Visible Light Mediated Photoactivated Sulfur Quantum Dots as Heightened Antibacterial Agents

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Synthesis of Sdots

Sdots were synthesized using our previously reported mechanochemical method. Oxalic acid (0.4 millimoles) and sodium thiosulphate (0.4 millimoles) was added to previously grinded 500 mg of NaOH moisture paste, and the whole mixture was grinded for 2-3 minutes. Then 500 μL PEG-400 was added to the reaction mixture and followed by continuously grinded for 10-15 minutes. The reaction mixture was kept for 1 h in water. The formation of Sdots was confirmed by photoluminescence spectroscopy (Figure S1) and transmission electron microscopy (TEM) (Figure S2). Sdots contain ~6% sulfur and 94% PEG (or other oxygenated functionalities), which was previously confirmed by the XPS study.\textsuperscript{1, 2}

![Figure S1: Excitation wavelength-dependent emission spectra of Sdots.](image)

Figure S2: TEM analysis revealed the formation of Sdots.

Antimicrobial activity

For antibacterial activity, ampicillin-resistant GFP-expressing recombinant *E. coli* and *B. subtilis* was cultured seperately in Luria Bertani (LB) broth using the standard method. Two different set of experiments were performed using different bacteria. Three sets of 5 x 6 mL of fresh prepared LB broth were taken in the test tubes. The three sets represent SBGC, Dark, and Sunlight treatment conditions. In each set, one control (without Sdots) and three dose-dependent treatment of Sdots were given. The doses were represented as 0.16 mg/mL (D1), 0.56 mg/mL (D2), and 1.13 mg/mL (D3). Later, each set was kept for incubation under the abovementioned treatment conditions, where SBGC set was kept in an incubator at 37 °C with proper shaking, the other set was kept in the dark condition, and the last one was kept under the sunlight for the duration of 6 hrs. The absorbance of all the samples was taken after
6 hr at 595 nm. Our previous XPS study confirmed that sulfur concentration in Sdots is only \~6\%, while 94\% is PEG (or other oxygenated functionalities).\textsuperscript{1,2} Therefore, effective MIC concentration of Sdots (based on sulfur concentration) is 0.01 mg/mL (D1), 0.034 mg/mL (D2), and 0.068 mg/mL (D3).

\textbf{Figure S3:} Normalized dose-dependent antibacterial activity measurement on \textit{B. subtilis} (Gram-positive bacteria) using Sdots under SBGC, dark, and sunlight. The values are represented as mean ± SD of results from three individual data.

\textbf{Figure S4:} Dose-dependent antibacterial activity measurement using Sdots under (a) SBGC; (b) dark; and (c) sunlight. The values are represented as mean ± SD of results from three individual experiments.
Figure S5: a) Percentage reduction of bacterial growth in a control sample of dark and sunlight conditions when compared with growth of control sample in SBGC; b) Percentage antibacterial activity observed at given MIC doses under dark and sunlight conditions; c) Actual percentage of antibacterial activity obtained in dark and sunlight conditions by removing the percentage deviation of growth in control [given in plot (a)] from percentage antibacterial activity in given MIC doses [given in plot (b)] under dark and sunlight conditions.

Figure S6: SEM image of a) control bacteria showing the healthy cell wall; b) treated bacteria showing the number of bacteria was decreased, and cell wall integrity was compromised. The scale bar is 1 µm.
**Table S1:** Table of comparison for the antibacterial performance in this work with previous literature reports.

| S. No. | Material Used          | Environment          | Bacterial cells | Antibacterial Activity                      | Time  | Ref. |
|--------|------------------------|----------------------|-----------------|----------------------------------------------|-------|------|
| 1      | Fe-ZnO                 | Sunlight             | *E. coli*       | 20 nm                                        | 16 h  | 3    |
| 2.     | TiO$_2$/ZnO/Zn materials | UV light             | *E. coli*       | Almost completely disinfected                | 60 min| 4    |
| 3.     | ZnO/Au Hybrid Nanostructures | Simulated Sunlight | *S. aureus* and *E. coli* | Upto 80 % for *S. aureus* and >90% for *E. coli* | 10 min| 5    |
| 4.     | MnS$_2$/reduced graphene oxide | UV light         | *E. coli*       | Not Available                                | 24-48 h| 6    |
| 5.     | Sdots                  | Natural Sunlight/Visible light | *E. coli* and *B. subtilis* | > 90% for *E. coli* and around 50% for *B. subtilis* | 6 h   | This work |

