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Surface modified silica nanoparticles for synchronous magnetic resonance imaging and drug delivery applications

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This study reports a simple and versatile method of tailoring surface modified silica nanoparticles (SMS NPs) *via* co-condensation technique. The particles were loaded with gadolinium oxide and an anticancer drug, colchicine, utilizing the aqueous core of the reverse micelle as "nano" host reactors. The surface of the silica NPs was modified with 3-aminopropyltriethoxysilane. Surface modification entails higher content of the drug and allows it to get released in a sustained manner. The particles exhibit spherical morphology with an average diameter of 60 nm as measured by TEM. Gadolinium oxide is paramagnetic in nature as observed from the NMR line broadening effect on proton spectrum of the surrounding water. Preliminary *in vitro* experiments on MCF-7 reveal good potential of these SMS NPs for cancer therapy. It is expected that these highly versatile multifunctional silica NPs could potentially be employed for simultaneous non-invasive imaging and therapeutic purposes.

1. Introduction

The ongoing research in the area of nanoscience and nanotechnology has fuelled a boom both among the academic and the scientific communities. One of the emerging areas for the biomedical applications of nanotechnology is in drug delivery systems for therapeutic purposes. Although conventional treatment methods such as chemotherapy and radiation technique have yielded some advantages over the past decades, cancer therapy is still far from optimal. The main challenges in the search of effective carrier in most drug delivery systems include *in vivo* stability, rapid clearance from the circulation, sustained and targeted delivery to site of action, plasma fluctuations of drugs which either fall below the minimum effective concentration or exceed the safe therapeutic level, solubility, biocompatibility, shelf life, therapeutic effectiveness and side effects.[1–4] Nanoscale materials alleviate the major issues accompanied with drug delivery system to a maximum extent, offering tremendous potential and a promising approach to deliver large payload of therapeutic agents into targeted tissues or cells. These nanomaterials have been actively developed primarily for efficient application in cancer therapy by modulating the characteristic behaviours of the loaded entity.[5–7] Among the inorganic based materials, silica NPs represent one of the excellent and versatile platforms for drug delivery owing to their straightforward synthesis as well as due to several attractive features exhibited by them. The ability to functionalize the surface of mesoporous silica based nanocarriers with stimuli responsive groups, other NPs, proteins, and polymer that work as caps and gatekeepers for controlled release of various cargos[8–10] has generated huge excitement. In particular, amino-functionalized silica base NPs can be used to target to specific cell types by conjugating a cell-line-specific ligand or antibody in a relatively easy way.[11] Recently, gadolinium based materials have been widely employed as positive contrast agents. This rare earth element is associated with seven unpaired electrons on its valence orbital, leading to a high magnetic moment (7.94 μ_B). Moreover, the electron spin relaxation time of Gd is long (1×10^{-9} to 1×10^{-8} s), maximizing dipole–dipole interactions of electrons and hydrogen protons (^{1}H) in the proximity of the contrast agent. Such interactions increase the proton relaxation time resulting in signal enhancement effects in MRI.[12,13] On the other hand, colchicine has the ability to inhibit polymerization of microtubules by binding to tubulin which is one of the main constituents of microtubules. During mitosis availability of tubulin is essential and therefore colchicine effectively functions as a "mitotic poison" or spindle poison.[14] One of the defining characteristics of cancer cells is to significantly increase rate of mitosis which indicates that cancer cells are more vulnerable to colchicine poisoning than are normal cells. Cauda V *et al.*[15] have recently shown the effectiveness of colchicine drug by delivering into HuH7 liver cancer cells in the form of colchicine loaded lipid bilayer coated with mesoporous silica NPs. Therefore, an amalgamation of different nanostructural materials will enable the development of multifunctional nanomedical platforms for

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multimodal imaging or simultaneous diagnosis and therapy techniques aiming towards clinical productivity.

