Electronic Supporting Information (ESI)

Near-Infrared Light-Responsive Graphene Oxide Composite Multilayer Capsules: A Novel Route for Remote Controlled Drug Release

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Experimental Section

Materials:

High purity graphite powder with average grain size 45 µm, PAH (M_w = 70 kDa), ethylenediaminetetraacetic acid (EDTA), doxorubicin hydrochloride (Dox), dextran sulfate sodium salt (DS) (Mw = 50 kDa), $CaCl_2$, $KMnO_4$ and Na_2CO_3 were purchased from Sigma-Aldrich and used without further purification. H_2SO_4, HCl, $NaNO_2$, NaOH and $NaNO_3$ were purchased from SRL India. The water used in all the experiment was obtained from Milli-Q system with resistivity greater than 18 MΩ cm.

Synthesis of exfoliated graphene oxide (GO)

GO was synthesized by using a modified Hummer's method followed by ultrasonication.[1, 2] Initially, 46 mL of H_2SO_4 (98%) added to 2 g of graphite powder (45 µm, Sigma-Aldrich) in a flask and stirred for 8 h, followed by addition of 6 g $KMnO_4$ slowly to above mixture while keeping the temperature below 20 ^oC. Now, distilled water was added to dilute the mixture and heated at 100 ^oC for 30 min. Then 350 mL of distilled water was gradually added and temperature was kept below 100 ^0C. Finally, the reaction was terminated by addition of 20 mL H_2O_2 (30%) and 300 mL of distilled water. Slowly, the color of the solution turned from dark brown to yellow. Repeated centrifugations (8000 rpm for 25 min) were carried out to wash the mixture using 5% HCl and distilled water. The mixture was then subjected to dialysis to remove residual salts, acids and metal ions from the graphite oxide suspension. The exfoliated graphene oxide was obtained by ultrasonication of the dialysed product of graphite oxide for 2-3 h. (Soniclean, 160 HT, 170W, Australia). Finally, centrifugation (3000 rpm for 30 min) was done to remove any unexfoliated graphite oxide. Then, exfoliated GO was characterized by various microscopy techniques such as AFM, TEM and SEM as shown in Figure S2, S3 & S4 respectively.

The lateral dimension of GO sheets varies between ~20 nm to 2 μm. The thickness of the exfoliated sheets was measured using AFM (Figure S2A and B), which varied between ~ 0.8 nm to 2.0 nm. Typically, the thickness of the monolayer GO sheets are in the range 0.7 nm -1 nm.[3] So, as prepared GO sheets most likely consist of single layer or two GO monolayers.

The X-ray diffraction spectra (XRD) of graphene oxide and graphite particles are shown in Figure S5, where graphite exhibits a typical sharp peak at 26.62^0 with a *d* spacing of 0.334 nm. After conversion of graphite to graphene oxide (GO), graphite peak shifted to a lower value 11.87^0 corresponding to *d* spacing of 0.734 nm, which is characteristic of the interlayer spacing between graphene oxide sheets.[3]

FTIR spectrometer were used to characterize functional groups present in GO as shown in Figure S6. The FT-IR spectrum of GO shows a absorption band at 3450 cm,$^{-1}$ which is corresponding to O–H groups, the peaks 1632 cm^{-1} and 1738 cm,$^{-1}$ which are attributed to presence of carbonyl and carboxyl groups in GO.

Raman spectra of the graphite powder and graphene oxide are shown in Figure S7. The spectra of GO contains prominent peaks at 1356 cm^{-1} and 1592 cm^{-1}, which correspond to well-known D and G bands of GO respectively.[4]

Synthesis of Dextran sulfate (DS) doped calcium carbonate $CaCO_3$ (DS) microparticles

The $CaCO_3$(DS) microparticles were prepared by co-precipitation method.[5] First, 2 mg/mL of DS was suspended in 0.33 M Na_2CO_3 solution under magnetic agitation for 30 min and equal volume of 0.33 M Na_2CO_3 solution was rapidly added and continued agitation for 30 sec. Then white precipitate of $CaCO_3$ particles was formed. The particles were filtered and washed using DI water through cellulose membrane filter with 0.45 μm pore size. The resulted $CaCO_3$(DS) microparticles were shown in Figure S8A.

