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Applying graphene oxide nano-film over a polycarbonate nanoporous membrane to monitor *E. coli* by infrared spectroscopy

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ABSTRACT

Nano-biosensors are excellent monitoring tools for rapid, specific, sensitive, inexpensive, in-field, on-line, and/or real-time detection of pathogens in foods, soil, air, and water samples. A variety of nano-materials (metallic, polymeric, and/or carbon-based) were employed to enhance the efficacy, efficiency, and sensitivity of these nano-biosensors, including graphene-based materials, especially graphene oxide (GO)-based materials. GO bears many oxygen-bearing groups, enabling ligand conjugation at the high density critical for sensitive detection. We have fabricated GO-modified nano-porous polycarbonate track-etched (PCTE) membranes that were conjugated to an *Escherichia coli*-specific antibody (Ab) and used to detect *E. coli*. The random distribution of nanopores on the PCTE membrane surface and the bright coating of the GO onto the membrane were confirmed by scanning electron microscope. Anti-*E. coli* β-gal Abs were conjugated to the GO surface via 1-ethyl-3,3-dimethylaminopropyl carbodiimide hydrochloride–*N*-hydroxysuccinimide chemistry; antibody coating was confirmed by the presence of a characteristic IR peak near 1600 cm^{-1}. A non-corresponding Ab (anti-*Pseudomonas*) was used as a negative control under identical conditions. When *E. coli* interacted anti-*E.coli* β-gal with Ab-coated GO-nano-biosensor units, we observed a clear shift in the IR peak from 3373.14 to 3315 cm^{-1}; in contrast, we did not observe any shift in IR peaks when the GO unit was coated with the non-corresponding Ab (anti-*Pseudomonas)*. Therefore, the detection of *E. coli* using the described GO-nano-sensor unit is highly specific, is highly selective and can be applied for real-time monitoring of *E. coli* with a detection limit between 100 μg/mL and 10 μg/mL, similar to existing detection systems.

1. Introduction

Infections caused by pathogenic bacteria pose serious health problems worldwide and are often borne by water, soil, and food [1–5]. Conventional bacterial detection methods [6,7] are mostly based on (i) culture and colony counting methods (which involves counting of bacteria) [8], (ii) immunology-based methods (which involve antigen–antibody (Ag-Ab) interactions) [9], and (iii) the polymerase chain reaction (PCR) method, which involves DNA analysis and requires highly skilled personnel or a long span of time, up to 7 or 8 days, to yield an answer [10]; however, currently available methods are limited by the time required to perform them and assay sensitivity, which led scientists to invent detection devices for the rapid screening of pathogens, including biosensors. Biosensors (also called immunosensors) are based on the principle of highly specific Ag-Ab recognition, and have been widely used for the sensitive and quantitative detection of disease-related proteins, which is critical for biomedical research and diagnostics [11–16].

In recent years, highly-sensitive biosensing materials have been explored to increase biosensor detection limits for pathogens in samples [17]. Among these, graphene and graphene-based nanomaterials (such as GO) are attractive candidates for biosensing [18]. GO is a chemically-modified form of graphene that contains oxygen functional groups, such as epoxides, alcohols, and carboxylic acids; these functional groups make GO more reactive than inert graphite and easier to intercalate with other partner groups [17]. The availability of several types of oxygen-containing functional groups on the basal plane and sheet edge allows GO to interact with a wide range of organic and inorganic materials in non-covalent, covalent, and/or ionic manners so that functional hybrids and composites with unusual properties can be readily synthesized [15,16,19–21], making graphene-based materials ideal for biosensing [18]. By exploiting abundant carboxylic groups (—COOH) on GO, covalent conjugation of amines on the protein molecules can be achieved by using well-known EDC–NHS (1-ethyl-3,3-dimethylaminopropyl carbodiimide hydrochloride–*N*-hydroxysuccinimide) chemistry.