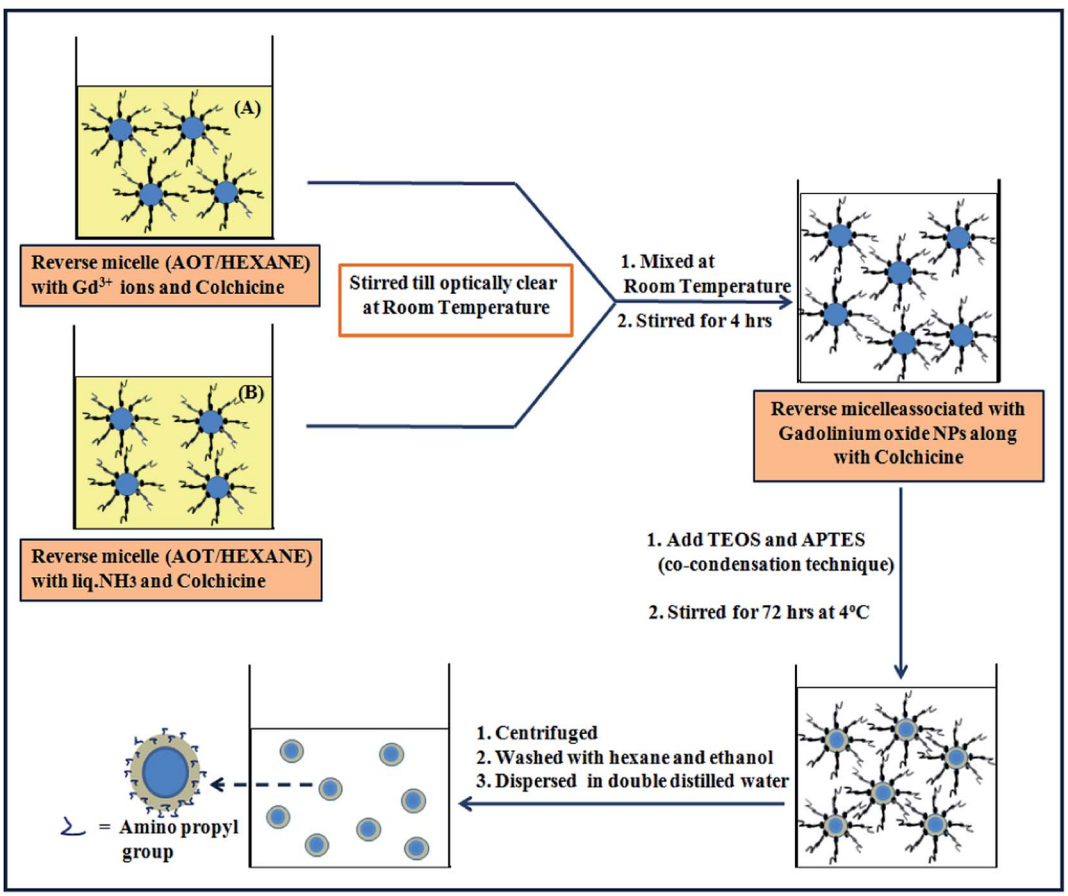
In the light of the above information, we have addressed the development of a simple route for the fabrication of surface modified silica (SMS) NPs for synchronous magnetic resonance imaging (MRI) and versatile drug delivery applications as our main endeavour in this particular report. Surface modification of particles was done *via* co-condensation technique utilizing the aqueous core of the reverse micelle as "nano" host reactors co-encapsulating gadolinium oxide NPs and colchicine drug. Subsequently, cytotoxic effect of these ensuing NPs on MCF-7 breast cancer cells was successfully evaluated. Designing of such multifunctional delivery system with potential release properties is still of paramount importance and may offer a new dimension to biomedical applications.

2. Results and discussion

The overall synthetic schemes employed for the fabrication of multifunctional composite SMS NPs *via* co-condensation technique has been illustrated in Scheme 1.