Synthesis of $(GO/PAH)_2$ composite hollow capsules:

The GO composite multilayer capsules were fabricated by step wise adsorption of positively charged PAH (0.5 mg/ml in 0.5 M NaCl) and aqueous GO suspension (0.1 mg/mL) onto negatively charged dextran sulfate doped $CaCO_3$(DS) template (4-8 μm). The alternation in the zeta potential value (ζ) for each deposited layer of GO and PAH, indicates the homogeneous coating of GO and PAH layers onto carbonate particles as also observed through scanning electron microscopy (SEM) (Figure S8B). The shell assembly was followed by template dissolution in 0.2 M ethylenediaminetetraacetic acid (EDTA) at neutral pH to obtain the hollow GO composite capsules. Finally, the hollow capsules were obtained by washing several times with pH 6 adjusted DI water as shown in Figure S9.

Encapsulation of Dox into GO composite capsules:

A 500 µl (1 mg/mL) of Dox solution in water was added to 200 µl of microcapsule suspension (9.6×10^5 capsules/1 mL) in an eppendorf tube and pH of the resulting capsule suspension was adjusted to 3. After incubation for 12 h at ambient conditions, the suspension was centrifuged (at 2000 rpm speed for 8 min) and concentration of Dox in the supernatant solution was measured using ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, USA). The amount of Dox loaded into the capsules was calculated from the difference in concentration before and after incubation. The absorbance of Dox was measured at 480 nm. Then the capsules were washed once with pH 3 water to remove unloaded Dox from supernatant.

The Dox was encapsulated into $(GO/PAH)_2$ capsules mainly due to the spontaneous deposition or charge-dependent loading and permeability of the weak polyelectrolyte (GO/PAH) capsule system at pH 3 as we reported earlier.[6, 7] After the dissolution of dextran sulfate (DS) entrapped $CaCO_3$ (DS) core particles using 0.2 M EDTA, DS molecules were retained in the interior of the capsules. These negatively charged DS molecules are responsible for spontaneous loading of the positively charged dyes and drugs into the capsules. Around, 0.63 mg of Dox was loaded in 1 mL of the capsules (9.6×10^5 capsules/1 mL).

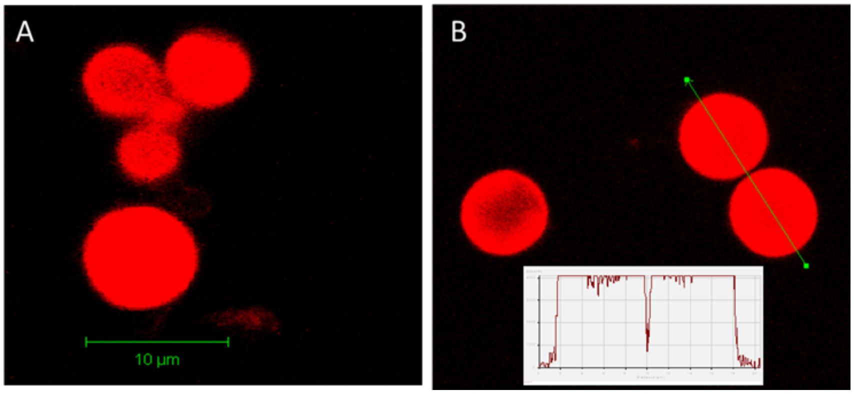

Figure. S1 (A) CLSM image of Dox encapsulated GO composite capsules after washing at pH 3. and (B) shows fluorescence intensity line profile of the Dox encapsulated capsules (in inlet).

Laser illumination experiments:

For laser treatment of the capsules, a pulsed NIR-laser light at 1064 nm wavelength (Nd:YAG, with 10ns pulses, 10 Hz) with different power (30 mW and 70 mW) were used. The laser illumination was performed by irradiating the laser source on the capsules suspensions in a 3 mL quartz cuvette for different time intervals.

NIR-laser induced release of encapsulated Dox:

For NIR-laser induced release studies, Dox encapsulated capsules at pH 3 were exposed to NIR-laser pulse with 30 mW power for 15 sec and 30 sec time intervals. After the NIR-laser exposure, the capsules were centrifuged and concentration of released Dox in the supernatant was determined at 480 nm using ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, USA). Each experiment was done in triplicate manner at room temperature.

Characterization techniques

Atomic Force Microscopy (AFM)

A drop of GO suspension was placed on newly cleaved mica sheet and dried in desiccator at room temperature. AFM images were obtained using Nanosurf Easy Scan2 AFM (Nanosurf AG, Switzerland) in contact mode at room temperature.

Transmission Electron Microscopy (TEM)

A dilute solution of GO sheets, (GO/PAH) hollow capsules and laser-treated capsules were drop cast on carbon coated copper grid and dried overnight at room temperature in a desiccator. TEM images were obtained using Technai T-20 machine (FEI, Netherlands) with an operating voltage 200 kV.

