Electronic Supplementary Information

Investigating the Evolution of Drug Mediated Silver Nanoparticles

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Experimental

Materials

Ciprofloxacin hydrochloride (Cp) was purchased from Sigma Aldrich. Silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were purchased from Rankem (India). All the chemicals were of analytical grade and were used without further purification. Mili-Q water was used throughout the experiments. All glass wares were washed with aqua regia and Mili-Q water before further use.

Synthesis of Ciprofloxacin mediated Ag nanoparticles

Synthesis of Cp-Ag nanoparticles were carried out under optimized conditions by adding 10 mL aqueous solution of $AgNO_3$ (1 mM) to a 10 mL aqueous solution of Ciprofloxacin Hydrochloride (5 mM) under vigorous stirring. After 2 minutes, 400 μL of NaOH (1M) was added to the reaction mixture in order to maintain the reaction pH~10 thereby activating the reducing ability of NH group present within the Ciprofloxacin moiety. The mixture was incubated at 40° C for 12 hours under constant vigorous stirring. The Cp-Ag nanoparticles formation was indicated by the change in the color of the solution from colorless to wine red when viewed under visible light.

Drug Release Study

Release of the drug Ciprofloxacin from the surface of Cp-Ag nanoparticles was studied under aqueous medium at pH (~7). 10 mL solution of Cp-Ag nanoparticles was mixed with 10 mL Mili-Q water (pH of the Mili-Q was monitored for over 10 minutes and was found to be constant at 7.1). Then the solution mixture was carefully dialyzed inside a beaker containing 10 mL of Mili-Q water which was kept under constant stirring. After every 30 minutes, 2 mL aliquot of the solution was withdrawn from the beaker and analyzed by UV-visible spectroscopy. After each such measurement, absorbance (monitored at 272 nm) of the aliquot solution was taken to be (A_t). When the release of drug was almost complete, which takes ~28 hours, the absorbance of the solution at that particular time was taken to be the final reading (A_0). From the values of A_t and A_0, the percentage of Cp molecules released was calculated.

Instrumentation

Absorption data was obtained using Perkin-Elmer Lambda 25 spectrometer by scanning in the range from 200 nm to 600 nm in a 1 cm path length quartz cuvette. Steady-state fluorescence measurements were recorded in a Horiba Jobin Yvon Fluorolog 3-111. The fluorescence spectra were measured with a 2 mm path length quartz cuvette. Both the emission and the excitation slits were kept at 1 nm. For fluorescence lifetime measurements, the Cp-Ag nanoparticles were excited at 375 nm using N-375 pico-second diode (IBH-NanoLED). The emission was collected at magic angle polarization using a

Hamamatsu MCP Photomultiplier (Model R-3809U-50). The time-correlated single photon counting (TCSPC) set up consist of an Ortec 9327 pico-timing amplifier. The Full Width at Half Maximum (FWHM) for the 375 nm diode was 145 ps. Transmission Electron Microscope (TEM) images were obtained from Jeol JEM-1400 operating at a voltage of 120 kV. Carbon coated copper grid was used as support material for sample imaging. Dynamic Light Scattering (DLS) experiments were carried out using Beckman Coulter Delsa Nano C instrument. FTIR spectra were obtained from Perkin-Elmer Spectrum BX spectrometer. Atomic Force Microscopy (AFM) images were obtained from Agilent 5500 instrument. Mica sheets were used to prepare thin film of the samples prior to image scanning in non-contact AFM mode. Thermogravimetric Analysis (TGA) was carried out on a Perkin Elmer TGA 4000.

