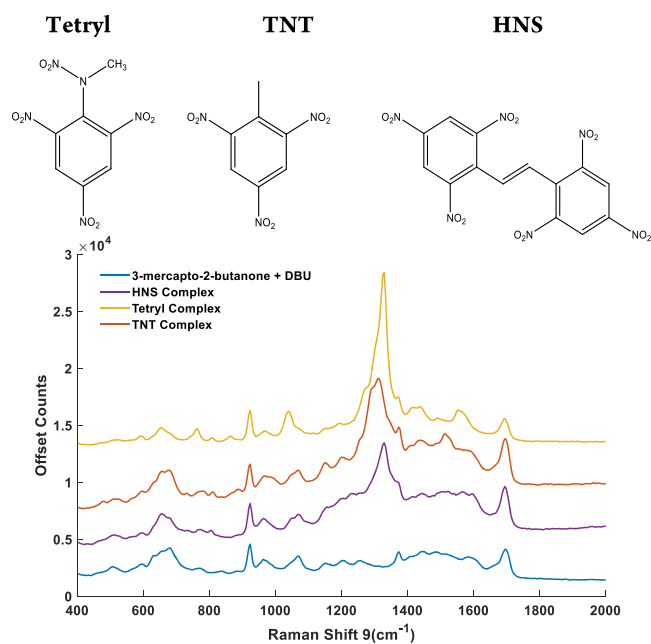

Figure 6. Structure of tetryl, TNT, and HNS and SERS spectra obtained from addition of TNT (red), hexanitrostillbene (purple), and tetryl (yellow) to a solution containing 3-mercapto-2-butanone and DBU (blue). All spectra were collected using a Renishaw Plate Reader with a 532 nm excitation wavelength (100 mW) and a 1 s acquisition time. Spectra are offset for clarity.

