

Electronic supporting Information

Graphene Oxide Composite Multilayer Capsules with Unique Permeable Properties: Facile Encapsulation of Multiple Drugs

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Experimental section

Materials:

High purity graphite powder with average grain size 45 μm , PAH (Mw = 70 kDa), ethylenediaminetetraacetic acid (EDTA), doxorubicin hydrochloride (Dox), FITC-BSA (Mw = 66 kDa), FITC-dextran (Mw = 3,000-5,000), 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 $\text{M}\Omega\text{ cm}$.

Synthesis of exfoliated graphene oxide (GO)

GO was synthesized by using a modified Hummer's method followed by ultrasonication.¹ 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 °C. Now, distilled water was added to dilute the mixture and heated at 100 °C for 30 min. Then 350 mL of distilled water was gradually added and temperature was kept below 100 °C. Finally, the reaction was terminated by addition of 20 mL H₂O₂ (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, FESEM as shown in Fig. S1.

The lateral dimension of GO sheets varies in between ~20 nm to 2 μm. The thickness of the exfoliated sheets is measured using AFM (Fig.S1A and B), which varies in 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.² So the shown 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 Fig. S2, where graphite exhibits a typical sharp peak at 26.62° 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° corresponding to *d* spacing of 0.734 nm, which is characteristic of the interlayer spacing between graphene oxide sheets.²

Raman spectra of the graphite powder and graphene oxide are shown in Fig. S3. The spectra of GO contains prominent peaks at 1356 cm⁻¹ and 1592cm⁻¹, which correspond to well-known D and G band respectively.³ It is well-documented in the literature that these G band and D bands

are attributed to first-order scattering of the E_{2g} mode observed for sp^2 carbon domains and structure defects, amorphous carbon or edges that can break selection rules.⁴

FTIR spectroscopy was used to characterize functional groups present in GO as shown in Fig. S4.

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. The AFM images were obtained using Nanosurf Easy Scan2 AFM (Nanosurf AG, Switzerland) in contact mode at room temperature.

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. All the major characteristics peaks for various functional groups of GO were labeled as shown in the spectra (Fig. S4).

Transmission Electron Microscopy (TEM)

A dilute solution of GO sheets and GO/PAH hollow capsules were drop cast on carbon coated copper grid and dried overnight at room temperature in a desiccator. The 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 coated particles, hollow capsules and drug loaded capsules were obtained using scanning electron microscope equipped with field emission gun (FEI sirion, Netherlands) at an operating voltage 10 kV. One drop of capsules suspension was applied onto silica wafer, dried under ambient conditions for

overnight and gold (Au) layer was coated before SEM analysis. EDS spectrum of the hollow GO/PAH casules after core removal was obtained as shown in Fig. S7.

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 K α radiation. The data were collected using the PAN analyticals Xpert Highscore software. The XRD pspectra of graphite powder and graphene oxide were shown in Fig. S2.

Raman Spectroscopy:

Raman spectra of graphene oxide and graphite powder were recored using LabRaM HR (UV) spectrophotometr with 514 nm excitation at room temperature. The raman spectra were shown in Fig. S3.

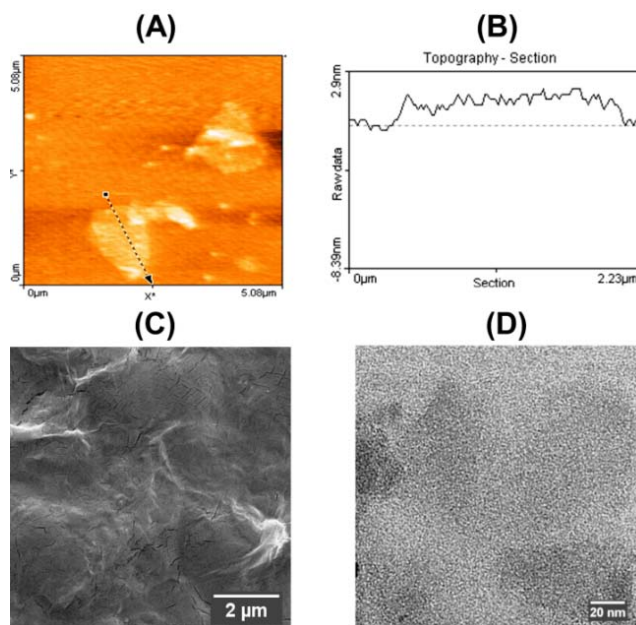


Fig. S1 AFM image of GO sheets along with line profile shown in (A) and (B) respectively and (C) and (D) are FESEM and TEM images of exfoliated GO