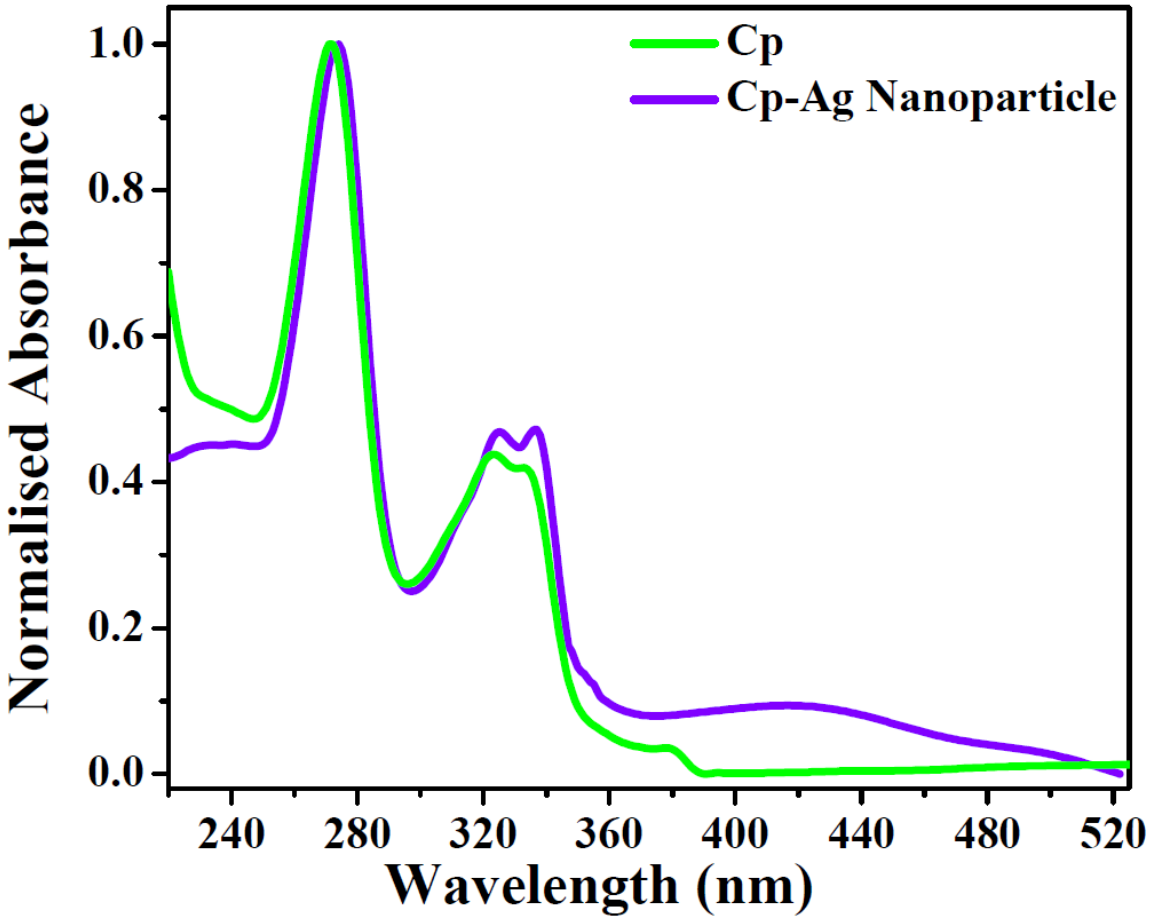

Figure S1: Absorption spectra of Cp alone (green) and Cp-Ag nanoparticles (violet).