Gadolinium oxide NPs and colchicine drug were co-encapsulated within the nanosized silica particles. In this exploration, co-condensation method was chosen as preparation technique for surface modification not only because of its simplicity but also offers a homogeneous coverage of functional group, large amount of drug loading and enhanced dispersion minimizing particles aggregation[16–20] which we have also observed. Since no harsh chemicals are employed during this co-condensation synthesis technique, the amine groups in the silica layer might not be hampered. The method involved preparation of silica coating at low temperature in order to control their morphology. Fig. 1(a) illustrates the UV-vis spectra of free colchicine and the silica particles loaded with the anticancer drug.

The absorption spectrum of the aqueous solution of free colchicine shows three characteristic absorption peaks at 201 nm, 246 nm and 353 nm. The two characteristic absorption peaks of colchicine at 246 nm and 353 nm is also exhibited by the spectra of the synthesized SMS NPs co-encapsulating gadolinium oxide and colchicine which depicts the presence of colchicine drug in the fabricated SMS NPs. On the other hand, colchicine in the presence of an aliquot H_2SO_4 hydrolyses to give colchiceine,[21,22] which exhibits prominent absorption peaks in ultra violet region at 200 nm, 253 nm and 378 nm as presented in Fig. 1(b). Such, peaks were also noted with the silica particles when dispersed in an aliquot of 30% H_2SO_4. It could be seen that the UV-vis spectra coincided very well, but absorption intensity corresponding to the loaded particles was



Scheme 1 Diagrammatic representation for the synthesis of SMS NPs co-encapsulating gadolinium oxide and colchicine drug using water-in-oil microemulsion.

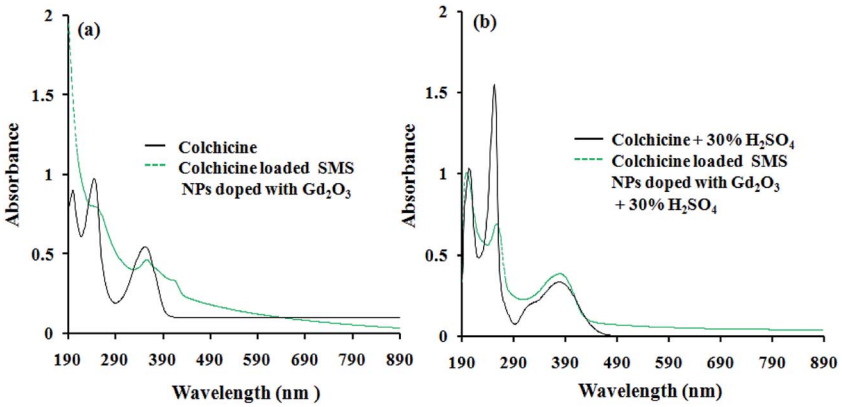


Fig. 1 UV-vis spectra of (a) free colchicine drug and SMS NPs co-encapsulating gadolinium oxide and colchicine in aqueous solution (b) free colchiceine solution and colchiceine obtained from the drug loaded silica NPs.

more apparently decreased. This observation further substantiates the above argument regarding the inclusion of colchicine drug in the so-synthesized SMS NPs doped with gadolinium oxide. The reaction of colchicine drug in the presence of an aliquot H_2SO_4 is displayed in Fig. 2.

Fig. 3(a) highlights the typical TEM micrograph of the SMS NPs co-encapsulating gadolinium oxide and colchicine drug. The particles have a narrow size distribution with uniform, discrete spherical morphology. The average diameter of the SMS NPs is about 60 nm while the HRTEM image of a particle shown in the inset displays a core shell like structure with a darker contrast at the central portions than at the periphery of the particle revealing the incorporation of a solid gadolinium oxide NPs within it.

