Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Dual Stimuli-Responsive Nano-Vehicle for Controlled Drug Delivery: Mesoporous Silica Nanoparticles End-Capped with Natural Chitosan**

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Contents

1. ABBREVIATIONS

MCM…….. ……Mobil Crystalline Materials

MSNPs……. ……. Mesoporous Silica Nanoparticles

DOX……Doxorubicin

RhB……..... Rhodamine B

RhB/ MSNPs…..... Rhodamine B loaded MSNPs

MSNPs-NH_2…….....3-aminopropyl trimethoxysilane modified mesoporous silica nanoparticles

RhB/MSNPs-NH_2.....…..... Rhodamine B loaded 3-aminopropyl trimethoxysilane modified mesoporous silica nanoparticles

DOX/MSNPs-NH_2..... Doxorubicin loaded 3-aminopropyl trimethoxysilane modified mesoporous silica nanoparticles

MSNPs-NH_2-COOH…….....3-aminopropyl trimethoxysilane and succinic anhydride modified mesoporous silica nanoparticles

MSNPs-NH_2-COOH-Chitosan…….3-aminopropyl trimethoxysilane and succinic anhydride modified chitosan end-capped mesoporous silica nanoparticles

RhB/MSNPs-NH_2-COOH-Chitosan…..... Rhodamine B loaded 3-aminopropyl trimethoxysilane and succinic anhydride modified chitosan end-capped mesoporous silica nanoparticles.

DOX/MSNPs-NH_2-COOH-Chitosan……Doxorubicin loaded 3-aminopropyl trimethoxysilane and succinic anhydride modified chitosan end-capped mesoporous silica nanoparticles

MTS …… (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium

2. EXPERIMENTAL SECTION

2.1. Instrumental Techniques

TEM images were taken from Techai G2 F20 S-Twin 200 KV Transmission Electron Microscope, FTIR spectrum was obtained from Burker Vertex 70 FTIR Spectrometer, Thermogravimetric Analysis (TGA) was carried out through Perkin Elmer Pyris TG Analyzer by using atmospheric air as an oxidant with continues heating from 30 ^{0}C to 796.84 ^{0}C with a heating rate 10 ^{0}C / min., X-ray diffraction was recorded through Bruker D4 Powder X-ray Diffractometer using Cu Kα radiation, N_2-absorption/desorption was performed by using ASAP 2020 sorptometer at 77K and absorbance readout was obtained through Agilent Carry 60 UV/Visible Spectrometer.

2.2. Materials and Reagents:

Tetraethylorthosilcate (TEOS), N-cetyltrimethylammonium bromide (CTAB), 1-ethyl-3-(3-dimethylaminopropyl) carbimide.HCl (EDC), rhodamine B and doxorubicin were purchased from Aladdin. Succinic anhydride (SA), chitosan (high molecular mass), (3-aminopropyl) trimethoxysilane (APTES), N-hydroxysuccinimide (NHS), nonporous SiO_2 and lysozyme were purchased from Sigma Aldrich. Sodium hydroxide (NaOH), 37% pure hydrochloric acid, methanol, anhydrous toluene, acetone, acetic acid and other laboratory reagents were provided by Sinopharm chemical reagents co. limited China. All these chemicals were used in original form as received from the relevant manufacturing company without any further purification. Ultra nanopure distilled water (18.2MΩ) was used in all experiments and in preparation of phosphate buffer solution (PBS). Chronic lymphocytic leukemia cancer cell line (CCL) was provided by the Center for Biological Preservation Wuhan. MTS reagent (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium was provided by Sigma Aldrich.

2.3. Synthesis of MCM-41 type Mesoporous Silica nanoparticles (MSNPs):

MCM-41 type mesoporous silica nanoparticles with the diameter around 150nm were synthesized according to previous study [1] by making some changes in the procedure. 1.0 g of N-Cetyltrimethylammonium bromide (CTAB) and 0.3g of NaOH were dissolved in 500 ml of distilled water and then heated up to 80^{o}C. Followed by addition of 5ml TEOS drop wisely with constant vigorous stirring. Subsequently, the mixture was stirred continuously at 80^{0}C for 2 hours until white precipitates were formed. Then, these white precipitates were centrifuged at 4000 rpm for 1 h and filtered; the centrifuged residue was rinsed with excessive water and methanol. This white solid product was dried in air for 24 hours. Subsequently, air dried product was refluxed in a solution containing 5ml HCl (37%) and 100ml Methanol for 24 h at 50^{0}C in order to remove the remaining surfactant Template (CTAB) and followed by filtration and rinsing with copious amount of distilled water and methanol. The obtained solid product was then dried under high vacuum (-0.01mPa) at 80^{o}C for overnight to remove residual surfactant and solvent molecules entirely from the pores of silica nanoparticles. Finally synthesized-mesoporous silica nanoparticles (MSNPs) MCM-41 type were obtained and Transmission Electron Microscopic images of synthesized mesoporous silica nanoparticles were taken to determine the size and to observe the mesoporous structure. The MSNPs were dispersed in methanol to make spot on the copper gird.

2.4. Synthesis of MSNPs-NH_2 nanoparticles:

In order to produce MSNPs-NH_2 silica nanoparticles, 1.0 g of silica nanoparticles was refluxed in a mixture containing 100 ml of anhydrous dry toluene and 1 ml of (3-aminopropyl) trimethoxysilane (APTES) for 24 hours. Subsequently, these silica nanoparticles were filtered off, rinsed with excess distilled water and methanol and dried at 80^{0}C under high vacuum overnight. The obtained product was ammonized or APTES modified mesoporous silica nanoparticles (MSNPs-NH_2).

2.5. Synthesis of MSNPs-NH_2-COOH Nanoparticles:

In order to synthesis MSNPs-NH_2-COOH, 500 mg of MSNPs-NH_2 were suspended in 50ml of acetone with constant stirring at room temperature for 4 hours, after that 20ml 2M succinic anhydride solution in acetone was added drop wisely with constant stirring and then allowed the mixture to stir for next 24 hours at ambient temperature. Subsequently, the stirred mixture was filtered off and residue was rinsed with copious amount of water and methanol. The resulting white solid residue was MSNPs-NH_2-COOH nanoparticles. Then these MSNPs-NH_2-COOH nanoparticles were dried under high vacuum (-0.01mPa) again at 80^{0}C to remove the solvent molecules from the pores of nanoparticles completely in order to increase the cargo loading capacity of mesopores.