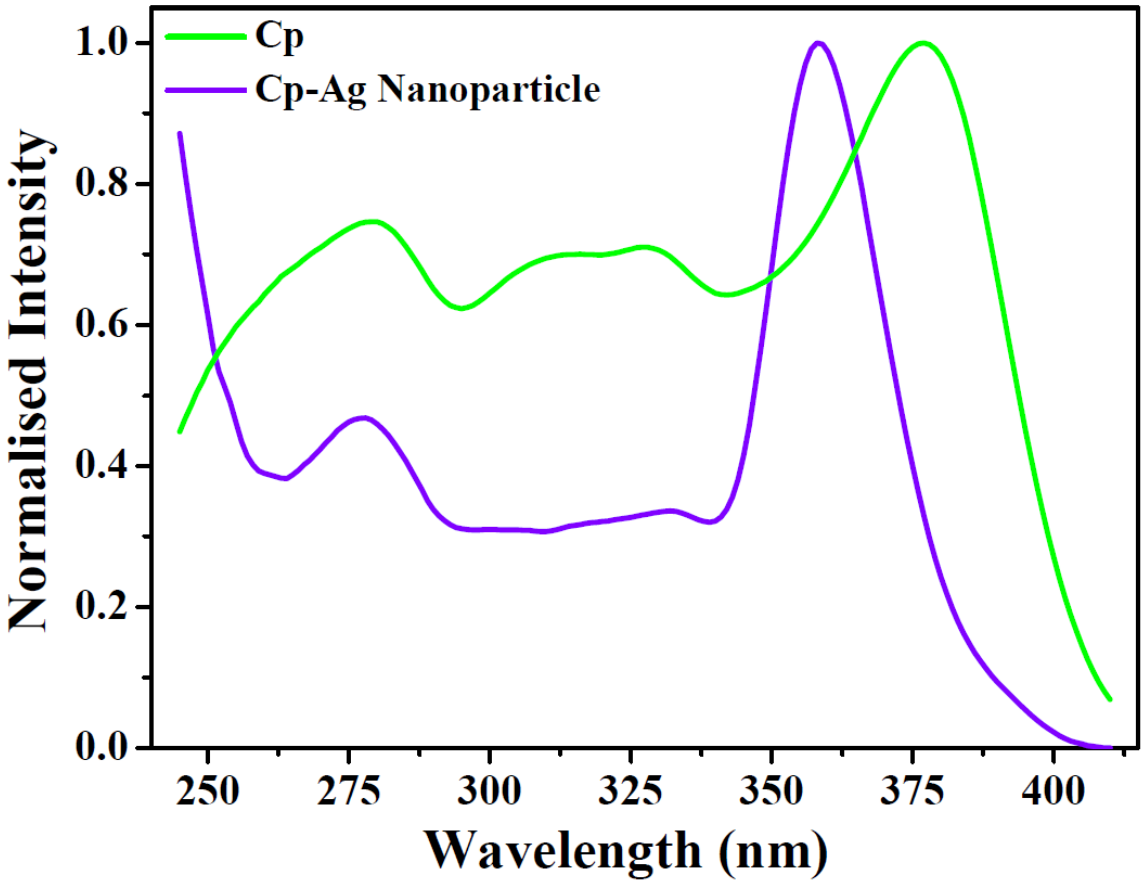

Figure S2: Excitation spectra of Cp alone (green) and Cp-Ag nanoparticles (violet).

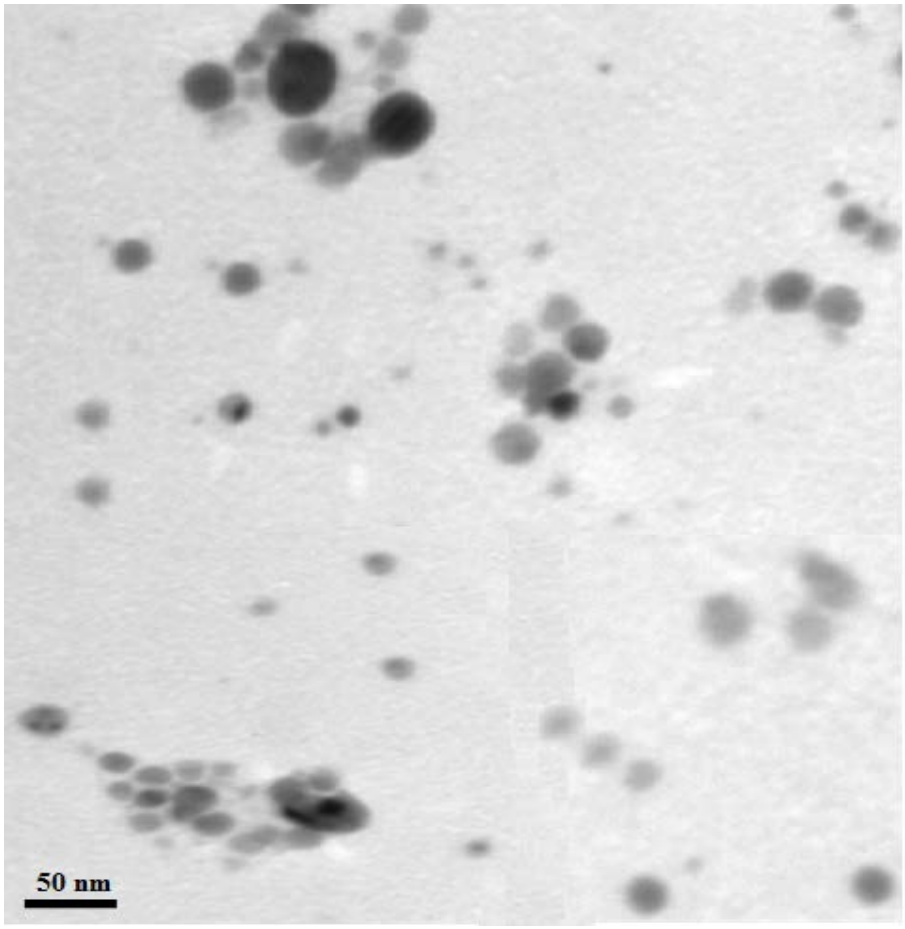

Figure S3: Transmission Electron Microscopy images for Cp-Ag nanoparticles.