trinitrophenylmethylnitramine) and HNS (hexanitrostilbene) also possess an electron-deficient aromatic ring and are therefore capable of forming a Janowsky complex with 3M2B. The resulting UV−vis absorption spectrum when each explosive is added to 3-mercapto-2-butanone and DBU is shown in Figure S2. The tetryl complex displayed absorbance maxima of 445 and 510 nm, and similarly, the HNS complex exhibited two absorbance bands at 470 and 560 nm, both of which are characteristic of a Janowsky-type complex. Formation of both the tetryl and the HNS complexes was observed over a period of 30 min. Similar to TNT, HNS formed a short-lived pseudo-Janowsky complex when only DBU was present in the solution, resulting in an absorbance spectrum with a broad peak at 680 nm. This peak disappeared after a period of 5 min upon addition of 3M2B and was accompanied by the emergence of new absorbance maxima at 470 nm due to formation of a true Janowsky complex between HNS and 3M2B. However, the peak at 470 nm continued to increase over a period of 15 min (Figure S3), whereas the TNT complex reached an absorbance maximum after 3.5 min. Tetryl exhibited similar results wherein a pseudo-Janowsky complex formed immediately after addition of tetryl to DBU as shown in Figure S2; however, addition of 3M2B resulted in a much less significant shift in absorbance from 435 to 445 nm. As a result of the minor change in absorbance maximum displayed when 3M2B was introduced to the solution containing tetryl and DBU, it was not possible to measure the rate of formation of the tetryl complex using the same method employed for the TNT and HNS complexes. The SERS spectrum of the solution containing only tetryl and DBU did not produce the same characteristic nitro stretching peak at 1329 cm^{-1} which was observed in the 3M2B tetryl complex and will be explained in more detail below. This supports the assumption that the complex formed between tetryl and DBU
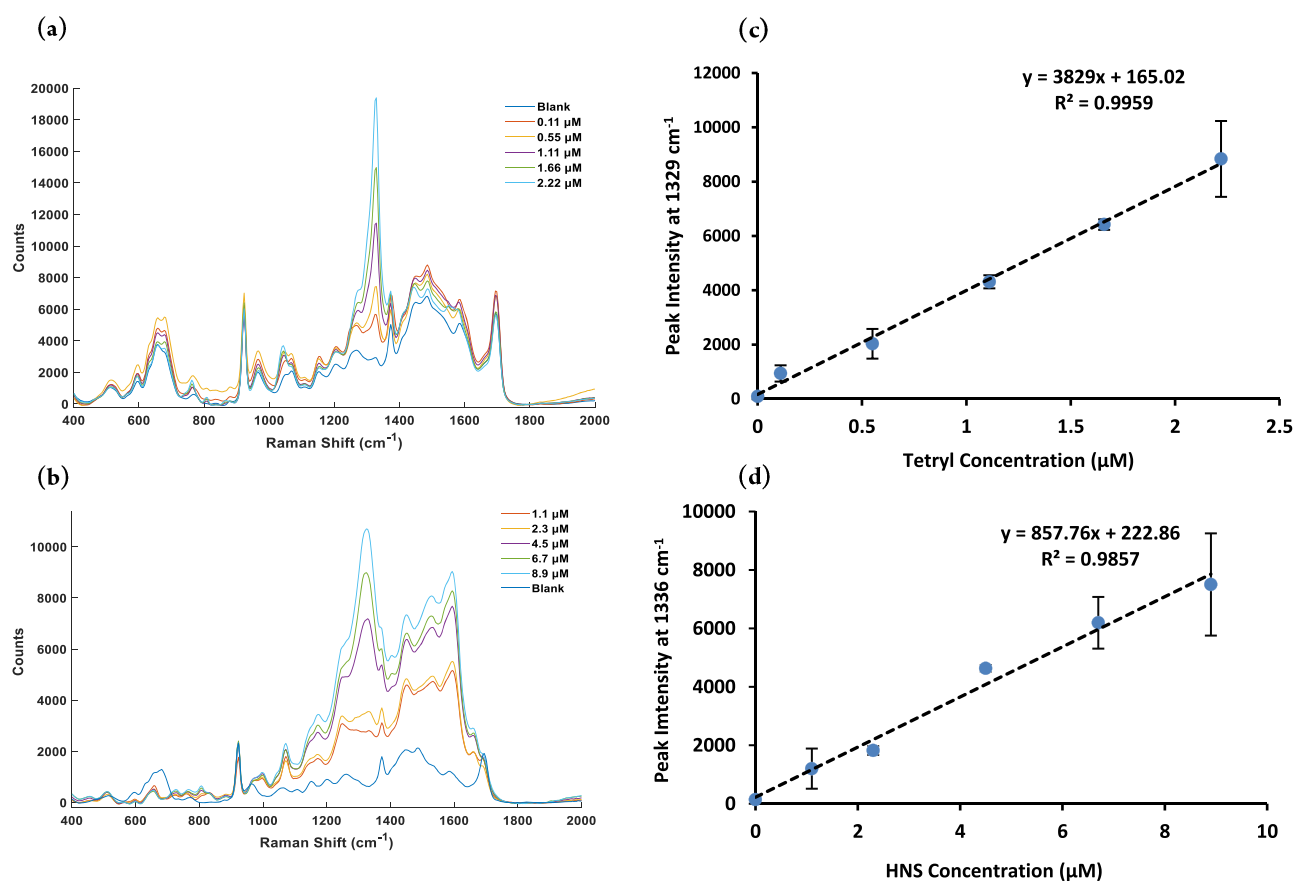
Figure 7. SERS spectra obtained upon addition of various concentrations of (a) tetryl and (b) HNS to 3-mercapto-2-butanone anion and subsequent addition to silver nanoparticles. All spectra were collected using a Renishaw Plate Reader with a 532 nm excitation wavelength (100 mW) and a 1 s acquisition time for tetryl and 0.5 s for HNS. (c) Plot of intensity of the 1329 cm^{-1} peak against tetryl concentration (μM) showing a linear relationship in the range 0.1−2.2 μM (coefficient of determination $R = 0.99$). (d) Plot of the intensity of the 1336 cm^{-1} peak against HNS concentration (μM) showing the linear relationship in the range 1.1−8.9 μM (coefficient of determination $R = 0.98$). Error bars indicate the standard deviation of 3 replicate samples with 5 measurements taken of each.

is a pseudo-Janowsky complex which has no affinity for the surface of the silver nanoparticles.