2.6. Binding of chitosan on the surface of MSNPs-NH_2-COOH:

300 mg MSNPs-NH_2-COOH were dispersed in 50 ml of pH 7.0 phosphate buffer solution. Subsequently, EDC/NHS mixture (EDC= 0.2M, NHS= 0.2 M) in 25 ml of pH 5.0 phosphate buffer solution was put in to activate the carboxylic group of silica nanoparticles and then the mixture was allowed to settle for 4 h to attain stable suspension at ambient temperature. After 4 hours, 5ml 2% chitosan (high Molecular mass/ Density) solution in 2% Acetic acid solution was added drop wise and the mixture was stirred for another 12 hours at room temperature. The resulting chitosan end-capped nanoparticles (MSNPs-NH_2-COOH-Chitosan) were centrifuged, filtered off, rinsed with excessive pH: 7.0 PBS solution and distilled water to remove the unbounded chitosan and remaining solvent then dried at 45^0C for overnight. TEM images of MSNPs-NH_2-COOH-Chitosan were taken to confirm end capping of pores through chitosan binding on the surface of nanoparticles. Chitosan end capped MSNPs were also dispersed in methanol to make spot on the copper gird to take TEM images.

2.7. Characterization of synthesized nanoparticles:

The synthesis of MSNPs, MSNPs-NH_2 and MSNPs-NH_2-COOH and end-capping of MSNPs nanoparticles with chitosan was performed according to scheme as illustrated in figure S1. The mean diameter of MSNPs was about 150 nm and surface area was about 840 m^2 /g. These large pore volume and pore size nanoparticles were synthesized by hydrolysis of TEOS in the presence CTAB as a template at 80^0C. Well-ordered array of pores on the surface of MSNPs could be observed in the TEM images figure 2A. The amino group was grafted on the surface of MSNPs by treating with APTES both on the external surface and internally as clear from the figure S2B N2-sorptiom isotherm (decrease in N_2-adsorption) and S4b FTIR spectrum. The grafting of carboxylic group on MSNPs-NH_2 was achieved by the reaction of the nanoparticles with succinic anhydride. The pores of MSNPs-NH_2-COOH nanoparticles were blocked completely in order to reduce the pre-mature release of the cargo molecules by the reaction with chitosan as shown TEM images figure 2B. As clear from the TEM images, the synthesized MSNPs with mean diameter 150 nm had well- ordered array of mesopores. These are the characteristics of MCM-41 MSNPs that was further confirmed by XRD patterns figure S6.

The surface area, pore size and pore volume of different synthesized nanoparticles was measured by using BET and BJH methods (see Table S1) from their respective N_2-adsorption/desorption isotherms (figure S2). The figure S4A, S4B and S4C show the characteristic type of IV N_2-sorption isotherm as per IUPAC characterization. The well characterized steps between the relative pressure P / P_0 at 0.5 - 0.7 suggest that nanoparticles have consistent mesoporous layers of pores. Notwithstanding, the nitrogen adsorption was decreased from MSNPs to MSNPs-NH_2-COOH but the form of hysteresis loop remained identical in case of MSNPs, MSNPs-NH_2 and MSNPs- NH2-COOH nanoparticles which is the

indication that pores shape was not significantly distorted during surface modification, however there was a little decrease in the pore size after treating with APTES and succinic anhydride to graft NH_2 and COOH groups respectively. The surface area of MSNPs, MSNPs-NH_2, MSNPs-NH2-COOH and MSNPs- NH2-COOH-chitosan was 840 m^2 / g, 753 m^2 /g, 629 m^2 /g and 142 m^2 /g respectively, the pore size of MSNPs, MSNPs-NH_2, and MSNPs- NH2-COOH was 3.94 nm, 3.45 nm and 2.92 nm respectively and the pore volume of MSNPs, MSNPs-NH_2, and MSNPs- NH2-COOH was 0.98 cm^3 /g, 0.86 cm^3 /g and 0.73 cm^3 /g respectively.

Figure S4 clarifies FTIR spectra of mesoporous silica nanoparticles before and after grafting with different functional groups. MSNPs exhibited strong absorption peaks at 1090 cm^{-1} and 964 $cm^{-1,}$ these peaks are assigned to stretching vibration of Si-O-Si bridges and skeleton vibration involving C-O bond stretching respectively (figure S4 a). Peaks at 3434 cm^{-1} and 1635 cm^{-1} are assigned to –OH groups present on the surface of MSNPs or due to water molecules adsorbed. In contrast, MSNPs, MSNPs-NH_2 nanoparticles displayed peak at 1642 cm^{-1} (Figure S4 b), that is assigned to- NH_2 bending, moreover, decrease in absorption peak at 3438 cm^{-1} suggests that APTES is successfully bonded on the surface of MSNPs that results decrease in -OH groups. After chemically modification with succinic anhydride (Figure S4 c), the intensity of absorption peak at 3458 cm^{-1}, that is assigned to –OH groups, is again increased due to -OH groups addition on chemical modification with succinic anhydride. On the other hand, a peak at 1722 cm^{-1} is observed which is attributed to (C=O) stretching of carboxylic group, it is obvious that succinic anhydride is covalently coupled with MSNPs-NH_2 nanoparticles. The absorption peak at 1643 cm^{-1} is observed (Figure S4 d) that is assigned to C=O stretching in amides and while the peak at 1602 cm^{-1} attributed to un-reacted amino groups decreased perceptibly, indicating that all amino groups of chitosan are not coupled with C=O group of MSNPs-NH_2-COOH because some monomer are not directly linked with C=O group but they are linked indirectly through another chitosan monomer by glycoside linkage that is covalently coupled with C=O of MSNPs by amide bond. Moreover, the broad less intensive absorption peak at 3434 cm^{-1} is also assigned N-H stretching in amides. It meant that chitosan has been covalently coupled with MSNPs-NH_2-COOH.

In the TGA curves (figure S5) MSNPs-NH2-COOH-Chitosan nanoparticles has shown 14.3% more weight loss than MSNPs-NH2 and 15.03% more weight less than MSNPs. Based on 14.3% more weight loss of MSNPs-chitosan compared with MSNPs-NH2, the grafting amount of chitosan on the surface of MSNPs is calculated to be 17.22mg / 100mg SiO_2.