Scanning Electron Microscopy (SEM)

The SEM images of exfoliated GO, bare $CaCO_3$(DS) microparticles, $(GO/PAH)_2$ coated particles and composite hollow capsules were obtained using scanning electron microscope equipped with field emission gun (FEI Sirion, Netherlands) at an operating voltage 10 kV. One drop of sample suspension was applied onto silica wafer, dried under ambient conditions for overnight and gold (Au) layer was coated before SEM analysis.

Powder X-Ray Diffraction (XRD)

Powder X-ray diffraction (XRD) was performed using Philips X'pert diffractometer operating at 40 kV and 30 mA, using Cu Ka radiation. The data were collected using the PAN analyticals Xpert Highscore software.

Fourier Transform Infrared (FT-IR) spectroscopy

The FT-IR spectra of graphene oxide (GO) was recorded using FT-IR spectrometer (Perkin-Elmer Spectrum one model) at room temperature.

Raman Spectroscopy:

Raman spectra characterization of graphene oxide sheets, graphite powder and GO composite capsules before and after NIR-laser irradiation was recorded using LabRaM HR (UV) spectrophotometer with 514 nm laser excitation (2 mW power) at room temperature.

The GO composite capsules and laser-exposed GO capsules were immobilized over a glass slide and dried for overnight then Raman spectra of both capsules were recorded. For the NIR-laser treated capsules, Raman spectrum was recorded at ruptured area in the capsules using optical microscopy attached to the Raman spectrophotometer.

Confocal Laser Scanning Microscopy (CLSM).

The doxorubicin (Dox) loaded capsules and the NIR-laser irradiated were analyzed using Zeiss LSM 510 META confocal system (Zeiss, Germany) equipped with a 100x oil-immersion objective with numerical aperture of 1.4. The samples were placed on a glass cover and excited with laser of wavelength 543 nm.

The rupturing of the capsules and changes in morphology of the capsules were analyzed by using transmission mode in CLSM with oil immersion objective (100 x objective lens).

Visible – near-infrared spectrophotometer: Visible – near-IR spectrophotometer (Ocean Optics) was used for recording visible-NIR absorption spectrum of graphene oxide by keeping pure water as reference.

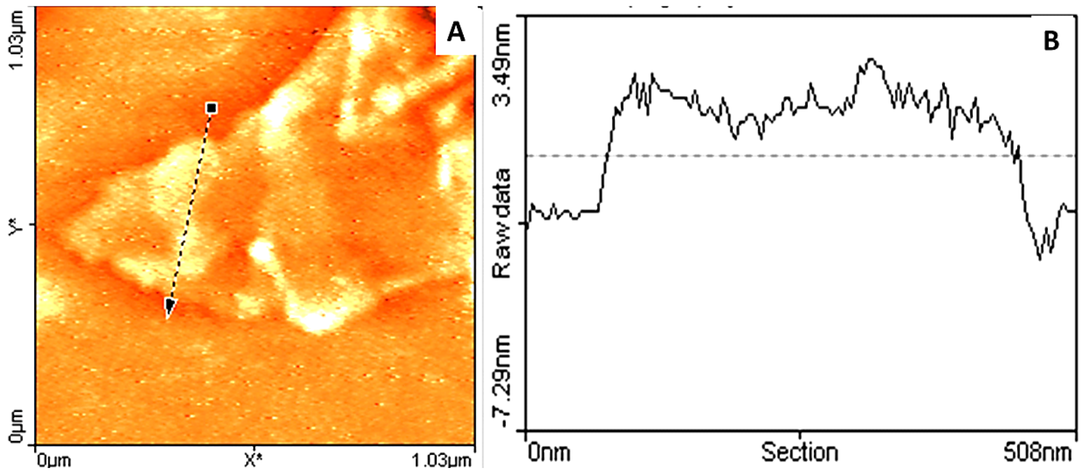

Figure S2. (A) AFM image of as prepared GO sheets along with line profile shown in (B).

