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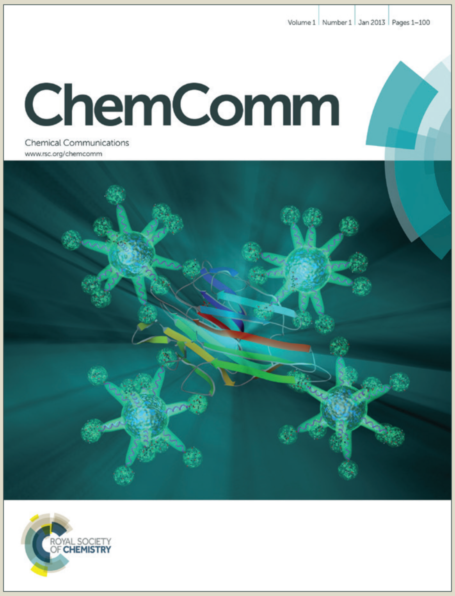

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ARTICLE TYPE

Ultrafast on-site selective visual detection of TNT at Sub ppt level using fluorescent gold cluster incorporated single nanofiber

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In this paper, the fluorescent gold cluster incorporated electrospun nanofibrous membrane and single nanofiber for selective and sensitive detection of TNT at sub-ppt level are demonstrated.

The advancement of an instant on-site visual detection of trinitrotoluene (TNT) is a critical need of the hour towards a secured society and a greener environment.[1] Despite momentous advances in the respective field, a portable and reliable method for quick and selective detection of TNT still poses a challenge to many, reasons attributing to inappropriate usage in subordinate areas and untrained personnel.[2] Recent past, the effort has been directed to the fluorescence quenching based sensors because of the acute sensitivity and simple on/off detection. As of now, fluorophores, conjugated polymers, and fluorescent quantum dots have been used as reporter materials.[3-4] Besides enormous attempts being made, the necessity of tagging makes it expensive for using these materials as sensors in real life applications. Also, appropriate exploration of fluorescent metal cluster-based fluorescence sensors remains at a premature stage. The main reason underlying this fact is the lack of durability under the demanding conditions, cost etc, all of which are necessary for perception of the day-to-day life requirements for on-the-spot detection of TNT. Here, we are fascinated in filling this gap and studying the possibility of preparing fluorescent nanofibrous hybrid system with superior selectivity and detection of TNT at sub ppt level.

We demonstrate in this article for the first time the selective, on-the-spot detection of TNT at sub ppt level using a single nanofiber embedded fluorescent gold clusters hybrid system. In this effort, the gold clusters are incorporated in the nanofiber (NF) using a simple and straightforward electrospinning method. Polyethylene oxide (PEO) has been selected as a matrix owing to its excellent electrospinnability. The most challenging step is the homogeneous distribution of clusters in NFs with the preservation of their optical properties.[5-6] The precise optimization and the effort has led to the accomplishment of the goal. Additionally, the utmost consequence of this assembly is its high stability, permitting better storage and instantaneous use. The clear change in the color of the electrospun nanofibrous membrane (NFM) could be used for the direct colorimetric visualization of TNT. As a result of which, a color change can be visualized by the naked eye in normal light conditions. Moreover, this nanofibrous hybrid system is relatively trouble-free and does not require any auxiliary alteration which makes it accessible even to an inexperienced person.

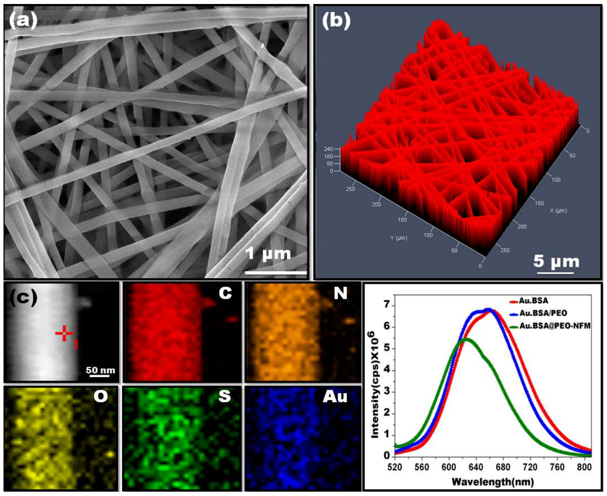

Figure 1: (a) SEM image of the Au.BSA@PEO-NFM (b) CLSM image of the Au.BSA@PEO-NFM in 3D view, excited at 488 nm (c) STEM image and EDS mapping of the C, O, N, S and Au elements presents in the Au.BSA@PEO-NF (d) Emission spectra of Au.BSA, Au.BSA/PEO solution and Au.BSA@PEO-NFM (λ_{ext} = 500 nm).