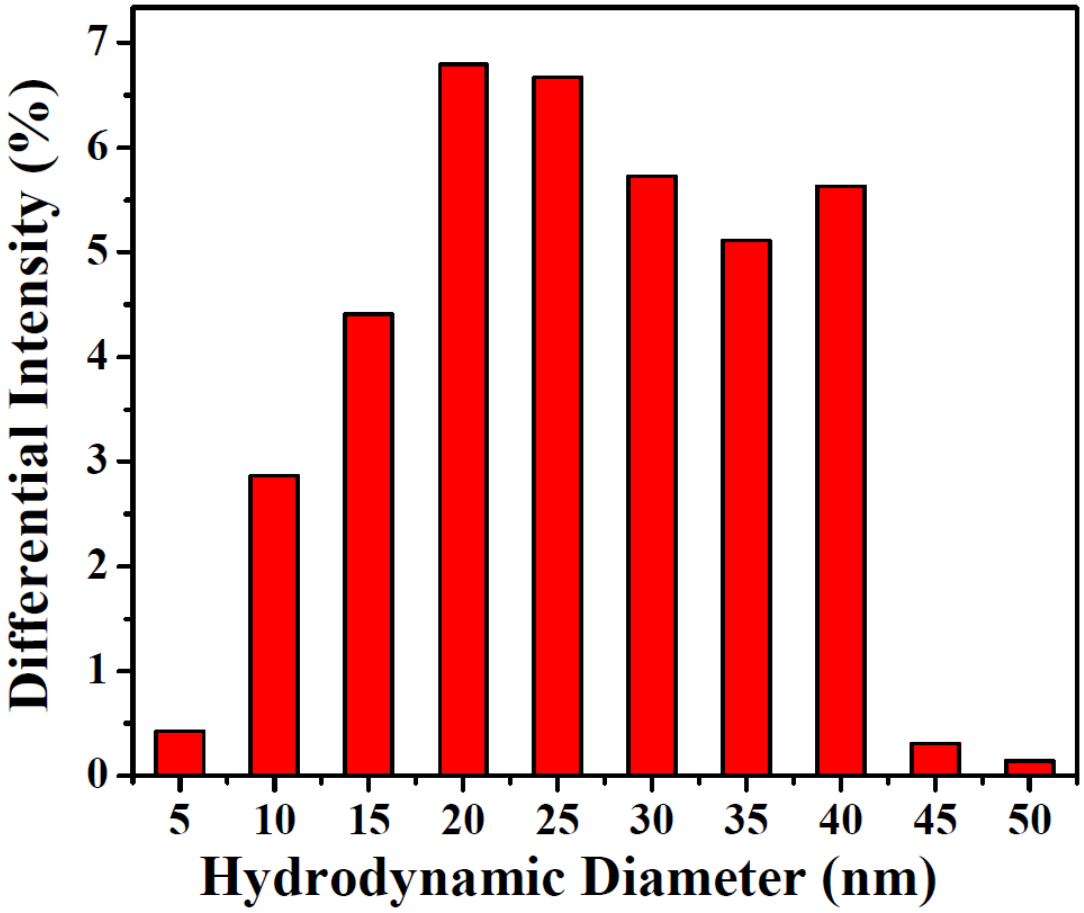

Figure S4: Dynamic Light Scattering plot showing the size distribution of Cp-Ag nanoparticles.