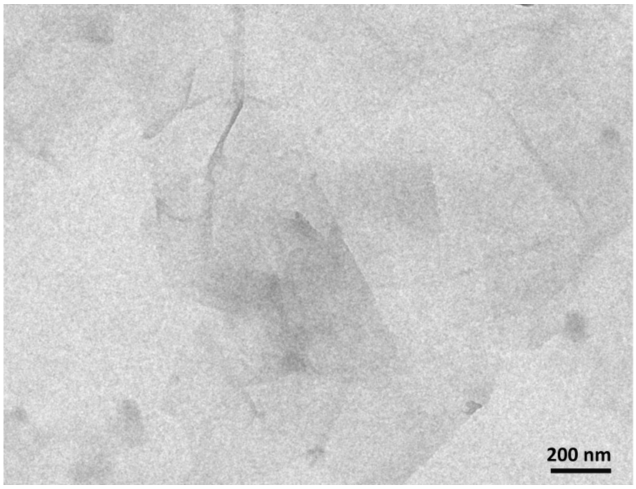

Figure S3. TEM images of exfoliated graphene oxide sheets.

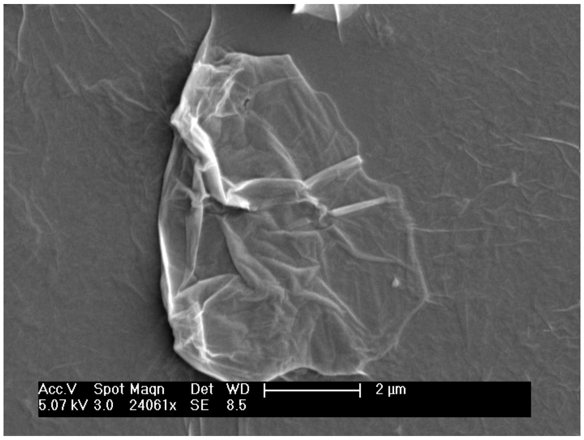

Figure S4. SEM image of the dried GO sheets in aqueous suspension.

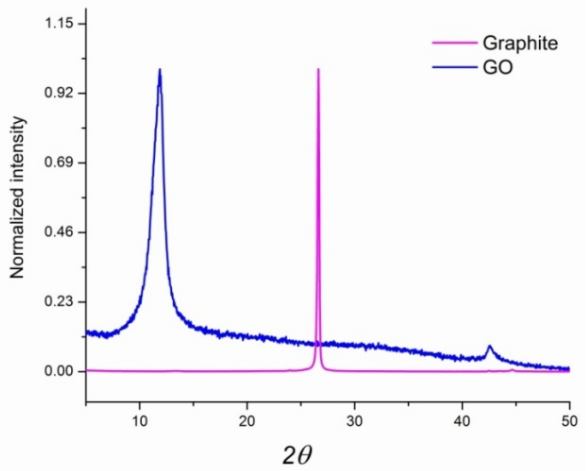

Figure S5. XRD spectra of exfoliated graphene oxide (blue color) and graphite powder (pink color).

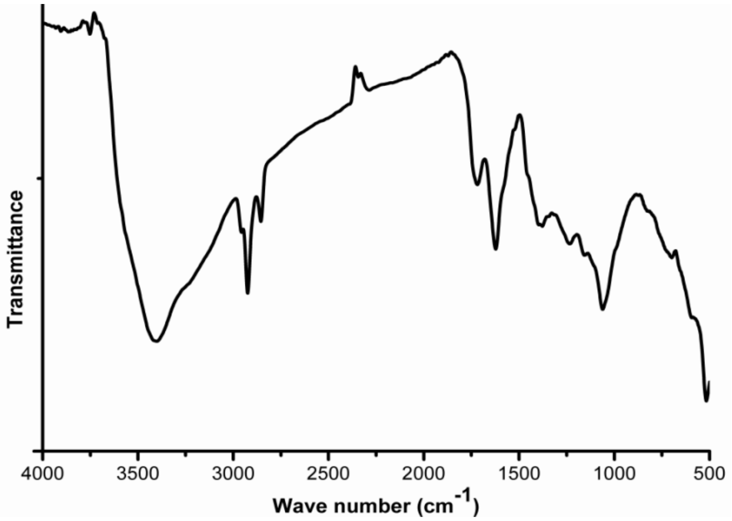

Figure S6. FT-IR spectrum of graphene oxide (GO).

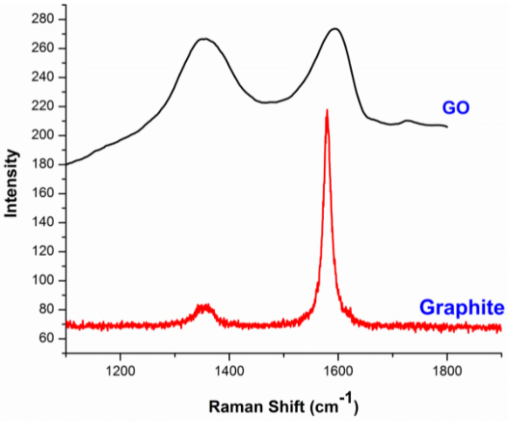

Figure S7. Raman spectra of graphene oxide (GO, black color) and graphite (red color).

