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Supporting Information

for

Triple signalling mode carbon dots-based biodegradable molecularly imprinted polymer as multi-tasking visual sensor for rapid and "on-site" monitoring of silver ion

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S1. Experimental section

S1.1 Reagents and instrumentation

All the chemicals were of analytical grade and were used as received. Silver nitrate, N-isopropyl acrylamide (NIPAm), and methanol was procured from Alfa Aesar (USA) and fluorescein dye, sodium lauryl sulphate (SLS), N,N'-Dicyclohexyl carbodiimide (DCC), ammonium per sulphate (APS), acryloyl chloride and other interferrents were procured from Loba Chemie Pvt. Ltd. (India) and Spectrochem Pvt. Ltd. (India). Standard stock solutions were prepared in milipore water and wastewater samples were collected from the coal mines area (Jharia, Dhanbad, India) and Durgapur industrial area (Durgapur, West Bengal, India). Human blood and urine samples were collected from local pathological laboratory (ISM, Dhanbad). Silver sulfadiazine 1% topical cream was purchased from local medical shop.

Fourier transform infrared spectroscopy (FT-IR) analysis and UV-Visible spectroscopic analysis was carried out on Varian FT-IR (USA) and Shimadzu UV-Vis spectrophotometer, UV-1800 spectrophotometers, respectively. X-ray diffraction (XRD) and transmission electron microscopic (HR-TEM) analyses were performed on Bruker D8 Focus X-ray diffractometer, and Tecnai T-30 (300 kV FEG TEM), respectively. The fluorescence spectra (FL) were recorded using a Perkin Elmer LS55 fluorescence spectrometer. The camera pictures were taken by Nikon Coolpix camera (16 mega pixel). All the experiments were performed at room temperature (25±2 °C).

S1.2 Preparation of CDs from fluorescein dye

The combination of microwave and hydrothermal synthesis was employed for the preparation of CDs. For this 0.5 g of fluorescein dye was dissolved in methanol and irradiated for 30 min in domestic microwave oven (power intensity = 600 W). After that, the solution was kept in stainless steel autoclave and the solution was heated to 150 ºC for 2 h. After cooling to room temperature, the solution was dialyzed against pure water through a dialysis membrane (1000 Da MWCO) to get the pure CDs.

S1.3 In vitro cytotoxicity and cell imaging

For the in vitro cytocompatibility study a standard methyl thiazoltetrazolium bromide (MTT) assay on MCF-7 (breast cancer cell line) was employed. MCF-7 cells were cultured in the Dulbecco's Modified Eagle's medium (DMEM) having 10% (v/v) bovine serum, 1% (v/v) antibiotics (penicillin and streptomycin) at 37 ºC in 5% CO_2 humidified atmosphere. The cells

were seeded in the tissue culture at an initial cell density of $1x10^5$ cells cm^{-2}. After incubation for 24 h at 37 ºC, the original culture medium was replaced with the fresh one containing various concentrations of CDs@MIP (0-1000.0 mg L^{-1}) and the cells were again cultured for another 24 h and 48h. Each concentration was added to three wells as parallel control and wells without cells as negative control. Wells without cell were treated as blank control. After the 24 h and/or 48 h incubation time, 20.0 µL of MTT solution was added to each well and the optical density was evaluated at 570 nm. Cell viability was calculated using the formula:

Cell viability (%) = $(A_{sample} - A_{blank}) \times 100\% / (A_{control} - A_{blank})$

Here A_{sample} denotes the absorbance of a well with cells, MTT solution, and CDs@MIP; A_{blank} and $A_{control}$ are stand for the absorbance of a well with medium and MTT solution, without cells and absorbance of a well with cells and MTT solution, without CDs@MIP, respectively. The data was expressed as the percentages of viable cells compared to the survival of control (untreated cells as controls having 100% viability). Three parallel wells were run for each concentration and each experiment was repeated three times.

For bio-imaging, cells were seeded in confocal dishes and incubated for 24 h in CO_2 incubator. Further the cells were treated with CDs@MIP and were kept for 24 h in CO_2 incubator. The cells were then washed with phosphate buffer solution (PBS, 1.0 M) thrice and the fluorescence images of MCF-7 cells were taken by using an inverted fluorescence microscope at excitation wavelength of 480 nm.