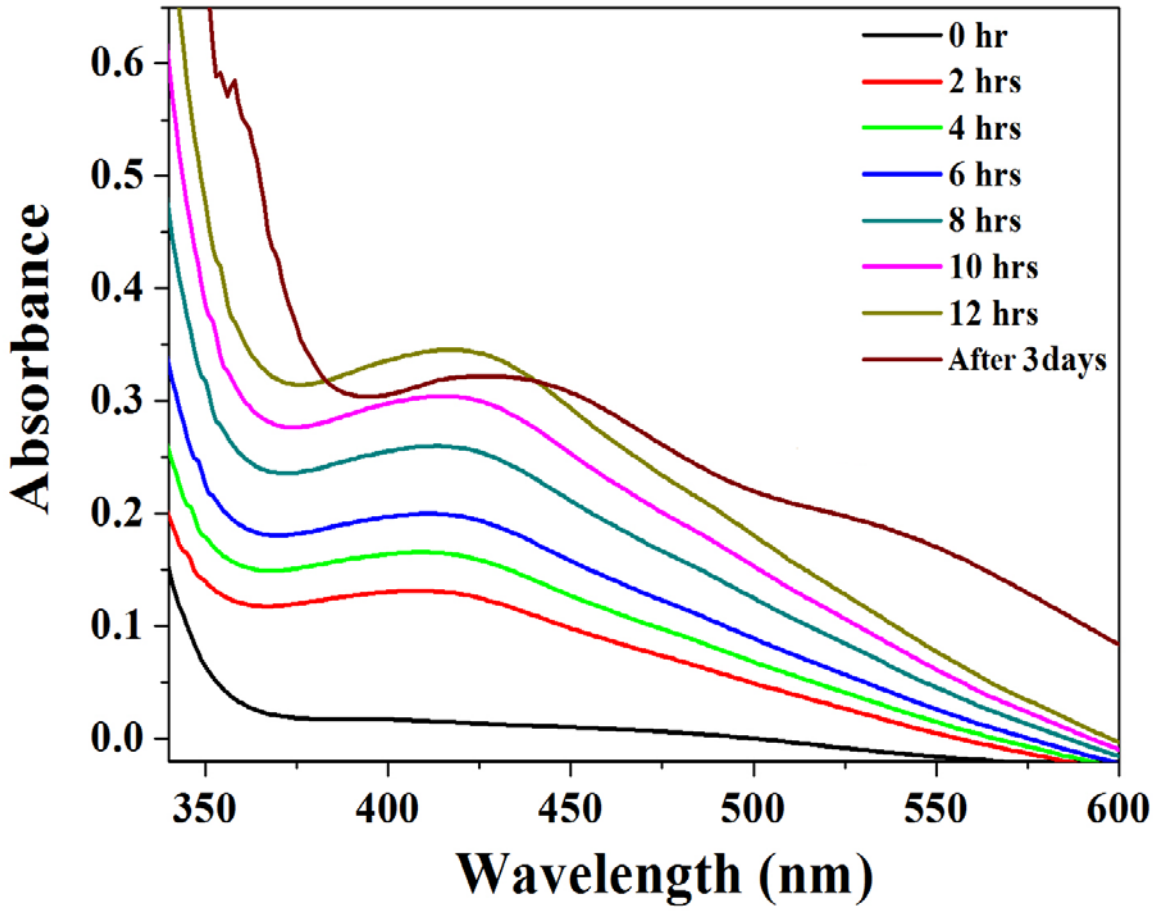

Figure S5: Time-dependent variation of the plasmon band for the Cp-Ag nanoparticles. The different times of measurements are mentioned in the figure. The profile after 3 days is a clear signature of the formation of bigger sized Cp-Ag nanoparticles most probably due to the process of agglomeration.