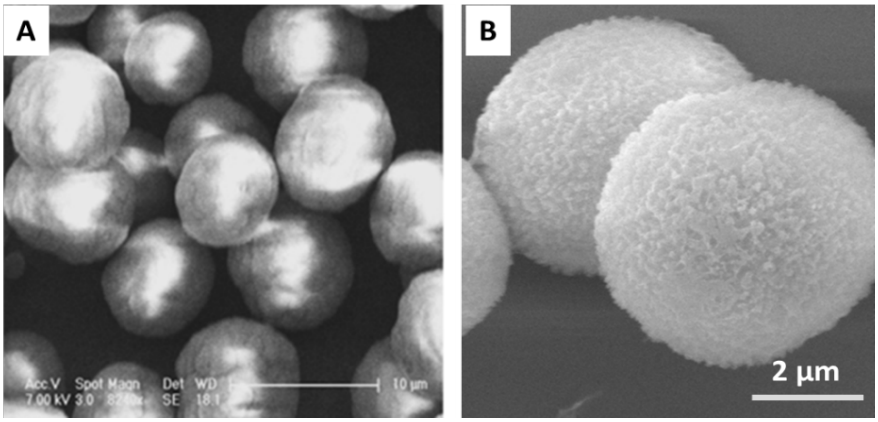

Figure S8. (A) SEM image of bare $CaCO_3$(DS) microparticles, and (B) SEM image of tow bilayer of $(GO/PAH)_2$ coated CaCO3(DS) microparticles.

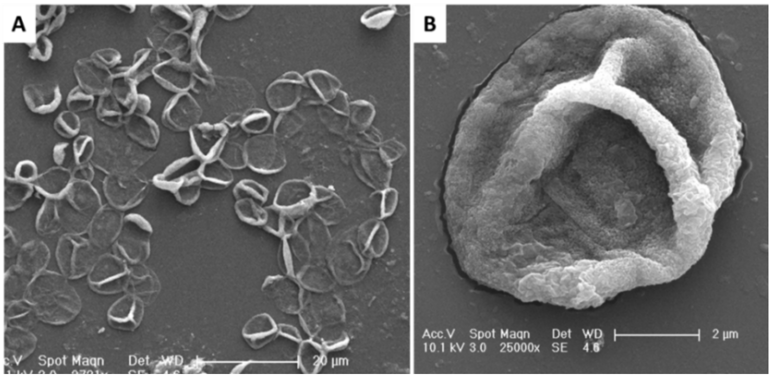

Figure S9. The low and high magnification SEM images of the GO composite capsules.

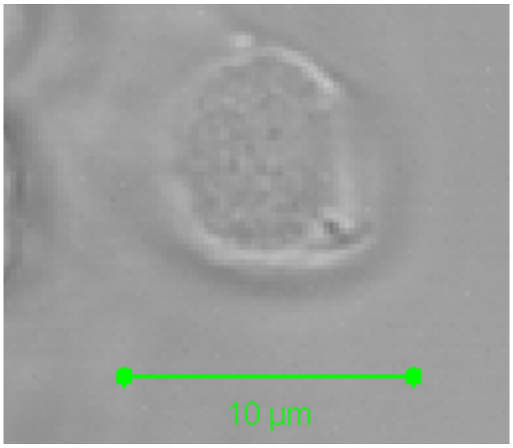

Figure S10. Shows transmission mode optical microscope image of NIR-laser irradiated GO composite capsules for 30 sec with 70 mW power.

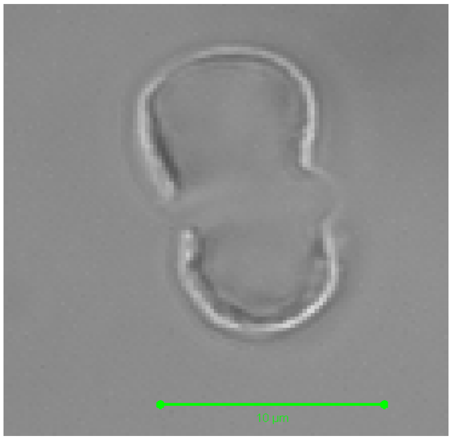

Figure S11. Shows transmission mode optical microscope image of NIR-laser irradiated GO composite capsules for 30 sec with NIR-laser (30 m W power).

References:

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