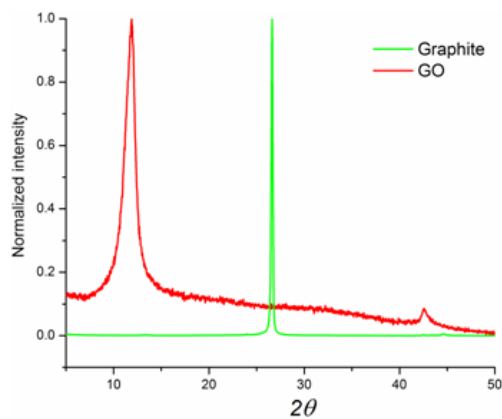


Fig. S2 XRD spectra of exfoliated graphene oxide (red color) and graphite powder (green).

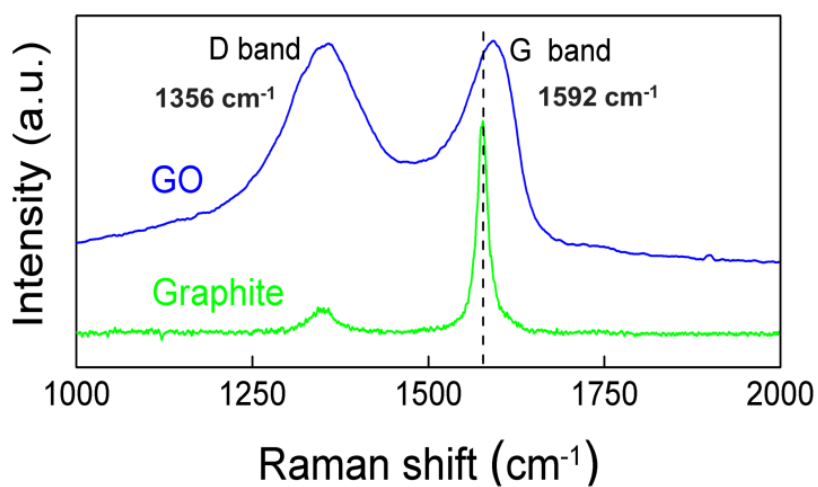


Fig. S3 Raman spectra of graphene oxide (blue color) and graphite powder (green color)

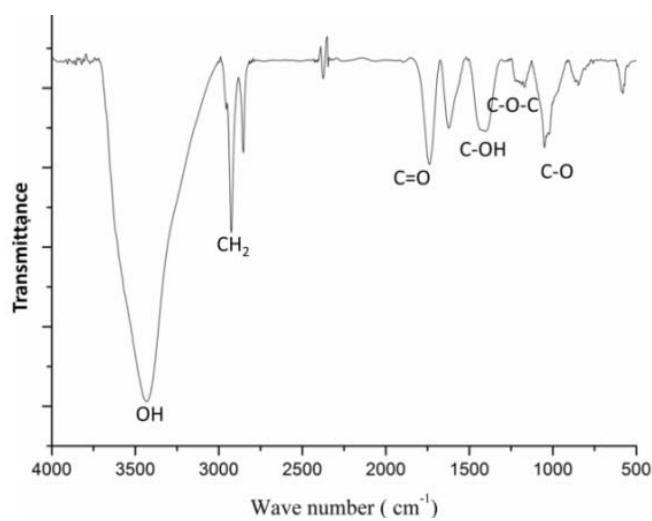


Fig. S4 FTIR spectrum of graphene oxide (GO)

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. the white precipitate of CaCO_3 particles were 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 Fig. S6A.

Zetapotential measurements

The LbL growth of PAH and GO on CaCO_3 (DS) microparticles was analyzed by measuring zeta potential (ζ) for each step of adsorption after three washing cycles with DI water by using Nanozetasizer machine (ZEN 3690, Malvern Instruments, UK). The measurements were done, right from bare template to next two bilayers at the same pH 6. Each zeta potential value is average of three parallel measurements at room temperature.