The tetryl and HNS complexes were analyzed by SERS in an identical format to the TNT complex, and the resulting SERS spectra are shown in Figure 6 along with the structure of each of the explosive compounds prior to formation of a Janowsky complex. It can be observed that there are clear differences between the SERS spectra obtained from the analysis of each of the complexes formed with the different explosives, TNT, HNS, and tetryl, under the same conditions even though all of these explosive compounds are structurally very similar. All complexes produced strong nitro stretching peaks between 1300 and 1340 cm^{-1}; however, it can be seen that the nitro peak for each complex varies and lies at 1300, 1329, and 1336 cm^{-1} for the TNT, tetryl, and HNS complexes, respectively. The TNT Janowsky complex also displayed a strong band at 1516 cm^{-1} likely due to the presence of aromatic ring-breathing modes. Aromatic C−H stretching peaks were also observed for the tetryl complex at 1560 cm^{-1}, and similarly for the HNS complex a broad band was observed at 1517 cm^{-1}, which can again be attributed to the 2 aromatic rings present in this complex. To determine the limit of detection of tetryl and HNS, the SERS intensity at 1329 and 1336 cm^{-1} was measured as a function of concentration over the range of 0−2.5 and 0−10 μM for tetryl and HNS, respectively. The limit of detection was calculated to be 17.2 ng mL^{-1} for the tetryl complex and

135.1 ng mL^{-1} for the HNS complex as shown in Figure 7a and 7b, respectively.