**ROS Measurement**

Oxidative stress measurement was performed using a standard NBT test. Herein, bacteria were cultured overnight at 37 °C in LB broth using the standard method. Later, in 5 different microcentrifuge tubes, 300 µL of bacterial culture was taken in each of them and was treated with the MIC concentration and individual controls. Then, 150 µL of NBT of strength 10 mg/mL was added to each sample and incubated at 37 °C for 30 minutes. Post-incubation the reaction was stopped using the 1M HCl (100 µL) solution. The samples were centrifuged at 6500 rpm for 10 minutes to collect the bacterial pellets. Then, 600 µL of DMSO was added to the pellet to extract the reduced NBT containing formazan blue, and their absorbance was taken at 575 nm.
Time-dependent fluorescence quenching of Sdots with p-BQ

Time-dependent fluorescence quenching of Sdots with p-BQ experiment was carried out using 2 mL of a solution containing 5 mg/mL Sdots and 0.2 mg/mL p-BQ. The solution was kept under visible light for 30 minutes. The emission spectra were recorded after 5 minutes intervals.

Time-dependent Uv-visible spectra of p-BQ with Sdots

Time-dependent Uv-visible spectra of p-BQ with Sdots were recorded using 3 mL of a solution containing 0.67 mg/mL Sdots and 0.02 mg/mL p-BQ. The solution was kept under visible light for 30 minutes. The Uv-visible spectra were recorded after 10 minutes intervals.

Figure S7: a) Time-dependent Uv-visible spectra of p-BQ after adding Sdots under visible light irradiation. b) Half diluted Uv-visible spectra of p-BQ and Sdots after 30 minutes of visible light irradiation. The inset image represents the Uv-visible spectrum at the 300-700 nm wavelength range. The absorption peaks at 245 nm and 289 nm of p-BQ are diminished, and two new peaks at 210 nm and 261 nm with broad absorption have appeared. The peak at 210 nm is due to the PEG of Sdots, however, the peak at 261 nm has emerged after interacting with Sdots under visible light.
Figure S8: Comparison of absorbance plot of Sdots original sample with sunlight treated Sdots sample post 30 minutes and 1 hour to observe the stability of the Sdots.

Bacterial growth inhibition test on polycotton fabric

For bacterial growth inhibition test on polycotton fabric in presence of the Sdots was conducted using control fabric and Sdots treated fabric on the agar plate. The standard protocol was followed for the agar plates experiment. For the antibacterial fabric experiment, polycotton fabric of average size of 5 mm ± 2 mm was made wet using 20 µL LB broth and placed on two different agar plates. In our case, we took three such polycotton fabrics placed at almost equal distances on each agar plate to observe the accurate growth of bacteria. However, any number of fabrics can be used based on the requirements of the experiment. Next, overnight cultured ampicillin-resistant GFP-expressing recombinant E. coli (20 µL) was evenly spread on both the agar plates. Then, one agar plate was kept as it was for the
control study, and in another one, three different concentrations of Sdots were added to each fabric. The Sdots were evenly distributed over the polycotton fabric based on their optimum retention capacity to obtain the best results. The sizes of the approximately rectangular-shaped polycotton taken were around 5 mm ± 2 mm. Sample A was the control fabric (without Sdots), while sample B represented the Sdots fabricated polycotton fabric. These concentrations were C1(10 µL), C2(20 µL), and C3(30 µL) of the Sdots (20 mg/mL). Later, the agar plates were kept in the incubator at 37 °C under light for 12 hours. Post-incubation agar plates were investigated for the zone of inhibition in the Sdots treated fabrics. Subsequently, we also found that the doses of the Sdots are proportional to the size of the fabric used for the antibacterial activity. Therefore, different sizes of the Sdots fabricated clothes represent different amounts of the Sdots loading. However, it does not affect the antibacterial activity of the Sdots fabricated fabric as the loading at the smallest part of the fabric (dx) and the interaction of the smallest part of the fabric at any given time (dx/dt) will remain the same for all the sizes for same fabric.

**Table S2:** Sdots amount coated on polycotton at different doses applied for antibacterial activity.

| Sdots Doses Applied on Polycotton | 10 µL | 20 µL | 30 µL |
|----------------------------------|-------|-------|-------|
| Sdots Amount Coated on Polycotton | 5.9 mg | 6.25 mg | 9.4 mg |

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