Real-time detection of microorganisms is possible by monitoring the changes in the IR patterns of samples. Hence, FT-IR spectroscopy can advance our basic knowledge of molecular interactions by characterizing the bonding pattern of the target sample structures at high spatial

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resolution [22,23]. In this report, we coupled the unique characteristics of GO with the FT-IR spectroscopy to test *Escherichia coli* binding and aimed to generate a highly sensitive method of *E.coli* detection. We have demonstrated that GO surfaces are suitable platforms for the conjugation of ligands, such as the *E. coli*-specific antibody (anti-*E. coli* β-galactosidase (β-gal) Ab) used in this study. Hence, GO can be considered the ideal material and component of the nano-biosensor system for pathogen detection. The interaction of *E. coli* with a surface-conjugated anti-*E.coli* β-gal Ab resulted in a clear shift in the FT-IR spectra, demonstrating the specific reaction of *E. coli* with the GO nano-sensor. The GO-coated nano-sensor system described here offers several novel features: (a) PCTE membranes contain well-defined cylindrical nanopores with a narrow pore size distribution, (b) deposition of GO (bearing carboxylic group) on the porous membrane offers the potential to further modify membrane characteristics, (c) ligand attachment can be directly achieved via the functionally active GO surface, and (d) the IR band analysis of the GO-coated PCTE membrane provides a direct and simple method to monitor the specific antibody-antigen interaction of the analyte being investigated. To our knowledge, we have described the first application of a GO-based nano-porous membrane surface for pathogen detection, presenting a novel platform for *E. coli* detection.

2. Experimental details

2.1. Chemicals, materials, and reagents

The solvents and chemicals were of laboratory and analytical grade, and procured from SRL, Merck, and Molychem (India). Sodium chloride (NaCl, RANKEM), potassium chloride (KCl, ANALAR), potassium dihydrogen phosphate (KH_2PO4, SRL), and sodium hydrogen phosphate ($NaHPO_4$, ACROS) were prepare phosphate-buffered saline (PBS). PBS (pH 7.2) was prepared by mixing NaCl (0.8 g), KCl (0.02 g), KH_2PO_4 (0.024 g), and $NaHPO_4$ (0.144 g) in 100 ml of double distilled water. GO was kindly provided by Dr. Rakesh K. Joshi, Marie Curie Fellow, University of Manchester, UK and 1.6 mg/ml GO was prepared by dilution in distilled water. The anti-*E.coli* β-gal antibody and the anti-*Pseudomonas* antibody were purchased from Bangalore Ge Nie. The gold-coated PCTE membrane was purchased from Whatman (USA).

2.2. Instruments

The GO coating on the PCTE membrane was initially confirmed by acquiring images using an inverted microscope (MAGNUS, Olympus, Chennai, India); for further detailed and in-depth analysis, the GO-coated membranes were examined by the scanning electron microscopy (SEM; LEO 438VP SEM equipped with an Energy Dispersive Spectroscopy (EDS/EDX) System, BRUKER, Germany). EDS/EDX which detects the X-rays emitted from a sample during electron imaging was used for the elemental analysis. Thermo Gravimetric Analysis (TGA) of the GO-coated PCTE membrane was carried out under N_2 flow using a Thermo Gravimetric Analyser Q50 (USA). The FT-IR study was done using a Nicolet 6700 spectrometer (Thermo Scientific, USA). The membrane holder was purchased from Thermo Scientific.

2.3. Modification and characterization of the PCTE membrane

A nano-porous polycarbonate track-etched (PCTE) membrane (100 nm) was used for surface modification. The drop coating method was used to add GO to the PCTE membrane surface. The round membrane was arranged on the circular planar base of a Petri dish and the surface of the membrane was coated by the pipetting method. The membrane to be coated was arranged with the help of forceps and GO was uniformly dropped onto the surface of the membrane by a micropipette; the coated membrane was kept overnight at room temperature. In this time period, the surface of membrane was covered by a thin layer of GO. The GO-functionalized membrane was characterized by inverted microscopy, SEM, EDC/EDX, and TGA (see Section 2.2) and used for the FT-IR, Ag-Ab interaction studies (described below, Section 2.4).

2.4. Antibody immobilization

An antibody solution was prepared from a 1.7 mg/ml stock by dissolving 100 μl of anti-*E. coli* (β-gal) in 900 μl of PBS to achieve a final concentration of 170 μg/ml, after which the GO-coated membrane was covered with this solution and incubated for 15 h at 4 °C.