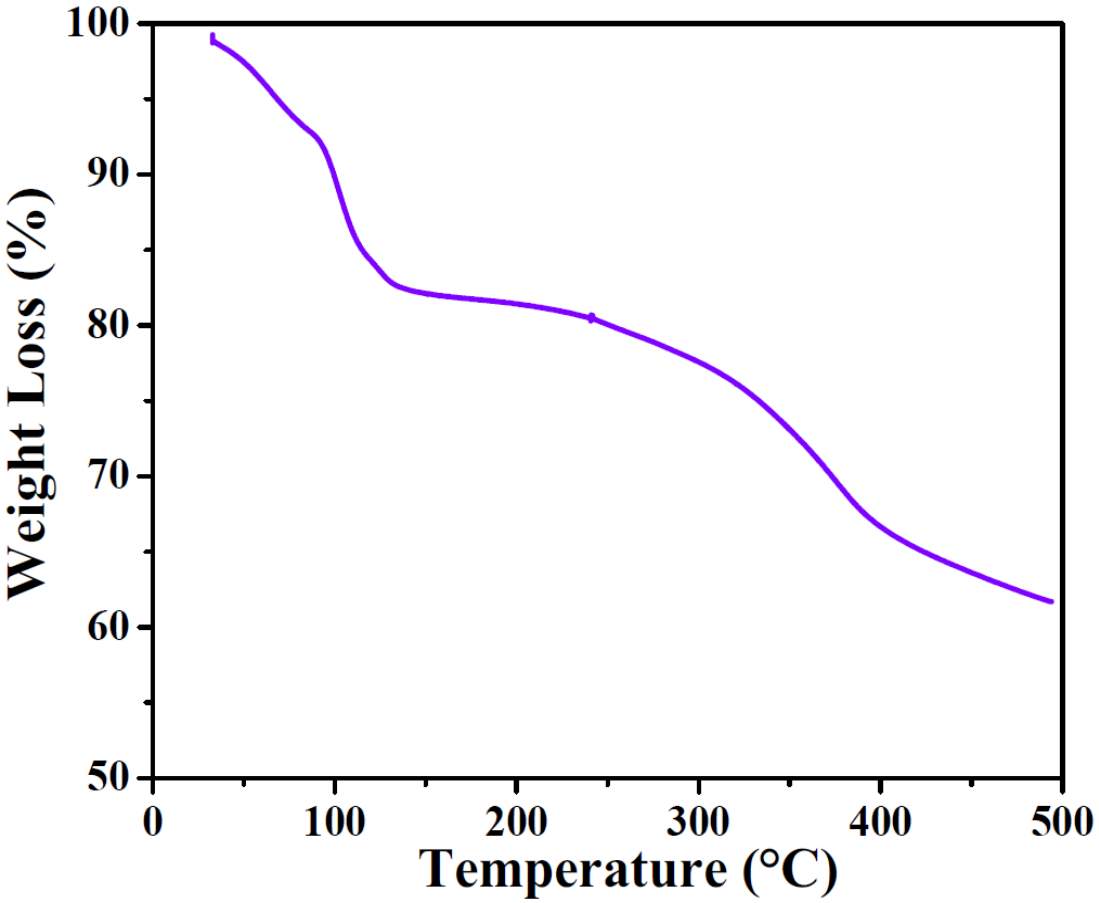

Figure S6: Thermogravimetric Analysis curve of Cp-Ag nanoparticles. The initial sharp fall in the percentage weight loss (in the temperature domain of 30 ºC to 135 ºC in the TGA curve) corresponds to the removal of water molecules from the system consisting of Cp-Ag nanoparticles. This desorption of water molecules from the surface of Cp bound nanoparticles is indicative of the fact that the nature of addition of water molecules may be physisorption. Since Ciprofloxacin hydrochloride exists as a mono-hydrate salt and hence the loss in weight may be due to the removal of this water of hydration as well.