2.8. Rhodamine B loading as a model drug and end-capping with chitosan:

1mg Rhodamine B was dissolved in 20 ml of 0.2 M PBS solution (pH 7.4). The aliquot was taken from the dye solution and absorbance was measured in the range of wavelength 500 - 600 nm in order to determine its initial absorbance in the solution. Subsequently, 200 mg MSNPs-NH_2-COOH nanoparticles were soaked in rhodamine B solution of known absorbance for next 24 h. After that suspension of MSNPs and dye solution was centrifuged then aliquot was again

taken to measure absorbance of rhodamine B in order to determine the concentration of remaining dye and loading capacity of nanoparticles. Followed by addition of 20 ml of Phosphate buffer solution (pH 5) containing mixture of EDC and NHS (EDC: 0.2M NHS: 0.2M) to activate the carboxylic group and allowed to settle for 4 h to achieve the stable suspension at ambient temperature. After 4 h, 5ml 2% chitosan solution in 2% Acetic acid solution was added drop wise and the mixture was again stirred for another 6 h at room temperature to complete the reaction between the amino group of chitosan and the surface carboxylic group of MSNPs. The resulting rhodamine B loaded MSNPs- chitosan nanoparticles were centrifuged, filtered off, rinsed with excessive PH: 7.0 PBS solution and distilled water to remove the unbounded chitosan and remaining solvent. The washing liquid was collected and aliquot was taken to measure the absorbance in order to determine the net loading capacity of MSNPs nanoparticles. The net dye loading capacity of MSNPs was calculated from difference in concentrations of initial dye and left dye by UV/Vis spectroscopy that was about 60μmol/g.

2.9. pH triggered rhodamine B releases:

10 mg of chitosan end-caped rhodamine B loaded mesoporous silica nanoparticles (RhB/ MSNPs) were dispersed in 3 ml of each four different pH PBS solutions (7.4, 6.0, 5.0, & 4.0). After different time intervals, the aliquots were obtained from the each suspension and the release of rhodamine B in PBS solution form the pores of MSNPs nanoparticles was measured through absorbance readout by UV/Visible spectrometer at λ max: 554 nm. The absorbance measurement was revised at all these pHs by three times and the mean of these results was considered as the final result. Photographs of samples were taken after 24 hours.

2.10. Lysozyme triggered rhodamine B releases:

10 mg of lysozyme was dissolved in 2ml of each pH 7.4 & 6.5 PBS solutions. Subsequently, 10 mg of chitosan end-capped RhB/MSNPs was dispersed in both lysozyme dissolved PBS solutions (pH 7.4 and 6.5); and also dispersed in third 2 ml PBS solution pH 7.4 in which lysozyme was not dissolved. Aliquots were taken from the each suspension at different time intervals and the delivery of dye from the pores of nanoparticles in the PBS solutions was observed via absorbance readout in three samples at λ max: 554 nm. This experiment was also revised three times and the mean of these results was considered as the final result. Photographs of all three samples were taken after 24 hours. Photographs of the samples were taken after 24 hours.

2.11. Doxorubicin loading and end-capping with chitosan:

20 mg of doxorubicin was dissolved in 10 ml distilled water. Subsequently, 100 MSNPs-NH_2-COOH nanoparticles were loaded with drug molecules by soaking them in doxorubicin solution for 24 hours. Afterward, the un-soaked solution of drug was removed through centrifugation.

The drug loaded nanoparticles were then suspended in the 10 ml of PBS solution (pH 5.0) containing mixture of EDC and NHS (EDC: 0.2M NHS: 0.2M) to activate the carboxylic group of DOX/MSNPs and allowed to settle for 4 h to get stable suspension at ambient temperature. Then 3 ml of 2% of chitosan solution was added drop wisely with constant stirring and mixture was stirred for next 6 hours at ambient temperature. The resulting DOX loaded MSNPs- chitosan nanoparticles were centrifuged, filtered off, rinsed with excessive PH: 7.0 PBS solution and distilled water to remove the unbounded chitosan and remaining solvent. The drug loading capacity of nanoparticles was calculated from the difference in the concentrations of initial and left drug by using UV/Vis absorbance readout at λmax. 482nm. The DOX loading capacity of MSNPs was 42μmol/g.

2.12. Confirmation of DOX loading inside the pores of MSNPs:

In order to confirm penetration of DOX molecules inside the pores of MSNPs, we soaked 100 mg of both mesoporous silica nanoparticles and nonporous dispersed silica nanoparticles in DOX solution of similar known absorbance for 24 hours to compare the loading of DOX inside or on the surface of nanoparticles. Following the centrifugation, the absorbance of remaining DOX concentration was measured again. The drug loading capacity of mesoporous and nonporous silica nanoparticles was calculated from difference in initial and left concentration of the DOX solution after soaking. The drug loading capacity dispersed nonporous silica nanoparticles was only 7.56 μmol/g as compared to drug loading capacity of mesoporous MSNPs 42μmol/g. It suggests that mostly DOX molecules penetrate inside the pores of mesoporous silica nanoparticles while only little adsorb on surface by weak bond interactions.

2.13. pH triggered release of doxorubicin:

10 mg of chitosan end-caped doxorubicin loaded mesoporous silica nanoparticles (DOX/ MSNPs) were dispersed in 2ml of each of four different pH (7.4, 6.0, 5.0, & 4.0) PBS solutions. Then different time intervals, the aliquots were obtained from the each suspension and the release of anticancer drug form the pores of MSNPs nanoparticles in PBS solution was measured through absorbance band by UV/Visible spectrometer at λmax. 482nm. Photographs of samples were taken after 24 hours.

2.14. Lysozyme triggered release of doxorubicin:

10 mg of chitosan end-capped DOX/MSNPs was dispersed in 2ml of each of two pH 7.4 PBS solutions samples; then the release of DOX in each suspension was observed till 5 hours. After 5 hours one sample was triggered with lysozyme by adding 10 mg of lysozyme while other sample was retained unchanged. Then again aliquots were taken from the each suspension at different time intervals and the release of doxorubicin from chitosan end-capped DOX/MSNPs in lysozyme containing PBS solution and without lysozyme PBS solution was observed via absorbance readout. Photographs were taken after 24 hours.