The proposed scheme here involves the incorporation of highly red fluorescent BSA-capped gold clusters[7](Au.BSA) into the electrospun nanofibrous membrane termed as Au.BSA@PEO-NFM for TNT detection. The essential characterization of Au.BSA and Au.BSA@PEO-NFM have been presented in the supporting information. The randomly oriented defect-free Au.BSA@PEO nanofibers with relatively uniform diameter of 150 ± 20 nm have been shown in Figure 1a as well as their photographs under UV light (λ_{ext} 366 nm) and white light have been presented in Figure S1. The SEM image of PEO nanofibers and BSA incorporated PEO nanofibers have also been studied and illustrated in Figure S2 as a reference. The use of polymeric matrix and the adopted method has allowed us to attain a uniform dispersion of gold clusters in the nanofibers while at the same

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time maintaining its morphology.

The confocal laser scanning microscopy (CLSM) image of the Au.BSA@PEO-NFs excited at 488 nm, shows the characteristic red emission, illustrated in Figure 1b. The distinct outline characteristic of the NFs, especially their length, makes it possible to utilize a single nanofiber (SNF) for sensing. Further, the intensity of the NFs was measured on their surface in diverse point of view, to ensure their exceptional ability, as presented in Figure S3. The STEM image and EDS (STEM-EDS) mapping of the Au.BSA@PEO single nanofiber clearly demonstrates the uniform distribution of gold clusters in the NF (see Figure 1C) and the size was found to be ~2nm as shown in Figure S4. The enhanced long term stability, with their definite morphology has touted to be an added advantage to the prescribed system. Thus, the unambiguous character of the nanofiber hybrid system is expected to provide a larger area for binding of the target analyte and in addition, enhance the performance of the sensor. The compared emission spectra of Au.BSA, Au.BSA/PEO solution and Au.BSA@PEO-NFM have all been clearly depicted in Figure 1d. The resultant fluorescence emission peak of the Au.BSA@PEO-NFM has been rarely found to change, shifting to the lower wavelength along with a decreased band width when compared to the results shown in the solution state. The observed results might be due to the strong confinement and well arrangement of the gold cluster in the nanofibers which could drives from the large specific surface area of the NFs and fast evaporation rate of the solvent during the electrospinning process.[9] The deconvoluted luminescence spectrum clearly shows that the existence of two bands (Band I and II), originates from the Au-core and Au-S stables respectively as demonstrated by others (see Figure S5).[8] There is no distinction in the emission spectra with differences in excitation wavelength as presented in Figure S6.

The FTIR and XPS spectra of Au.BSA and Au.BSA@PEO-NFM are illustrated in Figure S7 and S8. The stability of the Au.BSA@PEO-NFM has been tested for a long period of time in the typical atmosphere and their emission spectra have been taken after one and three months (see Figure S9), there is no discrete decline in the fluorescence intensity highlighting their long term storability and usability. Concluding, the nanofibrous hybrid system is void of the self quenching fluorescence and studies show its steadiness for a period of three months or more. In addition, no further growth of gold clusters in the PEO-NF has been detected, reasoning the fact that PEO polymer chains surrounding the gold clusters might possibly hinder the gold clusters to grow up. The sensing performance of Au.BSA@PEO-NFM has been tested upon exposure to different concentration of TNT. It is clearly observed that, the fluorescence intensity decreased as a function of increasing concentration of TNT which is demonstrated by CLSM images (2a-c). The fluorescence spectral changes of the Au.BSA@PEO-NFM for a wide range of TNT concentration has been picturised in Figure 2d. The relative fluorescence intensity (I_0/I, where I and I_0 are the fluorescence intensity in the presence and absence of TNT, respectively) versus the TNT concentration has been presented in Figure 2e. The visual fluorescence responses of Au.BSA@PEO-NFM at different concentrations of TNT by contact mode method have been tested by placing the nanofibrous membrane in the TNT solution for a second has been displayed in Figure 2f. Immediately, the color of the Au.BSA@PEO-NFM changed from white to either deep red or blue depending on the concentration and time in normal light condition which clearly illustrates their utility for the onsite instant visualization of TNT. The ocular noticeable response of the hybrid nanofibrous membrane towards the TNT solution has been found to be at one parts per billion (1 ppb) and such a level of sensitivity falls lower than the tolerable level of TNT in drinking water, recognized by the US EPA.[10-11]