The limit of detection established for tetryl was 17.2 and 135.1 ng mL^{-1} for HNS, which is comparable to the limit of detection obtained for the TNT complex (Figure 5). The HNS complex displayed an LOD significantly higher than that of the other two explosives, despite being structurally very similar. It was deduced that this was likely due to the size difference of HNS compared with the other two explosive compounds, as HNS is significantly larger (450.23 g mol^{-1}) compared to TNT (227.13 g mol^{-1}) and tetryl (287.15 g mol^{-1}). The larger size of the complex may hinder the attachment of the complex to the surface of the nanoparticles, and therefore, a less intense SERS response is obtained. It is also very likely that the reason for the higher detection limit obtained for HNS is a result of the slower formation of the 3M2B/HNS Janowsky complex as discussed previously and shown in Figure S3. Both the TNT and the tetryl complexes appeared to reach reaction completion after a period of less than 5 min, whereas the HNS complex did not reach an absorbance plateau until 15 min after the addition of 3M2B to the reaction mixture. It is possible the detection limit of HNS could be improved by increasing the time allowed for complex formation; however, in the interest of the development of a rapid detection method for multiple explosives, the time allowed for complex formation was kept to a minimum. The established detection limit for
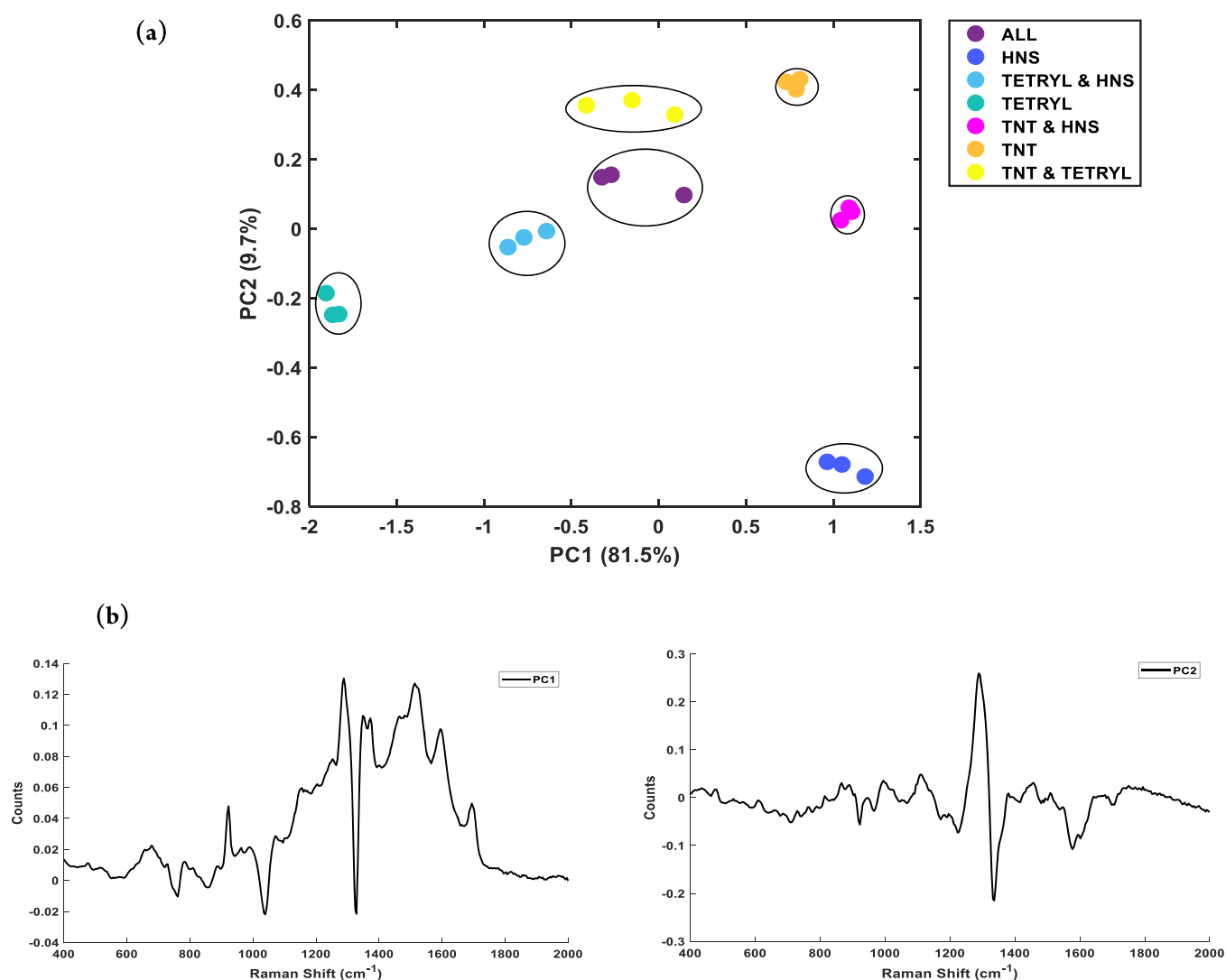
Figure 8. (a) Scores plot showing PC1 (which contributed 81.5% of the covariance) plotted against PC2 (9.7% of the covariance) for samples containing all explosive complexes (purple), the HNS complex (dark blue), a mixture of both the tetryl and the HNS complex (light blue), the tetryl complex (green), a mixture of the TNT and HNS complexes (pink), the TNT complex (orange), and a mixture of both the TNT and the tetryl complexes (yellow). (b) Loadings of PC1 and PC2.

each explosive compound is comparable with techniques which are already currently used for nitro aromatic explosive detection.

Despite all three complexes displaying strong nitro stretching bands, these bands differed slightly due to the variance in structure of each of the three explosives. This is hugely beneficial as the spectrum produced for each compound is therefore unique and allows structurally similar explosives to be identified using the same reagents and reaction. In order to reiterate the difference in the SERS spectrum obtained from the three types of explosive complexes, a multiplex study was carried out in which samples containing multiple explosives were added to a solution containing 3M2B (0.01 M) and DBU (0.01 M). The explosives were premixed before addition to 3M2B and DBU, and the resultant spectra are shown in Figure S4. Principal component analysis was carried out to determine the separation of each complex and mixtures containing two or more of each explosive, the results of which are shown in Figure S8. It can be seen from the scores plot that all three complexes showed significant separation, mostly along PC2, wherein the majority of the spectral difference can be

attributed to the TNT nitro stretching region around 1300 cm^{-1}. This would be expected as, although each complex is structurally very similar, the symmetrical nitro stretching peak varied for each complex, with TNT exhibiting a strong peak at 1300 cm^{-1}, the tetryl complex displaying a very sharp peak at 1329 cm^{-1}, and the HNS complex displaying a very broad peak at 1336 cm^{-1}. There was also some separation between the complexes along PC1, in which the variation in each spectrum can mostly be assigned to the aromatic region between 1500 and 1600 cm^{-1}. TNT and HNS displayed less separation on PC1, which could be expected as both compounds displayed similar aromatic peak stretches, likely due to their similarity in structure.