S1.4 Sample preparation

Human serum and urine samples were collected from local pathological laboratory and store below 4 ^oC before use. Before analysis, the samples were sufficiently diluted. The waste water samples were collected from local industrial and coal mines area. Prior to use, two drops of nitric acid was added and the samples were centrifuged at 16000 rpm to remove any solid particles before analysis. After that, 1.0 mL of the citrate buffer with pH 6.5 was added to 9.0 mL of the samples before PL study. For preparation of polymetallic ore samples, acid digestion method was used [S1]. For this, 0.3 g of the sample was weighed and transferred into a 15 mL polytetrafluoroethylene (PTFE) crucible. To this crucible, 3.0 mL of concentrated HF, 3.0 mL of concentrated HCl, 3.0 mL of concentrated HNO_3 and 0.5 mL of concentrated $HClO_4$ was added. The mixture was evaporated to almost complete dryness under gentle heating on a hot-plate at 120-150 ^oC. Then, it was leave to cool down to room temperature. After which, the residue was

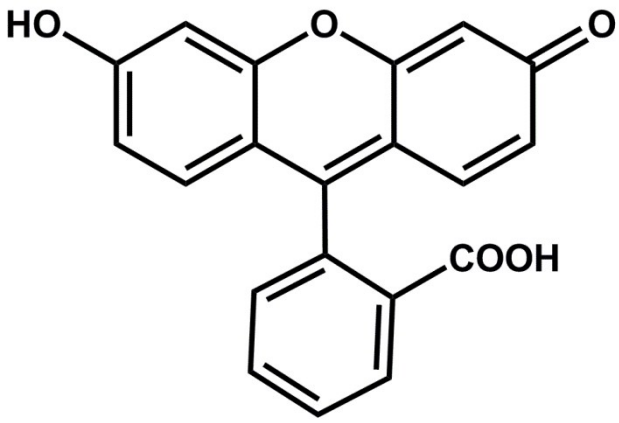
dissolved in water on a hot plate. The final solution was diluted to 100 mL by distilled water. From which, 0.1 mL of the solution was taken out and further diluted to 2.5 L with the citrate buffer (pH 6.5) prior to the measurement. For the determination of Ag (I) content in silver sulfadiazine 1% topical cream (Thermazene), the cream (0.01 g) was burnt to ash keeping the cream in an electric oven and then the ash was dissolved in concentrated HNO_3 (5.0 mL). Next, the solution was centrifuged and diluted by distilled deionized water to prepare a primary solution of 1.0 L. 1.0 mL of the primary solution was further diluted with distilled water (1.0 L) and by citrate buffer (pH 50.0 mL) prior to the measurement.

S2. Patterning and staining using CDs@MIP

In order to understand the property of prepared CDs@MIP to replace the commercially available fluorescent dye and their real time applications, the patterning and staining study was performed. For this, 0.1 mg mL^{-1} of CDs@MIP was filled in the lid of sketch pen used by school kids and they were used to write or drawing some patterns or words on a plane black sheet. Similarly, for staining the same concentrations CDs@MIP solution was used and the 3-D crafted paper arts work soaked in the solution. The corresponding camera pictures of different patterns and 3D craft works have been simply clicked by camera in the both day light as well as inside the UV chamber (365 nm).