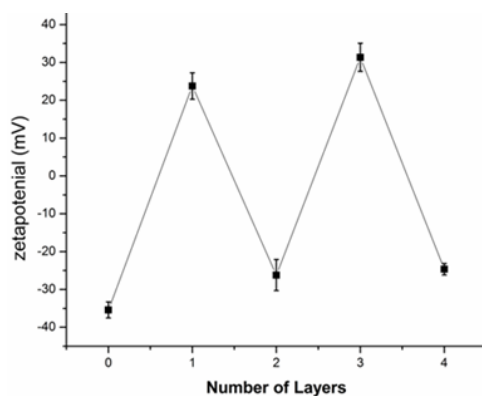


Fig. S5 The zetapotential measurements of $(\text{GO}/\text{PAH})_2$ coated CaCO_3 (DS) microparticles starting from bare carbonate particles. The point 0 in X axis is corresponding to bare CaCO_3 (DS) microparticles and followed by alternative layer of PAH and GO as shown in points 1, 2, 3 and 4 in the X-axis.

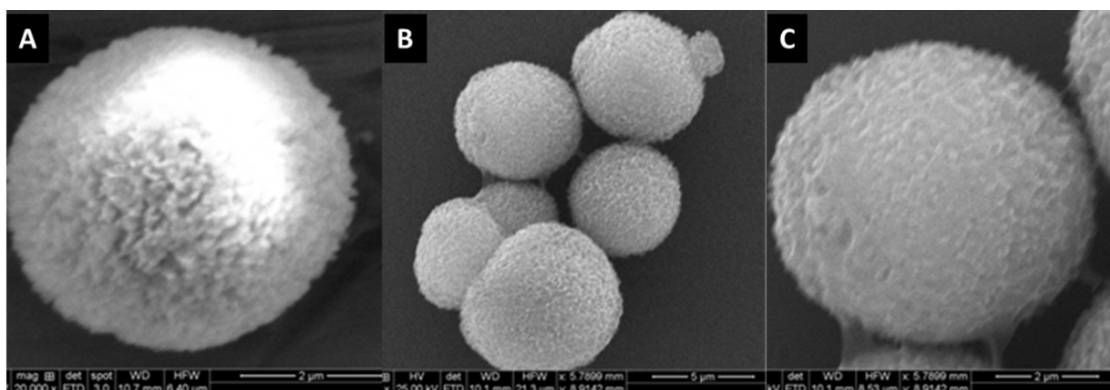


Fig. S6 Bare CaCO₃(DS) microparticles (A) and low and high magnification images of tow bilayer of (GO/PAH)₂ coated CaCO₃(DS) microparticles shown in images (B) and (C).

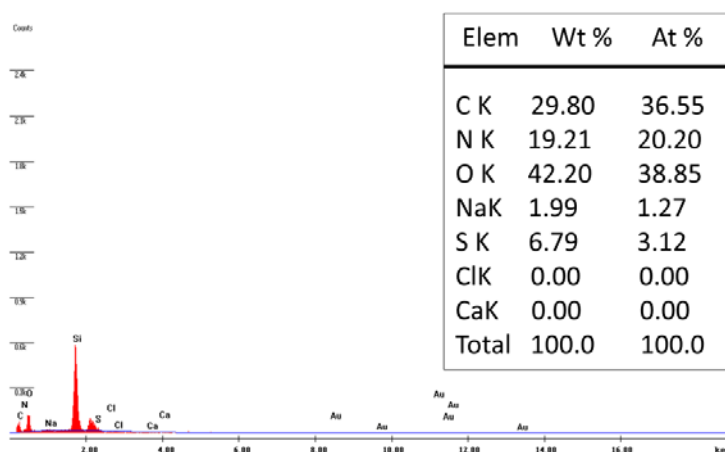


Fig. S7 The EDS spectra of the hollow capsules of GO/PAH after core dissolution along with elemental analysis shown in inset.

Confocal Laser Scanning Microscopy (CLSM)

Size, integrity and degree of filling of various dyes into capsules were investigated 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 for Rd 6G, TRITC-dextran and Dox and 488 nm for FITC-dextran, FITC-BSA and FITC-insulin respectively.