3. Results and discussion

3.1. Characterization of GO-coated membranes

The normal and GO-coated PCTE membranes were placed on a Petri dish (Borosil) one-by-one and the micrograph was recorded using inverted microscopy (40×), SEM, and TGA (see Section 2.2, Methods section). The results are presented in Figs. 1–5. Fig. 1 is a pictorial depiction of micrographical representation of GO-PCTE membrane fitted in membrane holder cell with different micrographs wherein Fig. 1a shows an image of the liquid cell used to hold the PCTE membrane. The micrograph in Fig. 1b depicts the grey surface structure of the normal membrane; whereas the micrograph of GO-coated membrane surface shows the golden brown colour of GO on the surface of the polycarbonate membrane as shown in Fig. 1c. Therefore, bright-field image analysis clearly demonstrates that the GO is uniformly coated over the surface of the membrane. The GO coating onto the PCTE membrane was further confirmed by SEM showed Fig. 1d. The SEM micrograph of GO-coated PCTE membrane is represented at 15.00 kX magnifications with a SE1 detector and EHT at 20.00 kV. The SEM micrograph of the normal PCTE membrane represented in Fig. 1e, at 1.00 kX magnification with a SE1 detector and EHT at 20.00 kV, shows a black uniform layer of GO over the surface of the nano-porous membrane and the absence of nanopores due to the healing of the surface by the

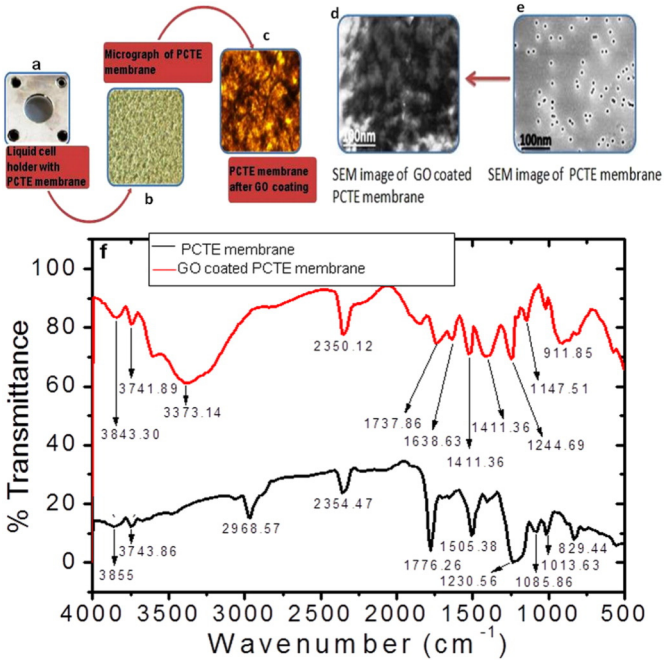

Fig. 1. (a) Liquid cell holder with mounted PCTE membrane. (b) Inverted microscopic micrograph (40×) of PCTE membrane. (c) Inverted microscopic micrograph (40×) of PCTE membrane after GO coating. (d) SEM micrograph of GO coated membrane (Mag = 1 kX). (e) SEM micrograph of normal PCTE membrane (Mag = 15 kX). (f) FT-IR spectra of GO coated PCTE membrane. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

