Supplementary Information

Exploring Fluorescence Switching Phenomenon of Curcumin Encapsulated Niosomes: In Vitro Real Time Monitoring of curcumin release on Cancer Cells

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Experimental Section

Materials and methods

All other solvents and chemicals were used analytical grade without further purification. Poly(ethylene glycol) methyl ether acrylate (PEGMEA-480), Tween 80, cysteine, curcumin and triethylamine (TEA) were purchased from Sigma Aldrich. Paraformaldehyde was purchased from Merck Pvt Ltd Bombay. MTT ((3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent and DMSO were obtained from Sigma Aldrich, USA. Dulbecco’s minimum essential medium (DMEM) medium, glutamine, penicillin, streptomycin and FBS were purchased from Himedia, Gibco and Invitrogen India, respectively. All other chemicals used in this study were of analytical grade.

Synthesis of PEG-Cys polymer

In brief, PEGMEA was separated from inhibitor by passing the sample through Al₂O₃ column it was eluted by 4:2 (v/v) petrol ether/ethyl acetate mixtures and subsequently after purification solvent from PEGMEA was removed through vacuum evaporation. Further reaction was carried to this sample (0.2mmol) along with 5 ml aqueous solution of cysteine (0.2 mmol) in 5 ml of methanol at room temperature for 8 h.¹ The resultant reaction mixture turns solid after removal of solvent under vacuum, which was re-dispersed in water followed by addition of isopropanol in order to obtain pure product and yield is around 87 %. The so-prepared product was characterized by FTIR and ¹H NMR studies (Figure A). FTIR (KBr, cm⁻¹) 665, 1647 and 1455 cm⁻¹. ¹H NMR (CDCl₃, ppm): 2.0278 (NH₂), 2.772 (SCH₂-C₇H₄-CO), 3.208 (O-CH₃), 3.051 (N-C₇H₄-C₇H₂-S), 3.652 (long chain glycolic CH₂), 3.705 (CO-OCH₂-C₇H₄), 4.254 (CO-OCH₂-C₇H₂).
**Figure A:** PEG-Cys sample (a) $^1$H NMR spectra and FTIR spectrum (b)

**Synthesis of Niosomes**

Based on the previous reported method niosomes were prepared with slight modification. In brief, a mixture of tween80, PEG-Cys and water were mixed at optimum weight ratio (i.e., 0.500:0.060:0.500) and vortexed for 5 min, followed by 30 min sonication. In addition, the resultant solution was diluted with 2 (w/v %) of PEG-Cys, followed by sonication for 30 min, resulted in formation of Niosomes and designated as PEG-Cys-NIO.

**Synthesis of curcumin encapsulated niosomes**

Concisely, we dissolved 2 mg of curcumin in 1 ml of chlorofrom and solvent was evaporated under vacum resulted in the formation of film. Then, we added a mixture of tween 80 (0.500 g), PEG-Cys (0.016 g) and water (0.500 g), and repeated the above niosome synthesis procedure for the establishment of curcumin encapsulated niosomes and designated as PEG-Cys-NIO-CU. Further, the mixture solution containing PEG-Cys-NIO-CU was subjected to centrifugation at 1100 rpm for 3 min to seclude curcumin encapsulated niosomes in the form of supernatant from pellet containing native curcumin. The loading efficiency of curcumin within niosome was determined, measuring the curcumin concentration in pellet through UV absorption spectrum and this value was deducted from the intial concentration of curcumin while the loading efficiency of niosome is found to be 86.4 %.

**Characterization**

$^1$H NMR spectra was used to detect the formation of PEG-Cys through BRUKER-AC 200 MHz spectrometer using CDCl$_3$ solvent. Absorption and fluorescence spectra were carried out through
SHIMADZU and HITACHI spectrometer. Elemental mapping was performed through FESEM. Noisome morphology and shape were recorded using JEOL JEM-210 transmission electron microscopy. Hydrodynamic size and surface charge were determined through nanozetasizer.

**Cell proliferation assay**

Cytotoxicity of free niosome, curcumin-loaded noisome and curcumin alone on MCF 7 cells was determined by conventional MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay. MCF 7 human breast cancer cell line obtained from National Centre for Cell Science (NCCS) which was maintained in Dulbecco’s minimum essential medium (DMEM) containing 10 % fetal bovine serum (FBS) at 37 °C and 5 % CO2. Cells were harvested in the logarithmic phase of growth; cell suspensions were dispensed (200 µl) into 96-well tissue culture plates at an optimized concentration of 1 X 10⁴ cells/well in complete medium. After 24 h, cells were treated in quadruplicate with 0.1 ml of incomplete media containing various concentrations of free noisome, curcumin-loaded noisome and curcumin. Control wells were treated with equivalent volumes of 0.1 % DMSO in incomplete media, and incubated for 48 h. Cell viability was measured by MTT dye reduction assay at 540 nm with slight modifications. The dose-effect curves were analyzed using Prism software (GraphPad Prism).

**Cellular drug release profiles**

Cellular uptake studies were carried out using the MCF 7 human breast cancer cell line. To study the cellular uptake of curcumin-loaded niosome, briefly MCF 7 cells (5×10⁴ cells/cover slip) were placed on cover slips in 60 mm plates and allowed to adhere for 4-6 hours. Cells were treated with IC₅₀ concentration of curcumin-loaded noisome in serum free medium for various time intervals for 24 h at 37 °C and 5 % CO2. The culture medium was removed and the cells were rinsed with PBS and fixed with 3.7 % paraformaldehyde for 20 min. The cells were then washed for 15 min with 0.1% Triton X-100 in PBS, followed with alcohol dehydration and permanently mounted with DPX. Changes in drug release mechanism in the cells were investigated with fluorescence microscopy using excitation wavelength of 350 and 420 nm for blue and green fluorescence, respectively. The emission wavelengths detected were
in the ranges from 440-495 and 500-550 nm for blue and green fluorescence, respectively. In order to obtain reliable estimates of apoptotic cells, the same experiment was repeated three times independently.

Figure S1A: FESEM image of PEG-Cys-NIO sample (a) and corresponding EDAX elemental mapping of Carbon (b), Nitrogen (c), Oxygen (d) and Sulfur (e)

Figure S1B: Hydrodynamic size distribution of PEG-Cys-NIO (a) and PEG-Cys-NIO-CU (b)
Figure S2. Fluorescence emission spectra of curcumin excited at 350 nm (a) and 410 nm (b).

Figure S3. PEG-Cys-NIO-CU sample various time intervals absorption spectra and their stability of curcumin release (a); PEG-Cys-NIO-CU sample fluorescent switch on/off route (b) and the corresponding samples emission spectra (c) $\lambda_{ex}$ at 350 nm (solution emitted blue color as inset) and (d) $\lambda_{ex}$ at 410 nm (solution emitted green color as inset).
Figure S4: Light irradiation after Figure S3 PEG-Cys-NIO-CU sample hydrodynamic radius spectra

Figure S5. Hydrodynamic size distribution of PEG-Cys-NIO-CU sample at pH 5
Figure S6. pH dependent surface charge of PEG-CYS-NIO-CU (a) and PEG-Cys-NIO (b)

References