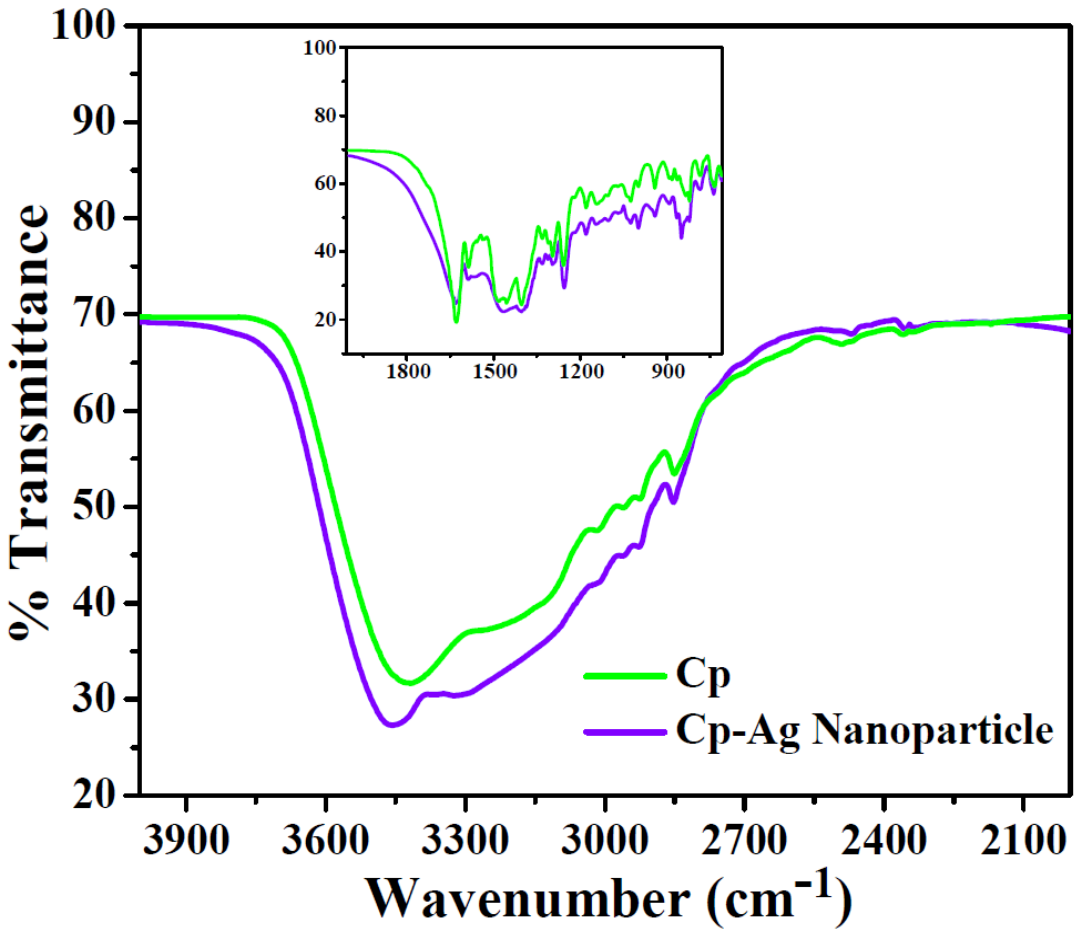

Figure S7: Infrared spectrum of Cp alone (green) and Cp-Ag nanoparticles (violet). The inset shows the region between 2000 cm^{-1} to 700 cm^{-1}.

Figure S8: Photograph showing images of Cp-Ag nanoparticles with time (A) initial Cp-Ag mixture (B) after 6 hours (C) after complete reaction, under (i) Visible and (ii) UV light.