2.15. MTS assay for cellular viability:

To determine cytotoxicity of doxorubicin loaded chitosan end-capped MSNPs (DOX/MSNPs-NH_2-COOH-Chitosan) nanoparticles on cancer cells, a MTS cell viability assay was performed, which seems at the decrease of (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium (MTS) to formazan in viable cells. Chronic lymphocytic leukemia (CLL) cells were plated at 1×10^4 cells per well in a 96-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (*V/V*) fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2/95% (*V/V*) air. Then cells were treated with different doses (10-200 µg/ml) of DOX/MSNPs-NH_2-COOH-Chitosan and MSNPs-NH2-COOH at 37 ^{0}C for 24 h. After treatment with different doses for 24 h, CLL cells were incubated with MTS reagent for 30 minutes and cell viability was calculated from the absorbance readout at 490 nm. The absorbance of non-exposed cells to nanoparticles was used as a reference for calculating 100% cellular viability.

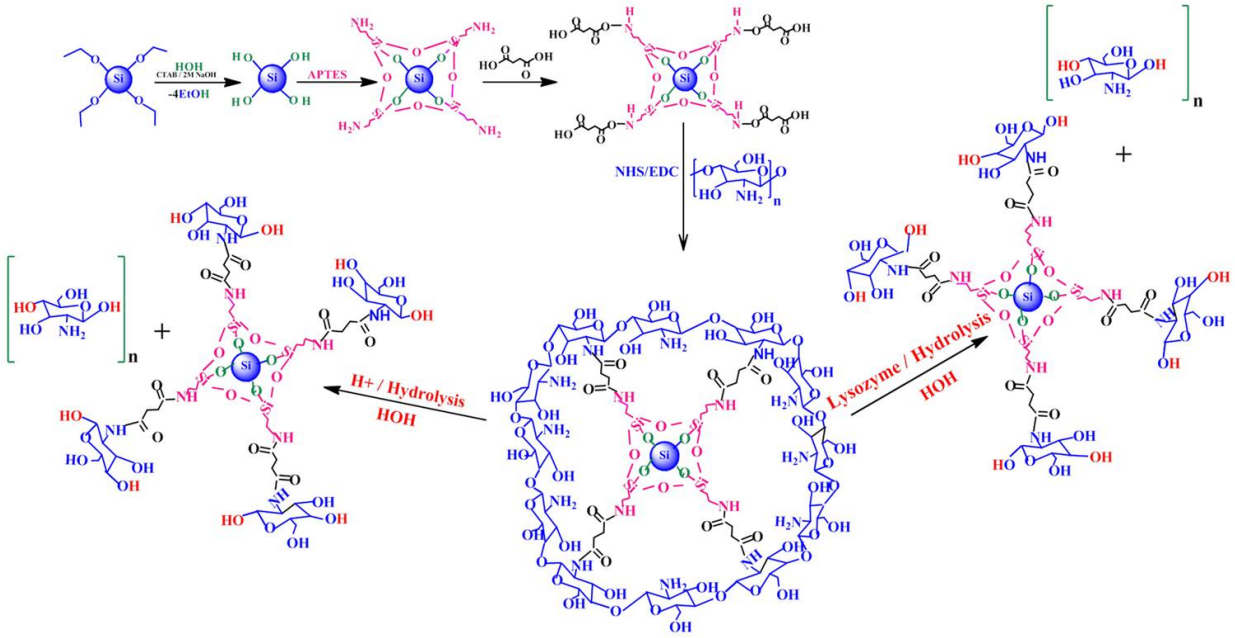

Figure S1: Stepwise bonding scheme of synthesis of mesoporous silica nanoparticles (MSNPs), APTES modified mesoporous silica nanoparticles (MSNPs-NH_2), Succinic anhydride modified MSNPs-NH_2 nanoparticles (MSNPs-NH_2-COOH, chitosan end-capped mesoporous silica nanoparticles (MSNPs-NH_2-COOH-chitosan), acid catalyzed hydrolysis and enzyme catalyzed hydrolysis of chitosan.