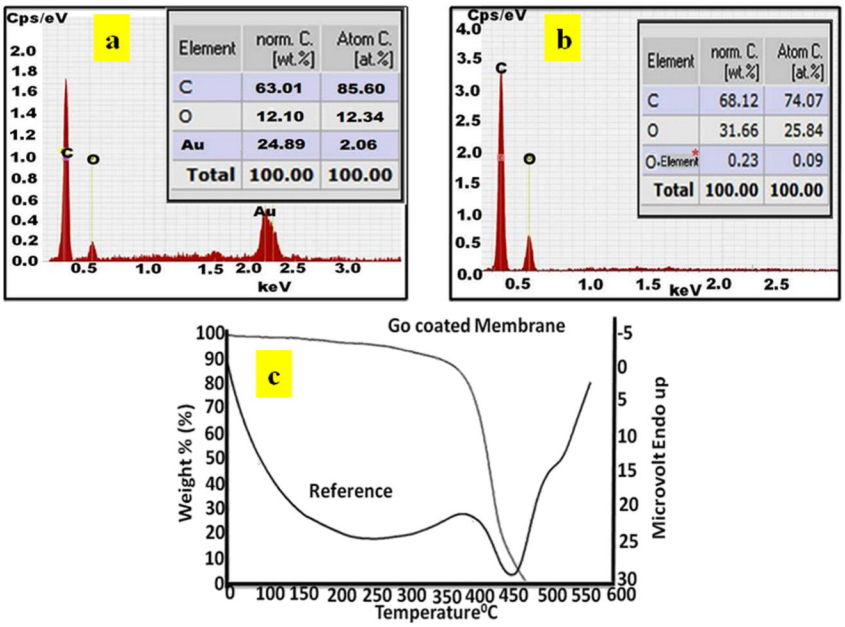

Fig. 2. (a) EDS/EDX spectra of Gold coated PCTE membrane. (b) EDS/EDX spectra of GO-coated membrane (c) TG/DTA of GO coated membrane.

GO coating. This SEM micrograph of GO-coated PCTE membrane confirmed the functionalization of the membrane for further study.

The FT-IR spectrum clearly shows the difference between the PCTE membrane and the GO-coated membrane. A change near 3000 to 3500 cm^{-1} (3373.14 cm^{-1}) was observed in the GO-coated membrane as shown in Fig. 1f. The modification of the PCTE membrane upon coating with GO was further examined by EDS/EDX to confirm the elemental composition of the modified surface of the membrane. The EDC/EDX spectra of the PCTE membrane with GO coating showed a higher amount of carbon with oxygen (Fig. 2); the presence of a higher amount of oxygen in the coated membrane than in the uncoated PCTE membrane confirmed that the membrane contained GO with oxygen functional groups on its surface. EDS/EDX of the gold-coated PCTE membrane showed that the normal carbon wt% was 63.01 and atomic carbon wt% was 85.60 as shown in Fig. 2a, whereas the GO-coated membrane had a normal carbon wt% of 68.12 and an atomic carbon wt% of 74.07 as shown in Fig. 2b. The normal oxygen wt% value of the gold-coated PCTE membrane was 12.10, but this was higher in the GO-coated membrane 31.66 as shown in Fig. 2a and b respectively. The atomic oxygen was also higher in the GO-coated membrane (25.84) than in the gold-coated (Au-o) PCTE membrane (12.34). This study provides valuable information about the coating of GO onto the PCTE membrane. In our next experiments, the TGA of the GO-coated membrane was done to measure the weight loss as a function of time and a pyrolysis study of the total oxygen present on GO was performed. The abscissa (X-axis) can be displayed as time or temperature and the ordinate (Y-axis) can be displayed as weight (mg) or weight percent (%). The samples were heated from room temperature to 600 °C at 5 °C/min. GO showed a slight mass decrease from room temperature to 150 °C and a significant decrease from 150 °C to 200 °C. The descending TGA thermal curve of the GO-coated membrane indicates that a strong weight loss occurred near 475 °C. The mass of GO slowly further decreased up to 600 °C (Fig. 2c). The major mass reduction at ~450 °C was caused by pyrolysis of the oxygen-containing functional groups, which generates CO and CO_2. The graph shows that when the temperature reached above 475 °C the membrane will no longer be stable because of decarboxylation, pyrolysis oxidation, decomposition, and weight % filler, among other variables.

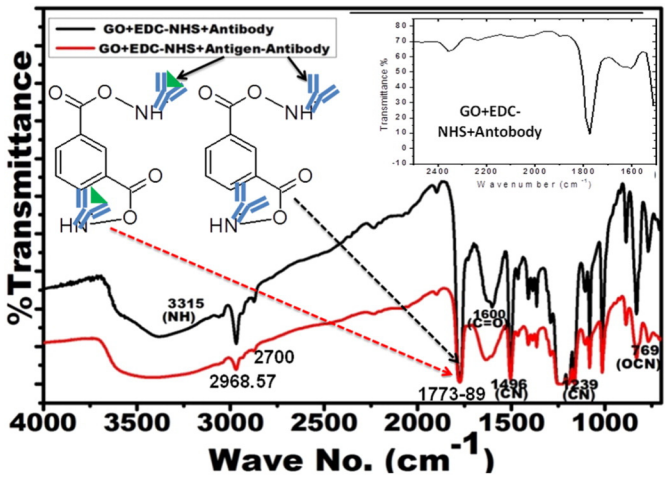

Fig. 3. FT-IR spectrum of GO-PCTE membrane after immobilization of antibody and corresponding antigen.