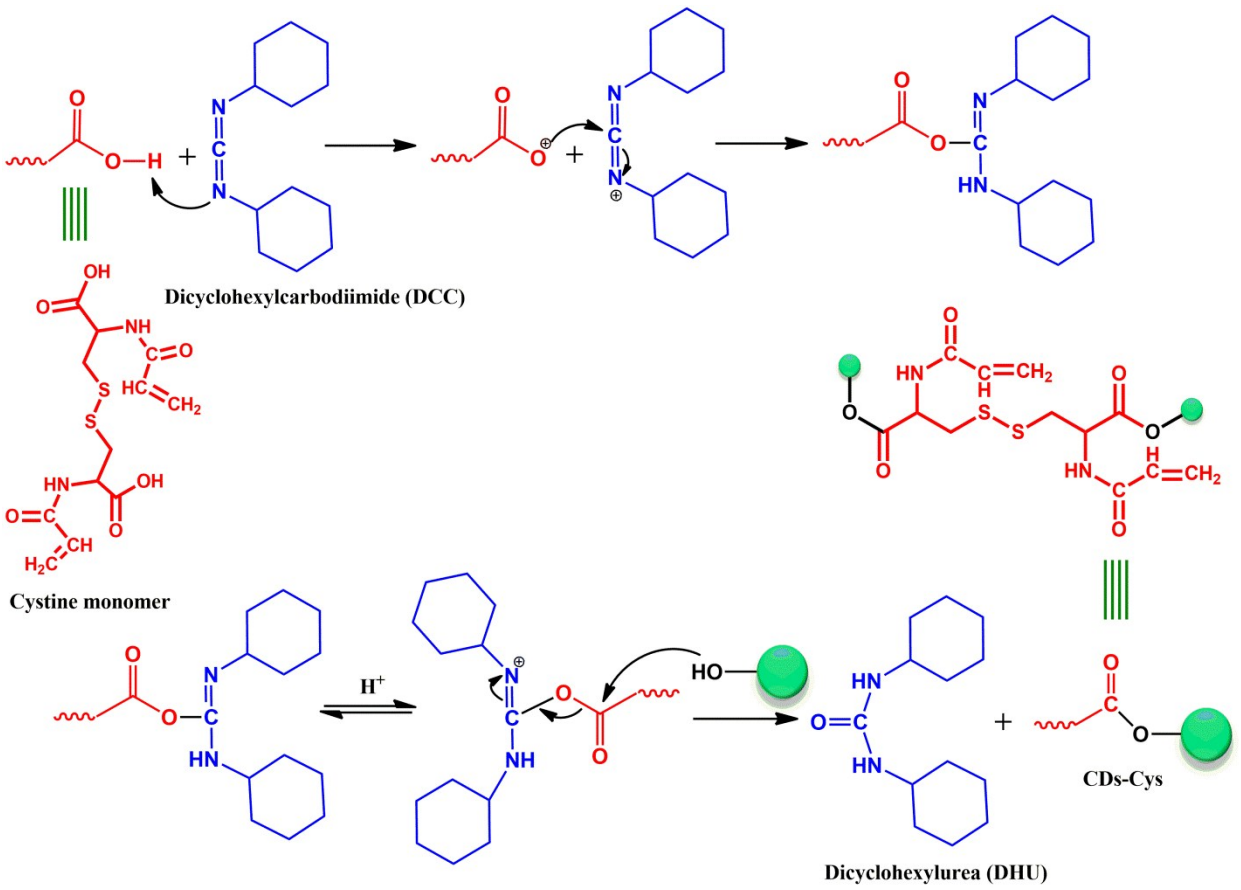
In recent years, fluorescent nanomaterials have gained much interest as potential competitors to traditional fluorescent dye, in which CDs could be placed at the top. Compared with the traditional fluorescent dye, CDs have quantum size effect, which can able to overcome many shortcomings of the dyes, such as the low stability, weak fluorescence intensity, and fast photo-bleaching [S2]. However, their combination with polymer moiety is not well explored yet. Therefore, here we have used the CDs@MIP for calligraphy as well as staining to explore their additional application. For this, CDs@MIP solutions filled sketch pens were used to draw some different patterns on a plane black paper and were placed in the UV light to record their camera pictures. Similarly, some 3D crafted paper works were soaked in CDs@MIP solutions and their camera pictures were clicked in both day and UV-lights. The paper works are invisible/white in daylight (Figure S1-A to D), however, gives intense green colour in the UV light (365 nm) (Figure S1-E to H). Similarly, the calligraphy patterns shown in the Figure S1-I, exhibit very bright green color in UV-light and supports the bright future of CDs@MIP in the field of calligraphy also.