SAED pattern shown in Fig. 3(b) indicates the particles are weakly crystalline in nature probably due to the coating of modified silica layer over the surface of gadolinium oxide NPs. The modified silica layer might have permitted the interaction of the electron beam with the encapsulated gadolinium oxide expressing its weakly crystalline nature. Furthermore, EDAX provides localized elemental information in case of multi-component nanomaterials. Subsequently, from the data obtained by EDAX analysis, shown in Fig. 4, we can precisely conclude the presence of gadolinium along with silica in the reported sample.

Fig. 5 demonstrates the XRD pattern of the particles. The diffraction pattern of the particles exhibits three sharp peaks at 18.98°, 29.28° and 33.86° corresponding to (211), (222) and (400) planes of cubic gadolinium oxide.[23,24] Therefore, XRD analysis further furnishes a strong evidence of the crystalline

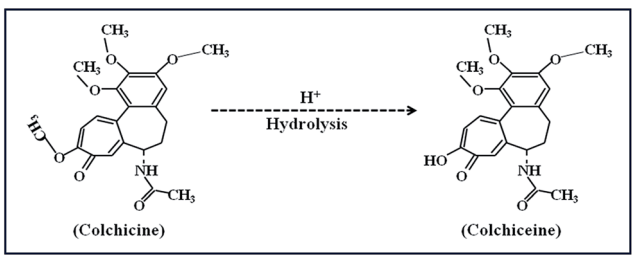


Fig. 2 Acid hydrolysis reaction of colchicine drug.

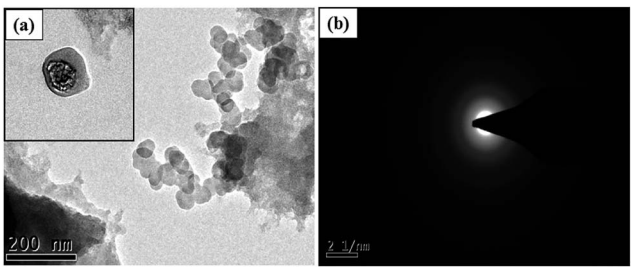


Fig. 3 (a) TEM picture of SMS NPs co-encapsulating colchicine and gadolinium oxide with an average diameter of 60 nm; inset shows HRTEM of the particle (b) SAED pattern of the synthesized particles.

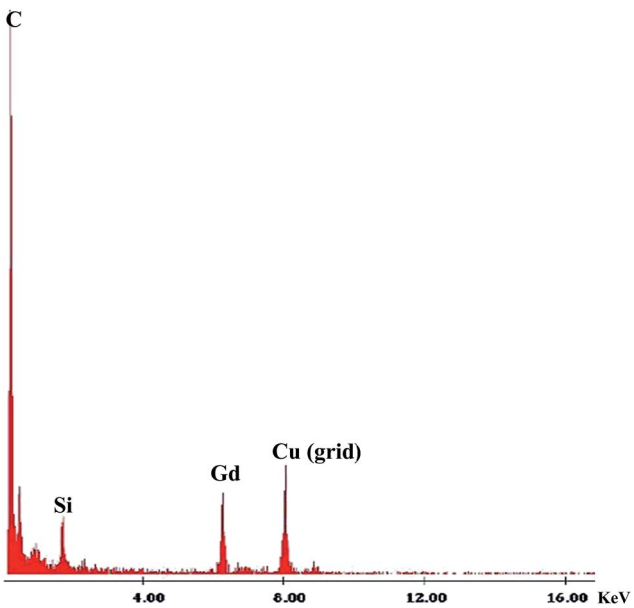


Fig. 4 Energy dispersive spectroscopy (EDS/EDAX) of silica NPs encapsulating gadolinium oxide NPs.

nature due to the inclusion of gadolinium oxide in the fabricated SMS NPs system.