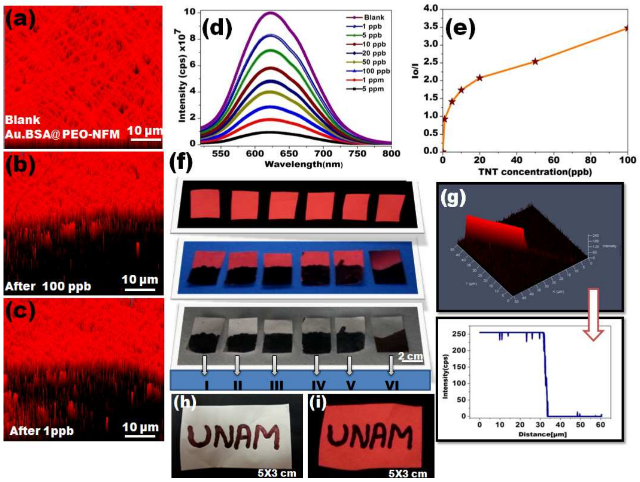

Figure 2: (a-c) CLSM images of the Au.BSA@PEO-NFM on glass slides before (a) and after (b-c) exposure to TNT (λ_{ext} = 488 nm, 20x magnification) d) Fluorescence spectra of Au.BSA@PEO-NFM upon treatment of various concentration of TNT e) Relative fluorescence intensity f) Photograph of the fluorescence quenching of Au.BSA@PEO-NFM strips by different concentration of TNT on contact mode when viewed under UV and white light (I-1 ppm, II-100 ppb, III-50 ppb, IV-20ppb, V-10ppb, VI-1 ppb). g) TNT treated Au.BSA@PEO-SNF and their fluorescence intensity profile. h-i) Hand written pattern on Au.BSA@PEO-NFM using TNT solution as ink.

As observed in the CLSM image, the fluorescence intensity of the Au.BSA@PEO-NFM completely decreases upon exposure to 1 ppb TNT, without affecting their NF morphology which has been confirmed by SEM analysis (Figure S10). Furthermore, half segment of the isolated single NF was treated with TNT and remaining at rest to ease of visual detection. The intensity profile collected from this single nanofiber is presented in Figure 2g. The complete disappearance of luminescence, devoid of any effect on their optical image (see Figure S11) is observed in the treated portion of TNT whereas there have been no decreases in the luminescence feature in the untreated portion. As well expected, the results were more straightforward. Figure 2h and 2i shows the pattern written on the Au.BSA@PEO-NFM using TNT solution as ink under normal and UV light. Another important aspect has been the thickness of the nanofibrous mat used for detection which is also directly interconnected to the expenditure of the devices. The electrospinning of Au.BSA@PEO NFs were carried out at different time periods of 1, 5 and 15 minutes in order to have different mat thickness. The observed result suggested that the sensing performance has not been profoundly dependent on the Au.BSA@PEO-NFM thickness, yet thin layers of NFs has been enough for detection as illustrated in Figure S12.

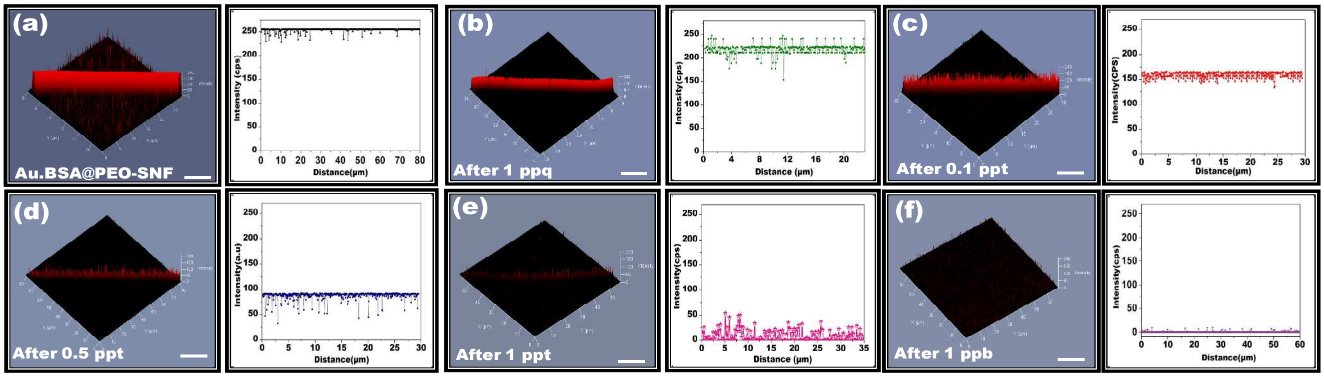

Figure 3: CLSM images of Au.BSA@PEO-SNF upon exposure to different concentration of TNT and their intensity profile collected from the surface of the nanofiber (scale bar 10μm)

The added flexibility of the hybrid system allows the NFs to be coated on any substrate as per our needs, such as cover slips, silicon wafers, aluminum foil, etc. A similar reduction in quenching efficiency has been observed in the Au.BSA@PEO-NFM, which had been stored for prolonged periods of 1 to 3 months as of now, suggesting further prolonging time. Further, the known factor in sensing performance in their bulk state has been known to be limited by their cross linked nature. Moreover, the complete decrease in the fluorescence intensity at one ppb level of TNT in bulk state further projected us to investigate their sensing performance on even lower concentrations using single nanofiber (SNF), due to which it will further reduce the outlay of the devices. As predicted, a SNF could sensitively detect the TNT molecules and showed enhanced detection limit of TNT that reaches a level up to sub ppt (0.1 ppt). The gradual decrease in the intensity, upon increasing the concentration is clearly observed at SNF level as presented in Figure 3. The decrease in the luminescence intensity occurred consistently throughout the SNF and such admirable characteristics of uniformity is significant in the development of a trustworthy method.