Figure S1: Different images of filter papers stained by CDs@MIP, (A-D) under daylight, (E-H) captured under UV light of 365 nm. (I) Different photographs of fluorescent patterns drawn by CDs@MIP solution filled sketch pens captured under 365 nm UV-light.

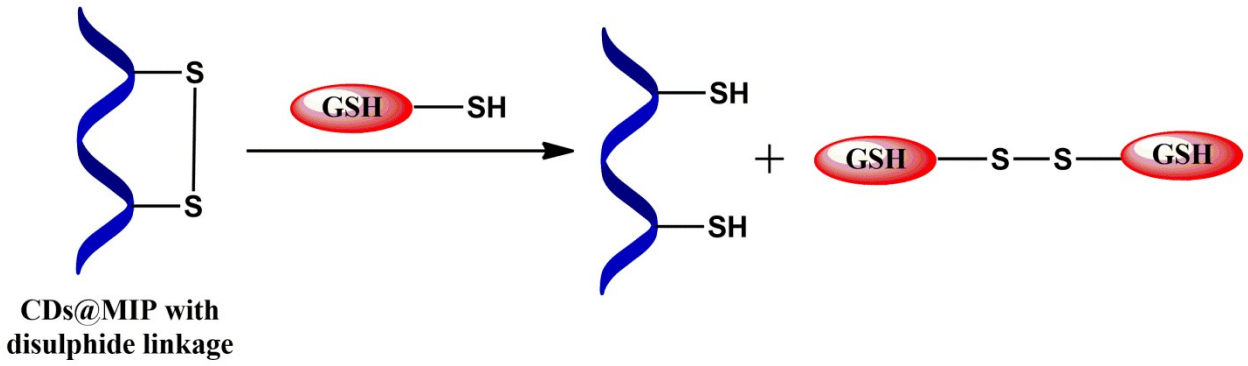


Fluorescein

Figure S2: Chemical structure of fluorescein dye.



Scheme S1: Mechanism involved in the preparation of CDs-Cys [S3].



Scheme S2: Graphical representation showing the breakage of disulphide linkage of polymer in presence of glutathione [S4].

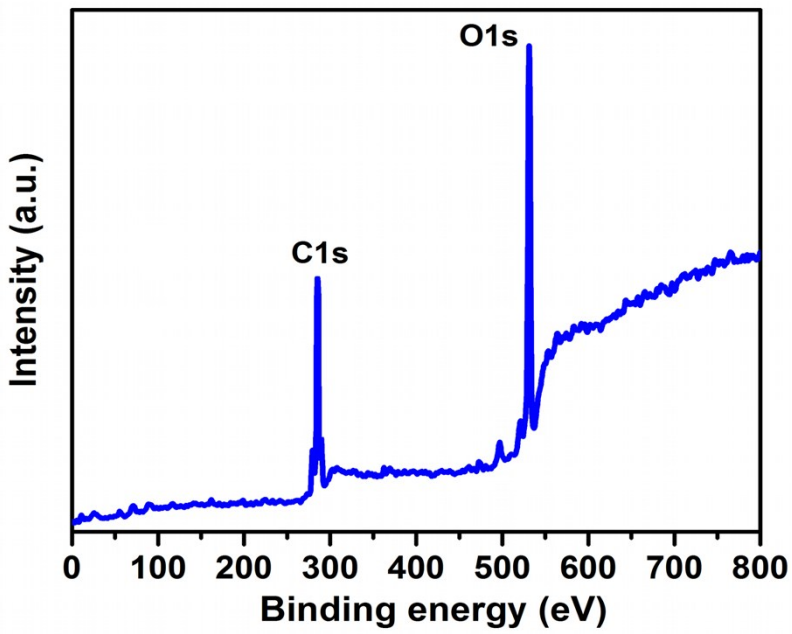


Figure S3: XPS survey spectra of CDs.