The FT-IR spectrum of the SMS NPs, presented in Fig. 6, exhibit a broad peak at 1108 cm^{-1} and a relatively weak peak at

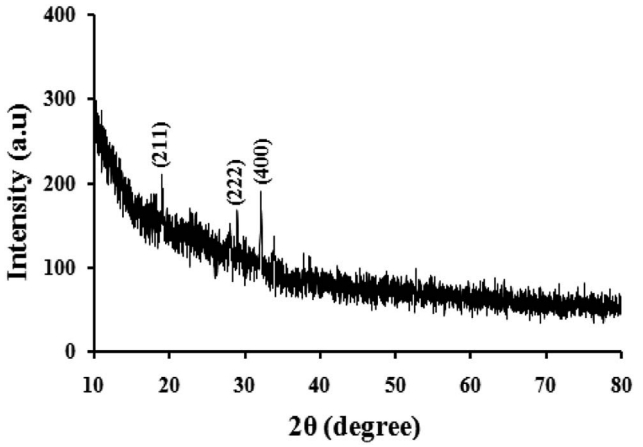


Fig. 5 XRD pattern of SMS NPs co-encapsulating gadolinium oxide and colchicine.

462 cm^{-1} which corresponds to Si–O–Si asymmetric stretching vibration and Si–O stretching respectively from the SiO_2 matrix.

The presence of amine group in the synthesized particles can easily be recognized from the peaks at 1592 cm^{-1} corresponding to the characteristic NH_2 asymmetric bending and 3436 cm^{-1} which has been assigned to –N–H and O–H stretching vibration. The stretching vibration of the methylene ($-CH_2-$) group obtained from the hydrolysis and condensation of $-Si-(CH_2)_2-NH_2$ of APTES is assigned to the peak at 2936 cm^{-1}.[25,26] This information validates successful grafting of the prepared silica particles. Additionally, the peak around 546 cm^{-1} is attributed to the stretching vibrations of cubic phase Gd–O bond which further confirms the existence of Gd_2O_3 in the composite nanodimensional SMS particles.[27,28] Furthermore, ^{1}H-NMR studies of SMS NPs with and without gadolinium oxide doping were carried out in D_2O using 400 MHz spectrometer (JNM-ECX-400P, JEOL, Tokyo, Japan) and are shown in Fig. 7. The unpaired electrons of gadolinium ion in gadolinium oxide is a good example of slow electron relaxation which results in line broadening effect of the surrounding water proton as displayed in Fig. 7(b). But such broadening effect of the heavy water protons in proximity with SMS NPs in absence of gadolinium oxide was not observed as indicated in Fig. 7(a). In this case only sharp normal peak of the water proton is seen due to the absence of gadolinium which exerts paramagnetic effect on the water proton resonance.[13] Besides, TGA was used to examine the thermal properties of the prepared silica particles. TGA studies of the colchicine drug and the SMS NPs co-encapsulating gadolinium oxide and colchicine were highlighted in Fig. 8. The thermograph shows a gradual decrease in weight loss initially in both the curves as the temperature increases which may be accounted to the release of the adsorbed moisture, slow decomposition of colchicine drug and dehydroxylation of silanol in case of silica particles.[29] Unlike the TGA curve of SMS NPs co-encapsulating gadolinium oxide and colchicine drug, TGA curve of free colchicine shows a sudden decrease in its weight at around 280 °C. Such rapid changes in the weight with rise in temperature were not observed in case of the nanodimensional silica particles co-encapsulating gadolinium oxide and colchicine. Thus, silica shell provides a protective environment of the materials within its core hereby increasing their thermal stability.