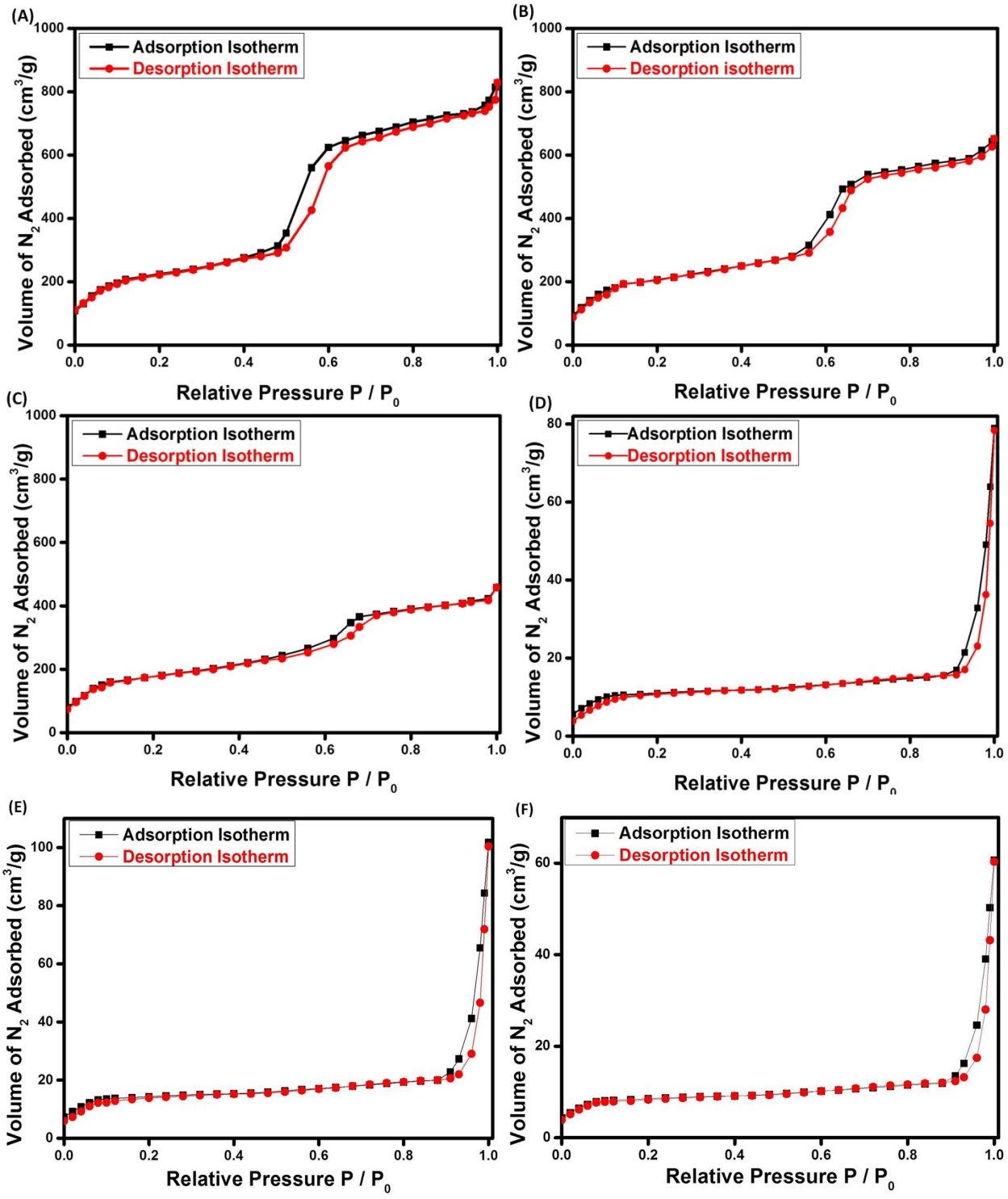

Figure S2: N_2-Adsorpion/Desorption Isotherms (A) MCM-41 MSNPs (B) MSNPs-NH_2 (C) MSNPs-NH_2-COOH (D) MSNPs-NH2-COOH-Chitosan (E) DOX/MSNPs-NH_2-COOH (F) DOX/MSNPs-NH_2-COOH-Chitosan

Synthesized Materials	BET surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Size (nm)
MCM-14 MSNPs	840	0.98	3.94
MSNPs-NH_2	753	0.86	3.45
MSNPs-NH_2-COOH	629	0.73	2.92
DOX/MSNPs-NH_2-COOH	453	0.16	0.69
MSNPs-NH_2-COOH-Chitosan	178		
DOX/MSNPs-NH_2-COOH-Chitosan	142		

Table S1: Table shows BET surface area of MSNPs, MSNPs-NH_2, MSNPs-NH_2-COOH DOX/MSNPs-NH_2-COOH, MSNPs-NH_2-COOH-chitosan and DOX/MSNPs-NH_2-COOH-chitosan and pore volume and pore size of synthesized MSNPs, MSNPs-NH_2, MSNPs-NH_2-COOH and DOX/MSNPs-NH_2-COOH, calculated from their respective N_2-adsorption/desorption Isotherms**.**

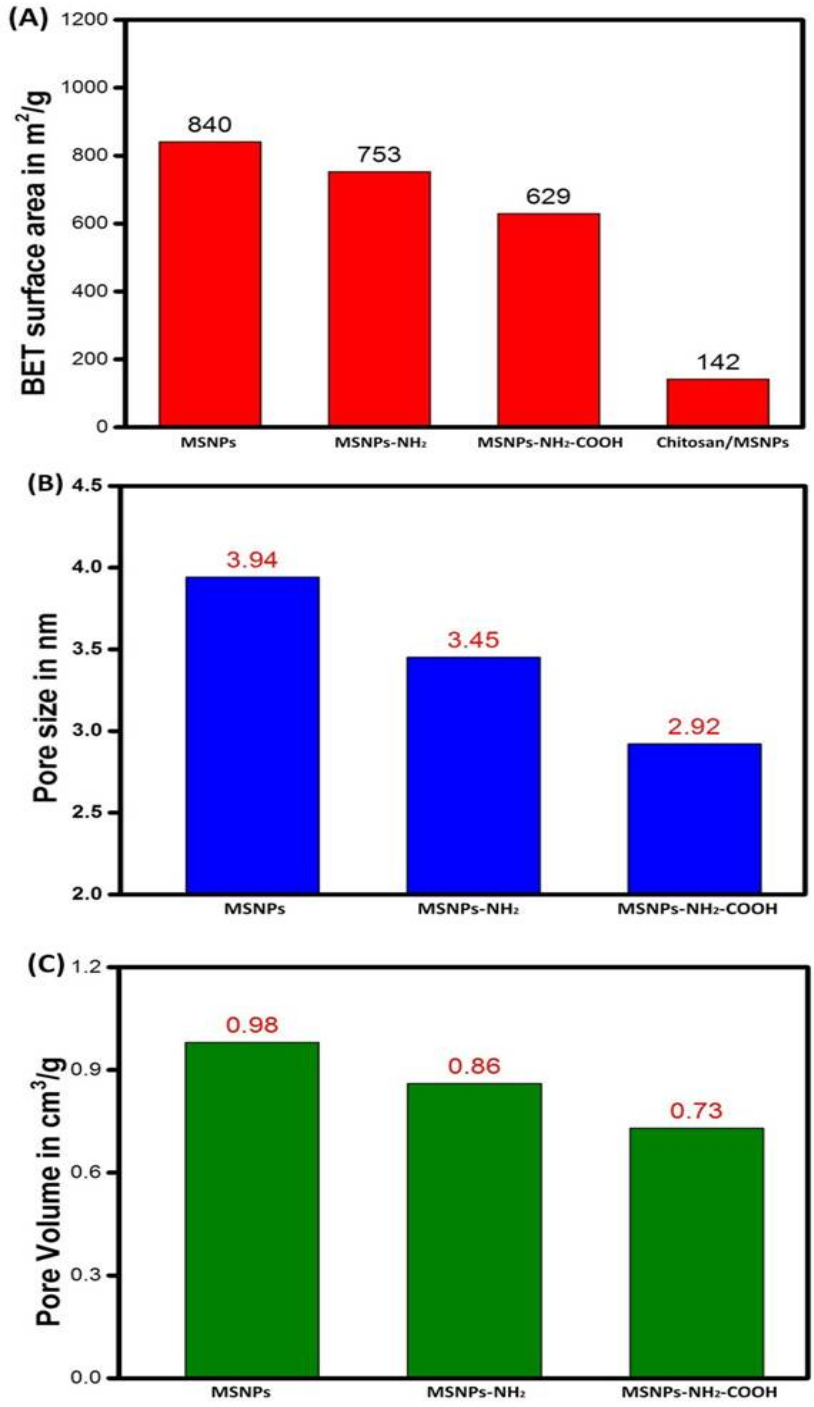

Figure S3: The surface area, pore size and pore volume distribution of synthesized nanoparticles. **(A)** The surface area distribution of MSNPs, MSNPs-NH_2, MSNPS-NH_2-COOH and chitosan end-capped MSNPS-NH_2-COOH **(B)** Pore size distribution of MSNPs, MSNPs-NH_2 and MSNPS-NH_2-COOH **(C)** Pore volume of MSNPs, MSNPs-NH_2 and MSNPS-NH_2-COOH.