Tetryl showed significant separation along PC1 when compared with the other two explosives, likely due to the very sharp nitro peak produced in the SERS spectrum of the tetryl complex, whereas TNT and HNS tended to display broader, less well-defined nitro stretching bands, Figure 8. The solutions containing mixtures of each explosive were found to lie between each individual explosive complex as would be expected, as did the mixture containing all three explosive

compounds. However, each sample containing TNT tended to fall closer to the TNT sample in the scores plot, which would be expected as TNT displayed a stronger SERS spectrum, which therefore dominated the multiplex spectrum when compared with the tetryl and HNS complexes, likely due to the fact that this method of detection was optimized for TNT. As each explosive was analyzed at equal concentrations it was expected that the TNT complex would therefore dominate the spectrum produced. Additionally, it was shown that HNS required a longer reaction time in order to reach completion of the HNS/3M2B complex, and therefore, this is likely the reason for the weaker SERS spectrum obtained for this complex when compared with the TNT and tetryl Janowsky complexes. It is also likely that competing reactions take place between the three explosive molecules and 3M2B when all are present in the reaction mixture, with TNT appearing to dominate the SERS spectrum obtained. This suggests that TNT forms a Janowsky complex with 3M2B faster than tetryl and HNS; however, this was difficult to discern as the resulting UV−vis absorption spectra produced very broad absorbance bands. It should also be noted that the detection conditions were optimized for TNT detection. Therefore, it is also possible that with further optimization and increased reaction times the detection limits of tetryl and HNS could be improved. Despite this, trace-level detection limits were achieved for all three explosive molecules with minimal sample preparation and analysis time, which is a priority for in-field detection of explosive compounds.

The use of principal component analysis to achieve separation between different explosive complexes, as well as samples containing multiple explosives, highlights the advantages of using SERS as a detection method as there is potential for the simultaneous detection of multiple explosives in a quantitative manner with detection limits similar to current methods. Quantitative detection of individual explosives within reaction mixtures was not attempted in this instance; however, we have previously reported quantitative multiplex detection using SERS.[26] Therefore, there is potential to develop this detection method further to quantify multiple explosives within a mixture at trace levels. These advantages are crucial for in-field detection where often more than one explosive substance is present in samples that pose a threat to health or security.

CONCLUSIONS

This work has shown that SERS-based detection of explosives has many advantages over the techniques which are currently in use, particularly for in-field detection. By employing 3-mercapto-2-butanone as a precursor in the formation of a Janowsky-type complex with TNT, sensitive and selective detection was achieved with a detection limit of 6.81 ng mL^{-1}, which rivals that of more well-established techniques such as mass spectrometry,[1] electrochemistry,[21] and colorimetry.[25] The time required for detection of TNT was less than 10 min overall, which is very short in comparison to many methods employed today and is a key advantage for in-field detection. Another major advantage of using this type of analysis is the unique fingerprint spectrum obtained, even for molecules which are structurally very similar. As a result, this detection method has also shown promise for the detection of multiple nitroaromatic explosives simultaneously, which is ideal for detection at the point-of-need. A detection limit of 17.2 and 135.1 ng mL^{-1} was achieved for tetryl and HNS, respectively. It was also shown, using principal component analysis, that

samples containing multiple explosives could be identified and displayed significant separation based on the nitro stretching bands and aromatic region vibrational modes. Additionally, this method of detection required minimal sample preparation as the same starting reagents were used to form each of the explosive complexes.

In this field many challenges still exist in achieving sensitive and selective detection of explosives in a portable format; however, this method shows promise in providing trace-level detection of multiple analytes, and with recent advancements in technology, the miniaturization of Raman instrumentation means this method would be ideal for in-field applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b05062.

> SERS measurements of control samples, UV−vis absorbance spectra for tetryl and HNS complexes, and SERS spectra of solutions containing multiple explosive complexes which were used in PCA analysis (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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