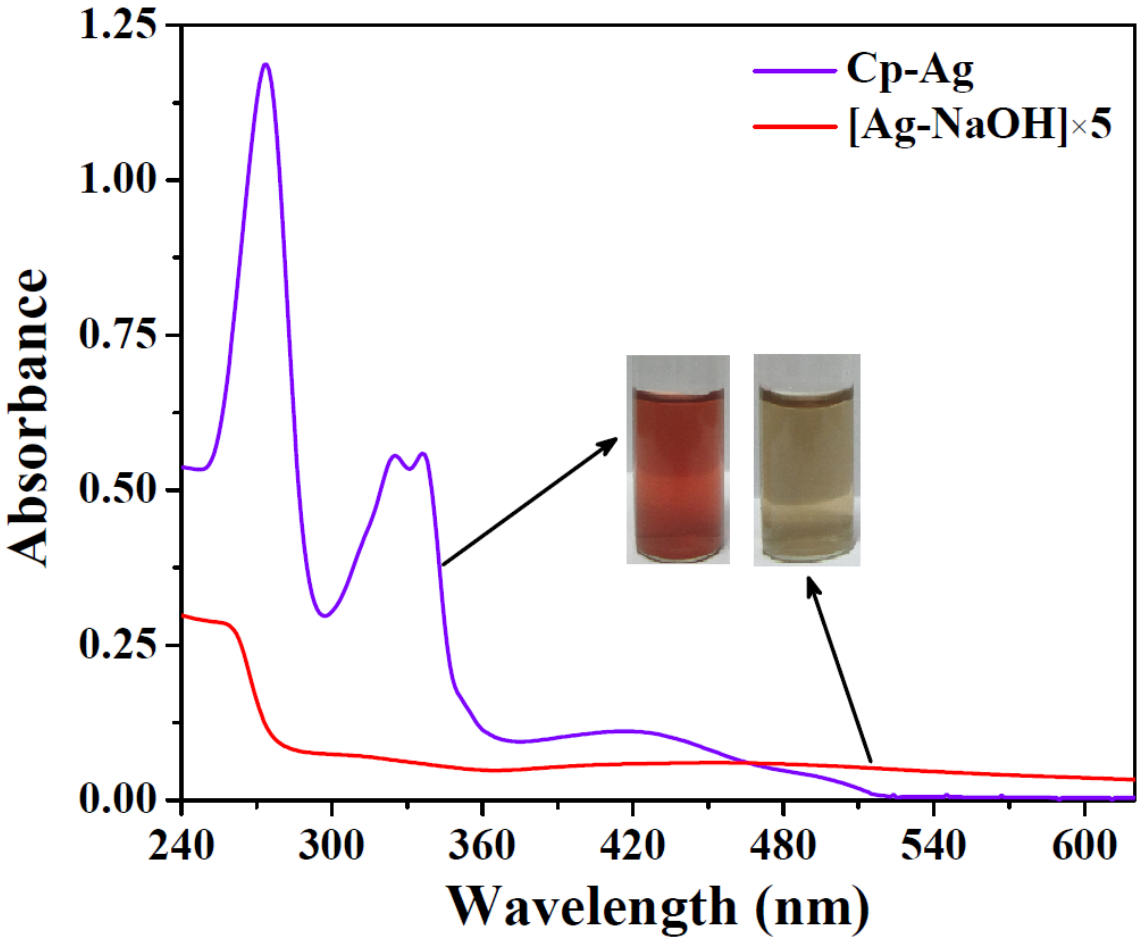

Figure S9: Control experiment showing the formation of nanoparticles in the presence and absence of Cp. The plasmon band in the absence of Cp is of very low intensity, broad and red-shifted exemplifying the fact that in the absence of Cp very few numbers of bigger-sized nanoparticles are produced whose spectral properties are totally different from Cp-Ag nanoparticles.

Estimation of the extent of binding of Ciprofloxacin on the surface of the Ag nanoparticle:

The most prominent absorption band for Ciprofloxacin is peaked at 272 nm. Therefore, we have recorded all the absorbance value for corresponding samples at 272 nm.

A_0, the absorbance of the Cp alone. (1.37)

A_c, the absorbance of Cp-Ag nanoparticles. (1.17)

A_s, the absorbance of free ciprofloxacin in the solution of Cp-Ag nanoparticles. (1.11)

A_b, the absorbance of Cp that is bound on the surface of Ag nanoparticles.

C_0 is the concentration of Cp alone and K is the proportionality constant.

C_b is the concentration of Cp bound to the nanoparticles.

Therefore, $A_b = A_c - A_s = 0.06$

Now, from Lambert-Beer law, the absorbance of a solution is directly proportional to its concentration.

Thus, $A_0 \propto C_0$ or, $A_0 = K \times C_0$

Similarly, $A_b = K \times C_b$

From the respective values from the absorbance as mentioned above and C_0 (which is 0.067 mM in the present case), it was found that $C_b = 0.0029$ mM.

We assume that 169.87 g of $AgNO_3$ on complete reduction gives 108 g of Ag. Therefore, 1.6987×10^{-3} g of $AgNO_3$ will give 1.08×10^{-3} g of Ag (W_{total}).

From TEM image, when size of 1 Ag nanoparticle ≈ 20 nm (smaller particles)

Weight of 1 Ag nanoparticle = $4/3\times\pi\times r^3\times\rho_{Ag} = 4.39\times10^{-17}$ g (W_{np}) [$\rho_{Ag} = 10.49$ g cm^{-3}]

Total number of Ag nanoparticles = $W_{total} / W_{np} = 2.45\times10^{13}$ (N_{np})

Total number of Cp bound on the nanoparticle surface = $C_b \times 6.023\times10^{23}$

$= 1.75\times10^{18}$ per liter of solution

Since we have used 10 mL hence, the total number of Cp bound on the nanoparticle surface = $N_{Cp} = 1.75\times10^{18}\ (10/1000) = 1.75\times10^{16}$

Therefore, the number of bound Cp molecules per Ag nanoparticle = $N_{Cp} / N_{np} \approx$ **720**

Similarly, taking nanoparticle size ≈ 40 nm (for larger particles), the number of bound Cp molecules per Ag nanoparticle = $N_{Cp} / N_{Ag} \approx$ **5760**

Hence the Cp bound to the nanoparticles vary from 720-5760 depending upon the size of the nanoparticles.