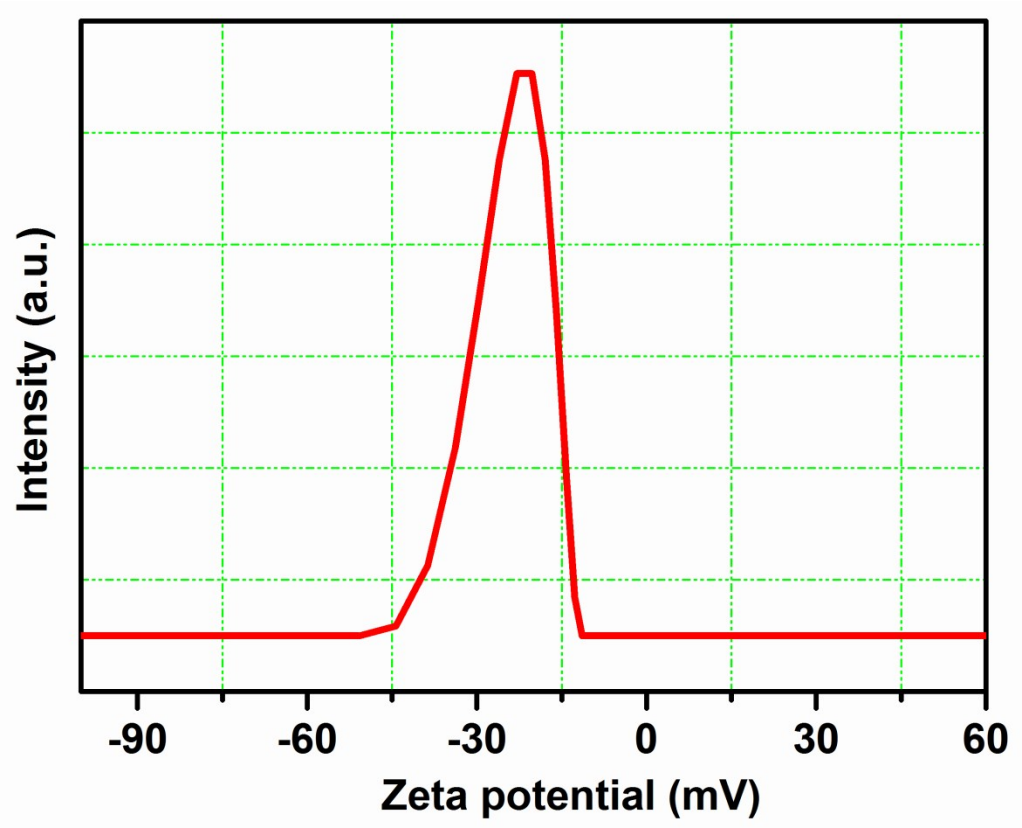


Figure S4: Zeta potential plot of the prepared CDs.