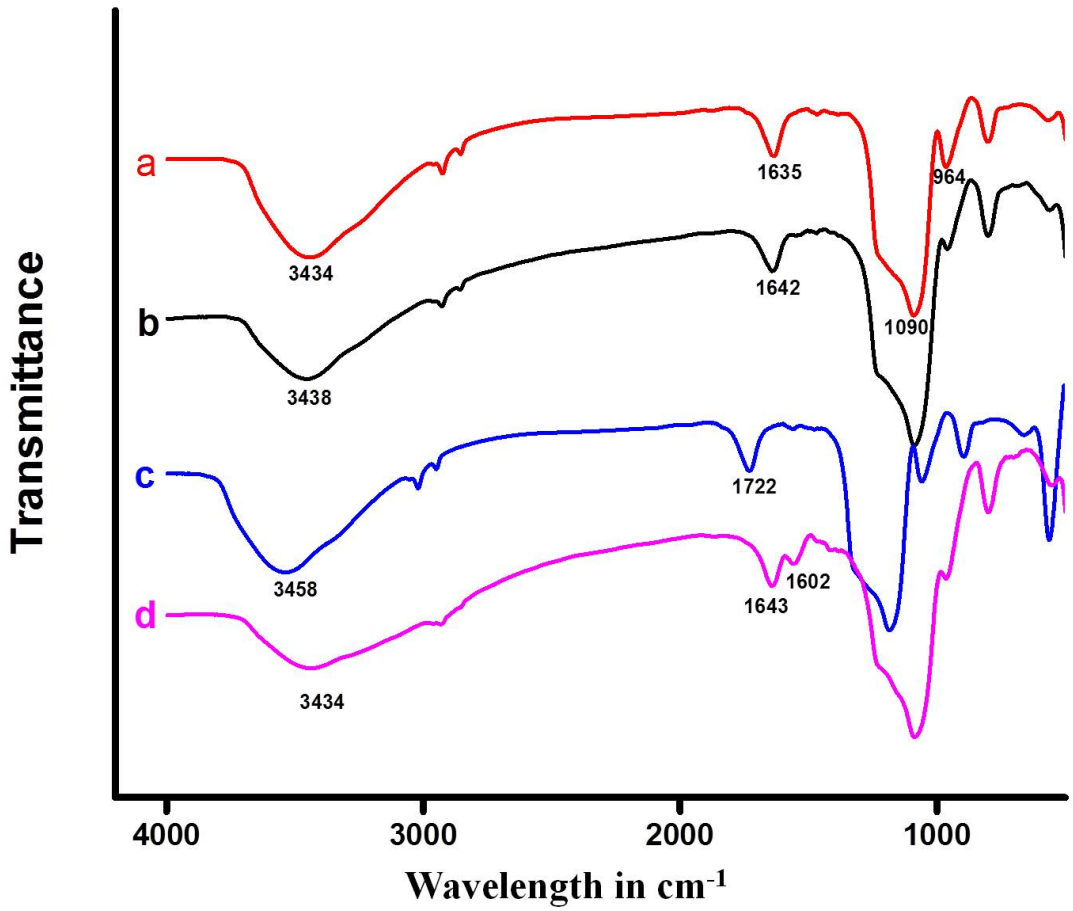

Figure S4*:* FTIR spectrum of **(a)** MSNPs nanoparticles **(b)** MSNPs-NH_2 nanoparticles **(c)** MSNPs-NH2-COOH nanoparticles **(d)** Chitosan end-capped MSNPs-NH2-COOH nanoparticles

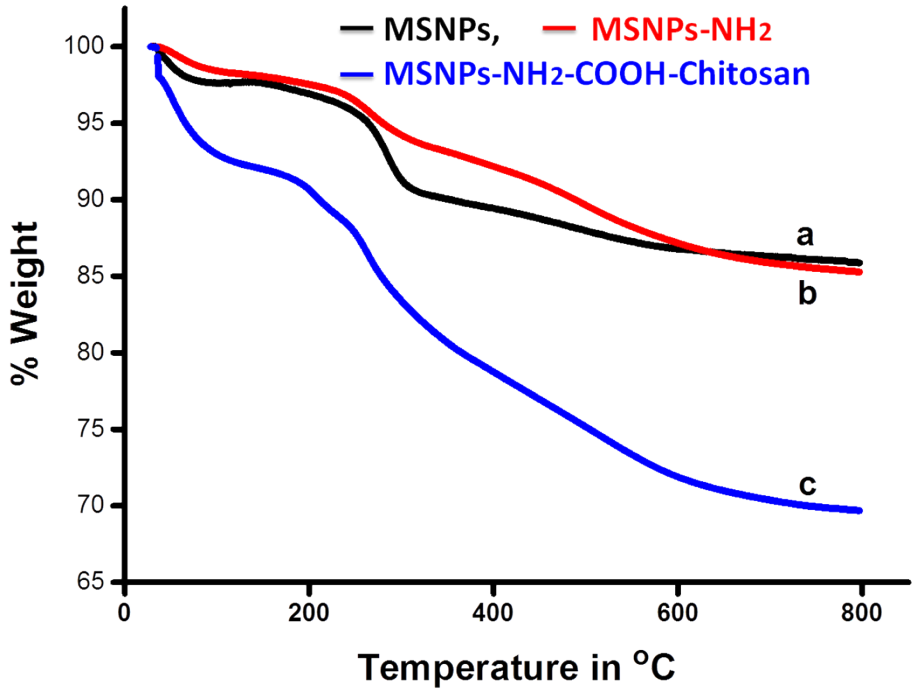

Figure S5: TGA curves of **(a)** mesoporous silica nanoparticles (MSNPs) **(b)** APTES modified mesoporous silica nanoparticles (MSNPs-NH_2) **(c)** Chitosan end-capped mesoporous silica nanoparticles (MSNPs-NH_2-COOH-Chitosan).