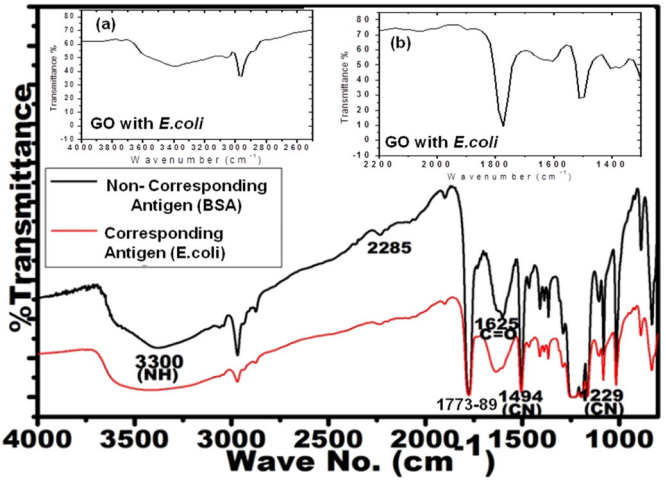

Fig. 4. FT-IR spectrum of GO- PCTE membrane after immobilization of antibody, corresponding and non-corresponding antigen. Inset (a) shows peak around 3300 cm^{-1} for N—H stretching and (b) 1650 cm^{-1} for amide bond between Ab-Ag.

For the IR study, the simple PCTE membrane fit into the liquid cell and sealed the cell before the observation; it was then placed between the light filters of the FT-IR instrument. GO exhibited an intense and broad peak centered at 3373.14 cm^{-1} attributed to the stretching mode of the O—H bond. The O—H groups in GO bonded to the various sites of the carbon skeleton varying from the sheet's center to its border, which may cause shifts in the frequency of O—H vibration and resultant peak broadening. A strong band at 1638.63 cm^{-1} was assigned to the C═O stretching vibrations of the —COOH groups. This spectral analysis clearly shows the presence of oxygen functional groups on the surface of the GO-coated membrane (Fig. 1f).

3.2. Interaction of E. coli with GO-modified PCTE membrane by FTIR analysis

FT-IR spectra of the GO-coated surface of the membrane were recorded before and after antibody immobilization which shows a shift of peak from 1638.63 cm^{-1} (Fig. 1f) to 1600 cm^{-1} (Fig. 3), indicating the binding of amino group of Ab to carbonyl group (C═O) of GO. Moreover, a peak 1737.86 cm^{-1} (Fig. 1f) is shifted to 1789.57 cm^{-1} and a peak 3373 cm^{-1} (Fig. 1f) shifted to 3315 cm^{-1} (Fig. 3). When the corresponding antigen of *E.coli* was exposed to GO-Ab, the intensity of spectral peak decreased to 1789.57 cm^{-1} (Fig. 3) due to specific AB-Ag interaction; and peak at 3315 cm^{-1} (Fig. 3) exhibited N—H stretching due to non-covalent Ag-Ab interaction. Infrared spectra had shown in Fig. 4 at the corresponding wave number evident the higher intensity of peak due to non-specific Ag-Ab interaction (Fig. 4). The inset image of Fig. 3 shows a clear peak at 1773–89 cm^{-1} and 1600 cm^{-1} of antibody attachment with GO-coated PCTE membrane. Furthermore, the inset image of a and b of Fig. 4 show the 3315 cm^{-1} and 1773–89 cm^{-1} at lower intensity of *E.coli* corresponding to the antibody, and non-corresponding antigen.

This confirms of antibody attachment on the GO-modified nanoporous membrane and detection of the Ab-Ag interaction on the surface through FT-IR analysis. The study justifies the use of the novel material GO as a new nano-material for development of IR-based nano-biosensors. The results are encouraging and indicate that FTIR spectroscopy could be developed into a routine analysis tool for pathogen identification. To determine specificity of the ligand-pathogen interactions in our system, we used a non-corresponding antigen (*Pseudomonas*). The FT-IR analysis of immobilized non-corresponding antigen (*Pseudomonas*) on the *E. coli* antibody attached surface of GO-membrane shows that there is absence of the peak from 3200 to 3400 cm^{-1}, this is due to the absence of binding between Ag-Ab. Peak at 1773.31 cm^{-1} arises from the carbonyl stretching and N—H bending and stretching vibrations of the peptide groups.