The detection of TNT in drinking water has triggered great interest as its intake may cause severe illnesses such as abnormal liver functions, anemia etc. With this objective, aqueous solution of TNT have been dropped on the Au.BSA@PEO-NFM which has been supported by aluminium substrate. The similar effect was observed for this case too, illustrated in Figure S13. The prepared Au.BSA@PEO-NFM hybrid system has been proved to be well suited for detection of TNT in aqueous media, knowing well that PEO is hydrophilic. A vital feature of the sensor is their selectivity towards the direction of the targeted analyte since the consequence of environmental interference problems encountered in water. Foremost, the attempt has been made towards 2,4-dinitrotoluene (DNT). No significant quenching effect of the fluorescence was obtained and the observed results confirmed that more apparent difference was seen in the quenching of fluorescence on exposure to TNT over DNT at the same concentration. Further, to exclude the interference of metal ions in water, the Au.BSA@PEO-NFM was tested towards the mercury, copper, and zinc ions which are the common toxic metal ions present in the aqueous media. The results confirmed that the common interfering ions in water have no significant effect on the fluorescence of Au.BSA@PEO-NFM the fluorescence quenching of TNT, when compared to DNT, has most probably been due to their lower electron accepting properties. In addition, they are not able to form effective Meisenheimer complex with amine groups similar to TNT resulted in subordinate response.[10] Though, the fluorescence was slightly quenched when treated at 50 ppm DNT concentration and the results was confirmed by CLSM image presented in Figure S15. Motivated by other groups, we have provided a comparison of our Au.BSA@PEO-NFM hybrid system to whatman filter paper for detection of TNT. [11] The highly fluorescent Au.BSA clusters were coated on the filter paper by dip coating method. The basic characterization and their sensing performance of TNT are presented in Figure S16. The limit of detection has been found to be 1 ppm, which has an outrange from the threshold limit and the detection definitely has not been as prominent as Au.BSA@PEO-NFM. The observed prominent disadvantages are listed here: (1) huge substances in need to coat the smaller area (2) stability is not superior as compared to Au.BSA@PEO-NFM which might be the environmental result and (3) constrained surface area available to act in response to the analyte. These facts highlight the merits of our nanofibrous hybrid system in terms of improved sensitivity and usage. The superior performance of Au.BSA@PEO-NFM towards TNT should be ascribed to the large energetic surface area for rapid interaction and more specifically, to the good adsorptive properties of membrane with nitroaromatic compounds.[12] Obviously, the larger surface area of the NF provides well-built contact space to TNT molecules and also, the entire surface of SNF has been assorted with Au.BSA, thereby the more active sites are readily available for interaction with TNT molecules with electron deficient effect. At the start, TNT molecules robustly bind to the surface of the NFs through an acid-base interaction between TNT and amino ligands on the NF surface.[13]

Generally, the binding of electron deficient TNT to the amino groups in the BSA has been expected to transfer the electron from amino groups to aromatic rings, which leads to the formation of Meisenheimer complexes between them.[14] The formation of TNT-amine complexes strongly suppresses the fluorescence emission, which is supported by our experiment output. The amplifying fluorescence quenching can sensitively detect the ultratrace TNT, and their specificity makes selectively distinguish the different types of nitro compounds.[15] Moreover, TNT–amine complexes strongly absorb the green part of visible light, and thereby the color of Au.BSA@PEO-NFM changes to deep red.

Note that the TNT solution does not absorb any visible part of the light. The annotations of visible absorption clearly demonstrate that the formation of TNT-amine complex. The experimental conception is fit in line with other groups also. The superior ability to bind analytes on their surface yields direct, label-free visual readout.

In summary, we have demonstrated an instant, onsite visual detection of TNT at sub ppt level using highly fluorescent Au.BSA incorporated electrospun PEO nanofibers. The observed changes in the color in the presence of TNT makes clear that their utility for onsite, instant detection of TNT. The compound exhibited selective recognition of TNT over a wide range of toxic metals in aqueous media. The extreme stability and maintained equivalent activity level, after a period of three months were well documented. The exceptional result on single nanofiber detection highlights their unique capability of the hybrid system which has great relevance to human health and greener environment.

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Single nanofiber embedded fluorescent gold clusters based sensor for selective detection of TNT at sub ppt level.