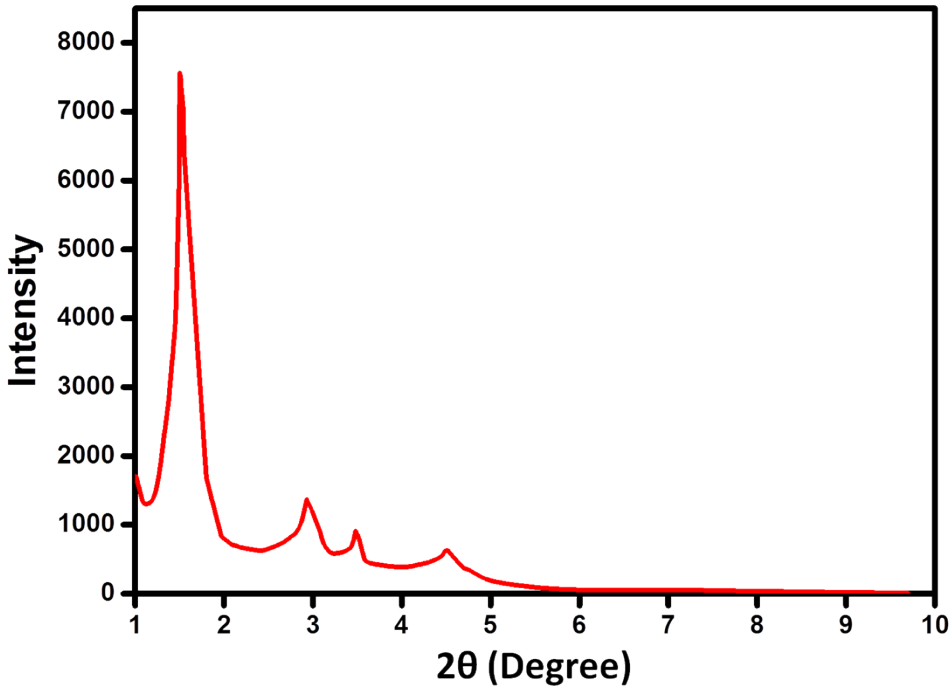

Figure S6: X-ray diffraction pattern of mesoporous silica nanoparticles (MSNPs), XRD pattern suggests that the synthesized nanoparticles were MCM-41 type MSNPs.

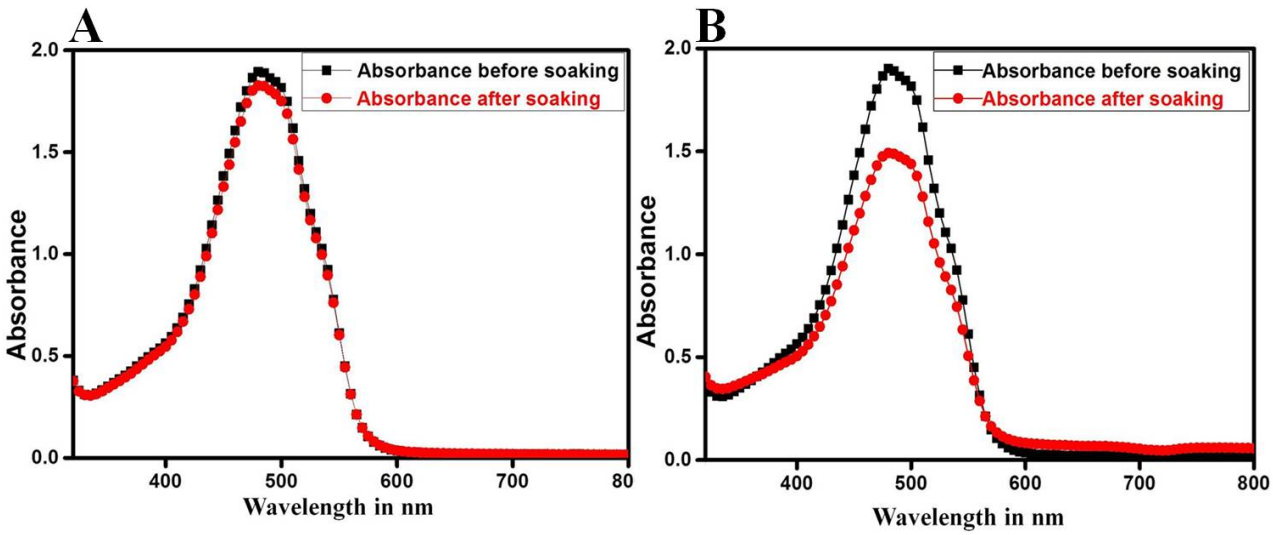

Figure S7: UV/Visible absorption spectra; (A) Absorption spectrum of DOX before (black —) and after (red—) by soaking nonporous silica nanoparticles, it confirms that very less amount of DOX was adsorbed on the surface of silica nanoparticles by weak bond interactions. The DOX adsorbed on surface was 7.56 µmol/g (B) Absorption spectrum of DOX before (black —) and after (red—) by soaking mesoporous silica nanoparticles, it confirms that DOX molecules penetrate inside the pores of MSNPs. DOX absorbed inside the pores of MSNPs was 42µmol/g.