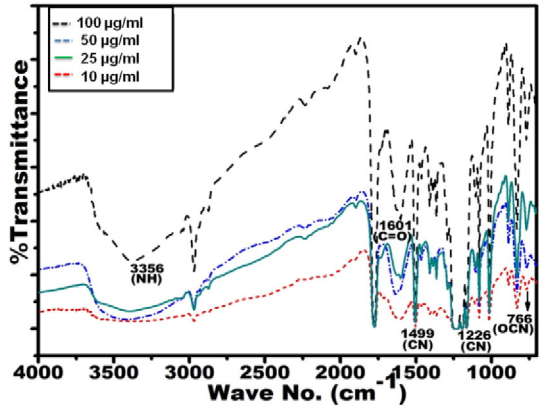

Fig. 5. FT-IR spectrum of graphene coated polycarbonate membrane after different concentration of antigen immobilization.

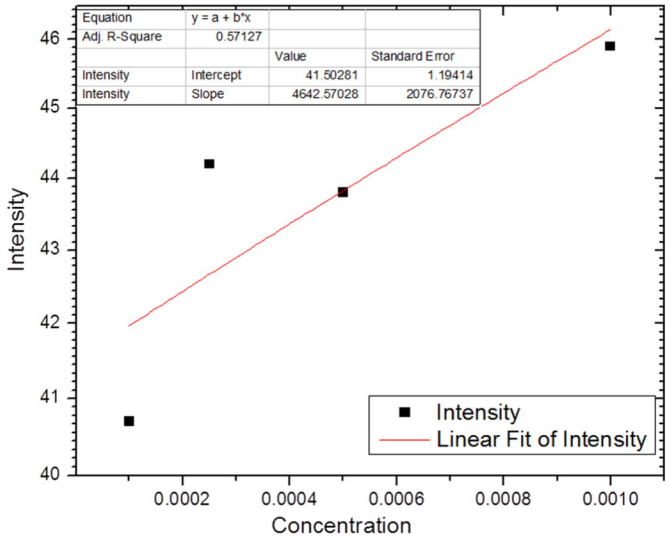

Fig. 6. Shows the linear relationship between the intensity vs concentration (g/ml).

3.3. Limit of detection

To determine the limit of detection of the FTIR based nanobiosensor for *E.coli* described here, we immobilized the different concentration of antigen as 100 µg/mL, 50 µg/mL, 25 µg/mL and 10 µg/mL on to the graphene membrane as shown in Fig. 5. It is clearly stated from Fig. 5. The film-based biosensors developed by Stefania Mura at el.showed a detection limit for *E. coli* of 1×10^2 CFU/mL [23]. Furthermore, a technique has been demonstrated to precisely detect *E-coli* by using Anti-*E. coli* β-gal antibody within a detection limit of 10 µg/ml. The calculated sensitivity is shown in Fig. 6 using linear relationship of intensity vs concentration. This linear curve is fitted by $y = 4642.5\times + 41.5$, where y is the intensity and x is *E.coli* concentration. The sensitivity of nanobiosensor platform was calculated 0.035 cd-gml^{-1} cm^{-1} at biosensing surface area of 1.32 cm^2.

4. Conclusion

The study concludes that the GO coated nano-film on the nanoporous PCTE membrane provides a novel platform for the detection of *E. coli,* probably used for the first time by a FTIR based nanobiosensor. The beauty of this approach is the rapid, label free and robust detection of *E. coli* by recording the specific PCTE-GO-Ab-Ag interaction, as evident from specific peaks on 3315 cm^{-1}, 1773–89 cm^{-1}, and 1600 cm^{-1} and the characteristic fingerprint of the target pathogens. Therefore, our IR based biosensing system provides a relatively quick and specific detection through a FTIR measurement. The developed nano-immunosensors device is selective, does not allow the linking of other subspecies of *E. coli,* specific due to IR absorption, have a limit of detection between 100 µg/mL to 10 µg/mL, and sensitivity up to 0.035 cd-gml^{-1} cm^{-1}. The system has potential to detect other analyte and ease in fabrication.

Conflict of interest

The authors declare that they have no conflict of interest.

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