Simultaneous loading of dual drugs

A 300 μl (2 mg/mL) of FITC-BSA solution in water and 500 μl (1 mg/mL) of Dox solution in water were added to 200 μl of microcapsule suspension (9.6×10^5 capsules/1 mL) in an eppendorf tube and adjusted pH to 3. This capsules suspension with Dox and BSA was incubated for 12 h at 25°C and mixture solution was centrifuged and measured the concentration of FITC-BSA and Dox in supernatant solution using ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, USA). The capsules were washed once with pH 3 adjusted water to remove unloaded BSA and Dox. The amount of FITC-BSA and Dox loaded into the capsules were measured from the concentration difference between supernatant obtained after centrifugation and initial feeding concentration. The absorbance of Dox and BSA were measured at 280 nm and 480 nm respectively. The CLSM image of (GO/PAH)₂ single capsule loaded with Dox and FITC-BSA was shown in Fig. S6. The CLSM image shows clearly loading of two drugs into core and shell of the GO composite capsules.

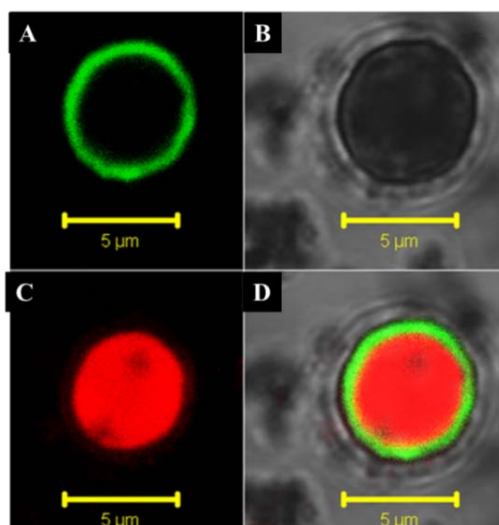


Fig. S8. CLSM images of the single capsule encapsulated with Dox and BSA simultaneously. (A) green fluorescent channel image shows BSA loaded in shells of the capsule, (B) bright filed

image(C) red fluorescent channel image shows Dox loaded mainly in the core of the capsule and (D) is the overlay image.

MTT assay:

The cell viability tests for GO/PAH capsules were done using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay in human breast cancer MCF-7 cell line.⁶ Initially, MCF-7 cells (human breast carcinoma) were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 IU/mL streptomycin and cultures were grown at 37 °C in humidified 5 % CO₂ incubator. The MCF-7 cells were plated at 1.5x 10⁴ cells per well into 96-well plates. After overnight adherence, cells were exposed to GO/PAH capsules by varying the number of capsules, starting with capsules/cells ratio 25:1, 50:1, 100:1 and 200:1. The cells along with the capsules were incubated at 37 °C in a 5 % CO₂ incubator for 48 h. After treatment for 48 h, the fresh medium was replaced by 20 µl of medium containing MTT (5 mg/mL) and cells were incubated for further 3-4 h. The resulting blue formazan crystals were solubilized in 200 µl of DMSO, which are only produced by live cells. The absorbance of formazan in DMSO was measured at 595 nm by using a BIORAD ELISA plate reader and this absorbance was proportional to live cells. The cytotoxicity was measured as the percentage ratio of the absorbance of the treated cells to the untreated controls.

References:

- 1 (a) N. I. Kovtyukhova, P. J. Ollivier, B. R. Martin, T. E. Mallouk, S. A. Chizhik, E. V. Buzaneva and A. D. Gorchinskiy, *Chem. Mater.*, 1999, **11**, 771; (b) W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.
- 2 D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu and J. M. Tour, *ACS Nano*, 2010, **4**, 4806.
- 3 K. N. Kudin, B. Ozbas, H. C. Schniepp, R. K. Prud'homme, I. A. Aksay and R. Car, *Nano Lett.*, 2007, **8**, 36.

4 A. C. Ferrari and J. Robertson, *Physical Review B*, 2000, **61**, 14095.

5 D. V. Volodkin, A. I. Petrov, M. Prevot and G. B. Sukhorukov, *Langmuir*, 2004, **20**, 3398.

6 M. Roy, B. V. S. K. Chakravarthi, C. Jayabaskaran, A. A. Karande and A. R. Chakravarty, *Dalton Transactions*, 2011, **40**.