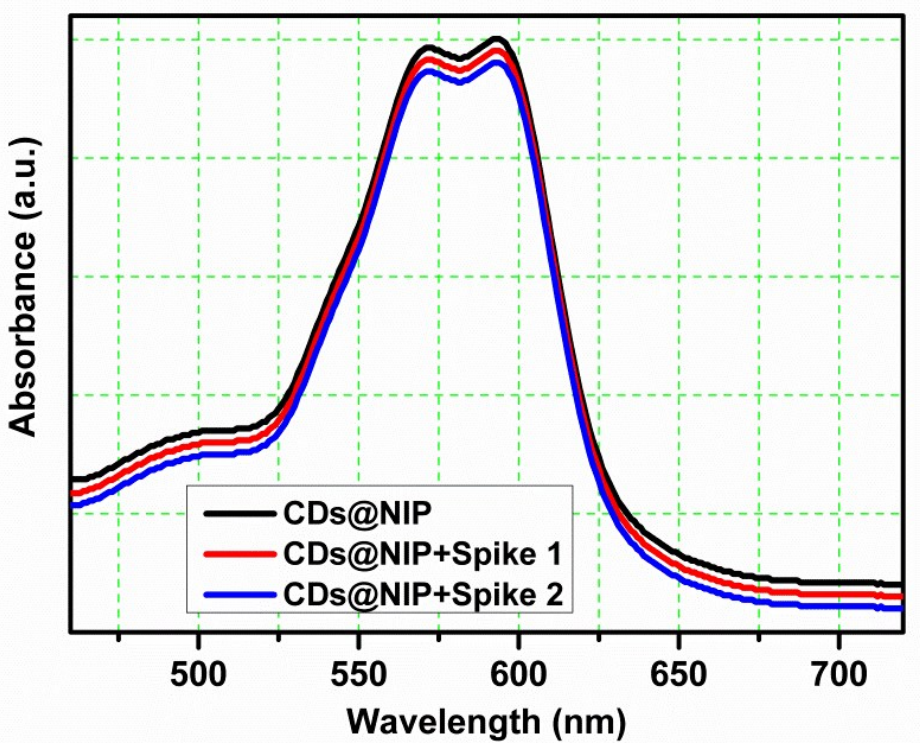


Figure S5: UV-visible spectra of CDs@NIP, in the absence and presence of silver ions.

S3. Optimization of pH

For the detection of Ag^{+}, pH is the most important parameters to be optimized before their detection. The performance of the sensor is dependent on the pH values of the environment around the sensor. This is mainly due to the protonation or deprotonation reaction and the hydrolysis of metal ions under basic condition. The effect of pH values was therefore investigated. Solutions that contain a specific concentration (200.0 µg L^{-1}) of Ag^{+} were adjusted to a pH range from 2.0 to 7.0. It has been observed that the acidic pH values have a minimum effect on the sensory performance and shows a good response at neutral pH. In basic pH value (pH<8), a black precipitate of silver hydroxide have always precipitated out. The results can also be found also in the blank test (in absence of CDs@MIP). So, the visual or quantitative determination can be performed in acidic or neutral pH condition. The camera photo at each pH in both UV-light and in day light can be found in Figure S6.

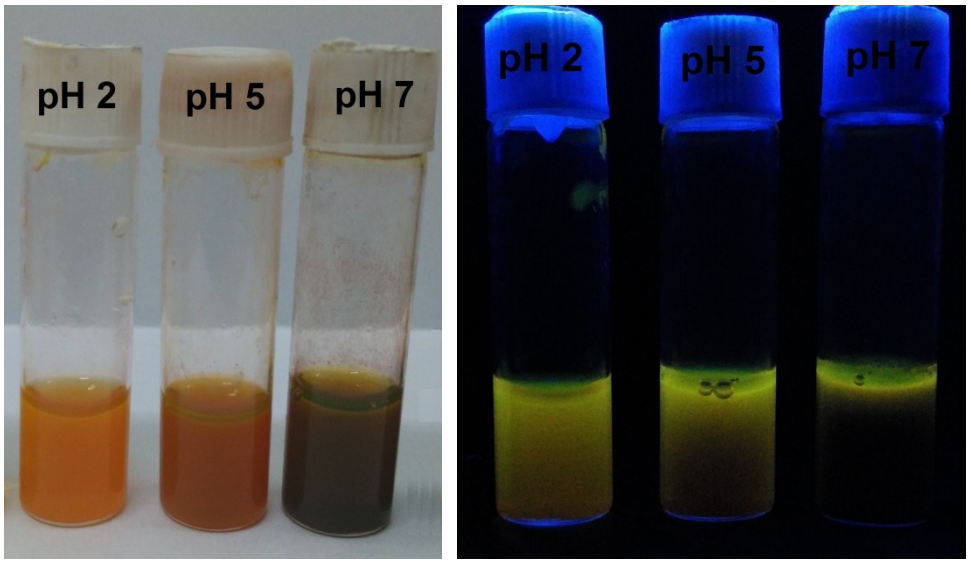


Figure S6: Optimization of pH for the detection of Ag^{+}.

Reference:

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S2. S. Khan, N. C. Verma, A. Gupta and C. K. Nandi, *Sci. Rep.*, 2015, **5**, 11423, DOI: 10.1038/srep11423.

S3. B. Neises and W. Steglich, *Angew. Chem. Int. Ed.*, 1978, **17**, 522-524.

S4. M. V. de Paz, F. Zamora, B. Begines, C. Ferris and J. A. Galbis, *Biomacromolecules*, 2010, **11**, 269-276.