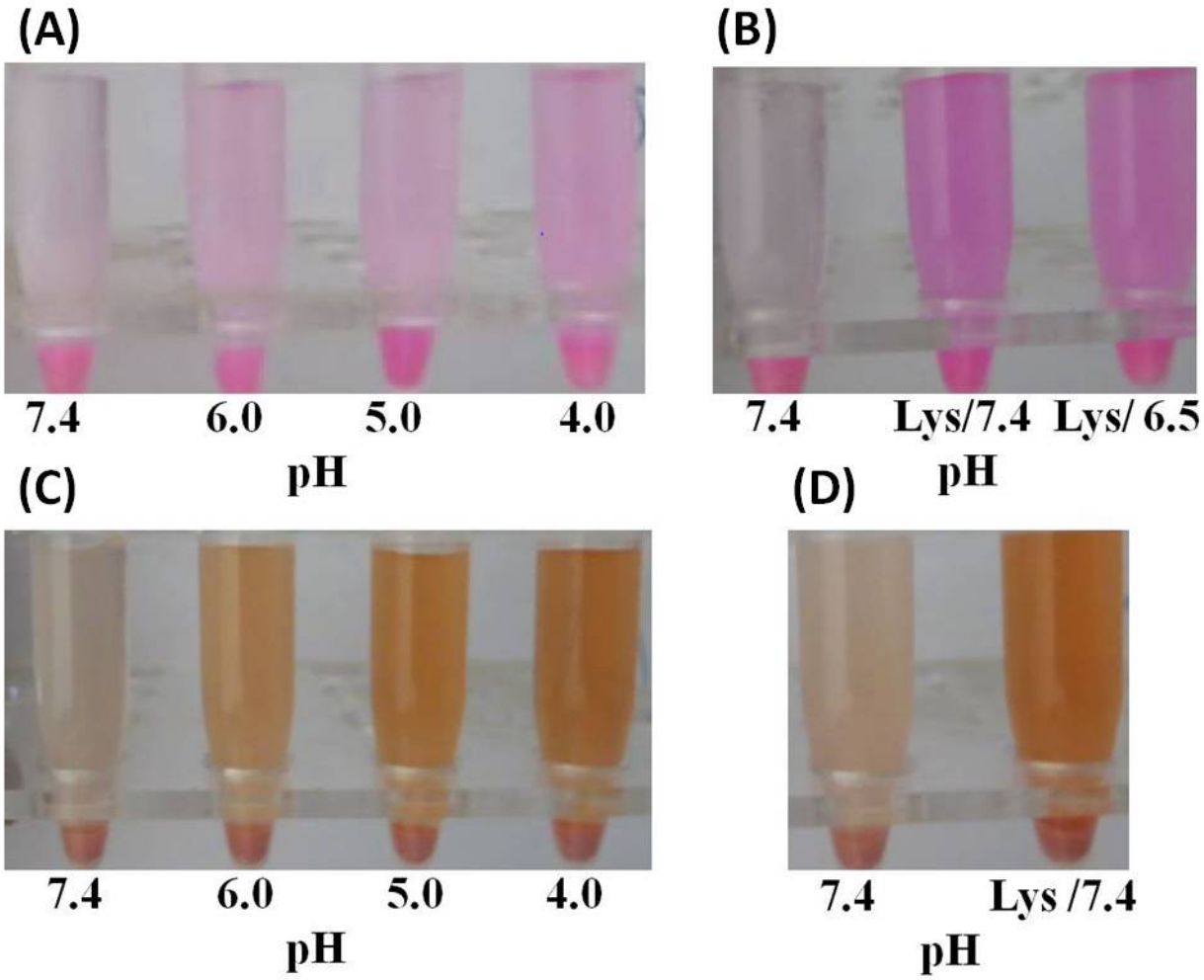

Figure S8: Photographs of cargo release for the chitosan end-capped MSNPs after 24 hours; **(A)** Rhodamine B release from chitosan end-capped RhB/MSNPs in different pH PBS solutions **(B)** Rhodamine B release for RhB/MSNPs in pH 7.4 PBS solution and in lysozyme triggered 7.4 and pH 6.5 PBS solutions **(C)** DOX release from DOX/MSNPs in different pH PBS solutions **(D)** DOX release from DOX/MSNPs in pH 7.4 PBS solution and in pH 7.4 solution triggered with lysozyme after five hours from the starting time.

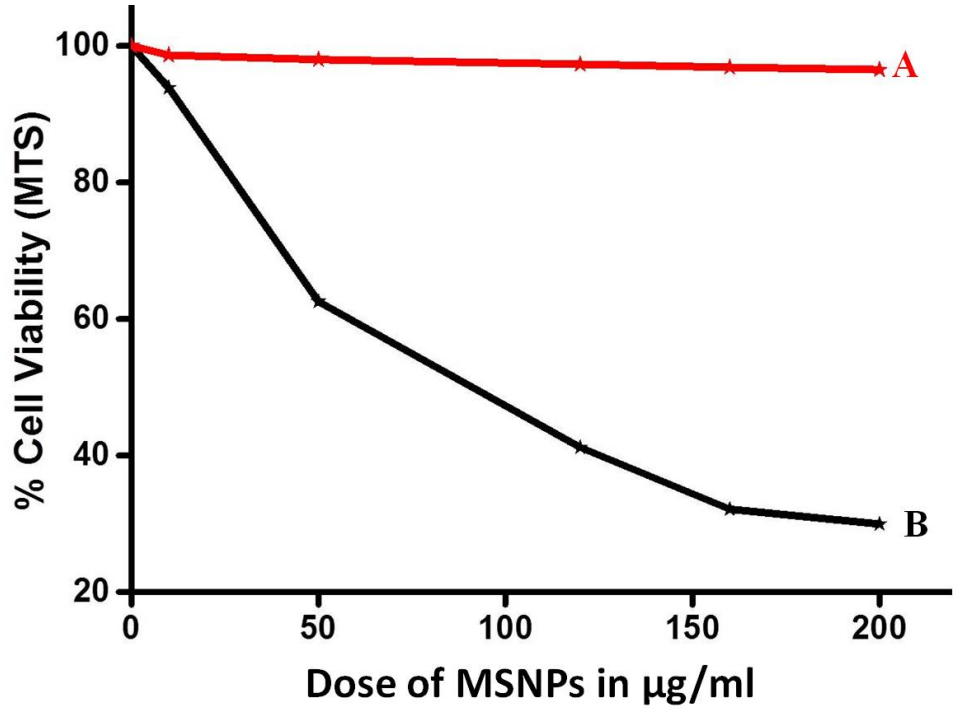

Figure S9: Cell viability determination CLL Cells by the MTS assay; cell viability of CLL cells was determined after treatment with different doses of **(A)** MSNPs-NH_2-COOH and **(B)** DOX/MSNPs-NH_2-COOH-chitosan for 24 hours at 37^0C. Following treatment, cells were incubated with the MTS reagent for 30 min and absorbance was measured at 490 nm while absorbance of non-exposed cells to nanoparticles was used as reference for 100% viability.

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