From the concentration of the unloaded drug we have calculated the entrapment efficiency indirectly using the following equation

$$\text{Entrapment efficiency}(E\%) = \frac{\text{Total colchicine added at the time of synthesis} - \text{Total colchicine present outside the particles}}{\text{Total colchicine added at the time of synthesis}} \times 100$$

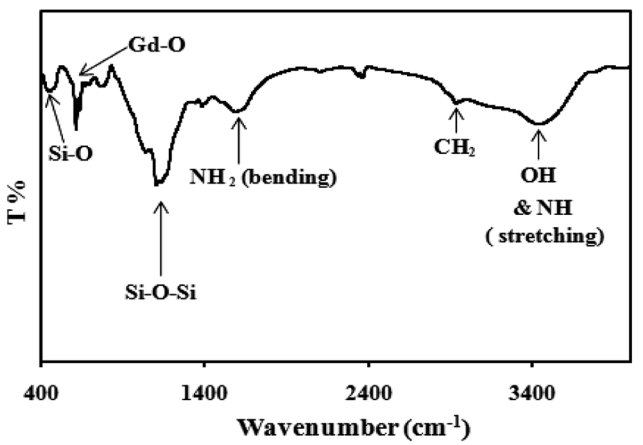


Fig. 6 FT-IR spectrum of the SMS NPs.

The entrapment efficiency was found to be about 72% as determined spectrophotometrically from the absorption of colchicine in different conditions. In view of the afore mentioned discussion, drug release profile of the SMS NPs co-encapsulating gadolinium oxide and colchicine was estimated by dialysis method using UV-vis spectrophotometer in 0.1 M phosphate buffer solution of pH 7.4 at room temperature. The amount of the anticancer drug, colchicine, released from the particles *via* diffusion at different point of time was calculated spectrophotometrically at 353 nm, a prominent characteristic peak of colchicine, and the percentage of the drug release was plotted against the duration taken as displayed in Fig. 9. From the release profile it can be noted that after 24 h and 48 h of dialysis about 40% and 70% respectively of the loaded drug gets released from the SMS NPs. Such release kinetics may have been controlled by extended interaction between the functional group(s) of the drug and the introduced amine on the silica layer *via* H-bonding and/or Vander Waals interaction.[18,30] Generally, functionalized group(s) *via* co-condensation technique also anchored covalently to the pore walls as direct

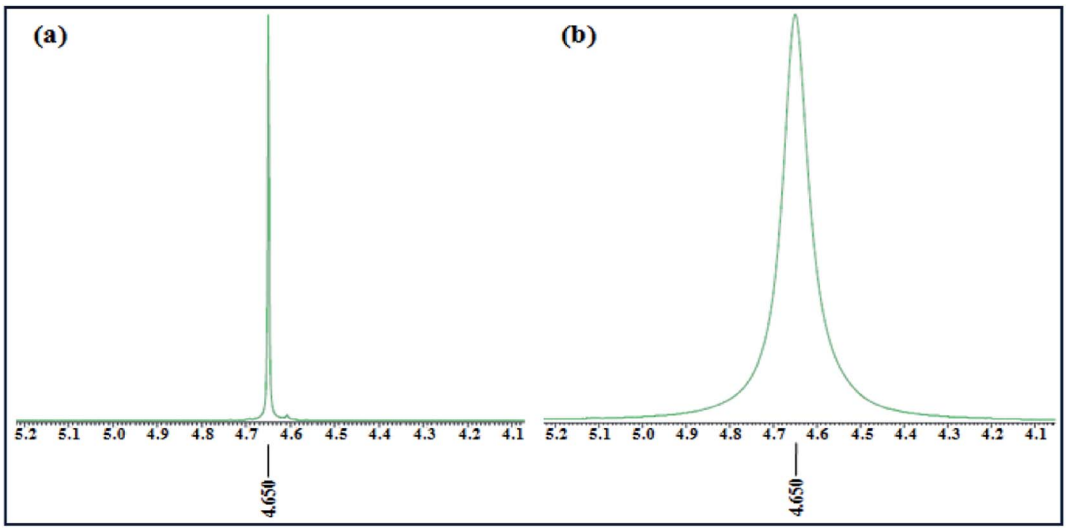


Fig. 7 ^{1}H-NMR of SMS NPs (a) without and (b) with gadolinium oxide doping.

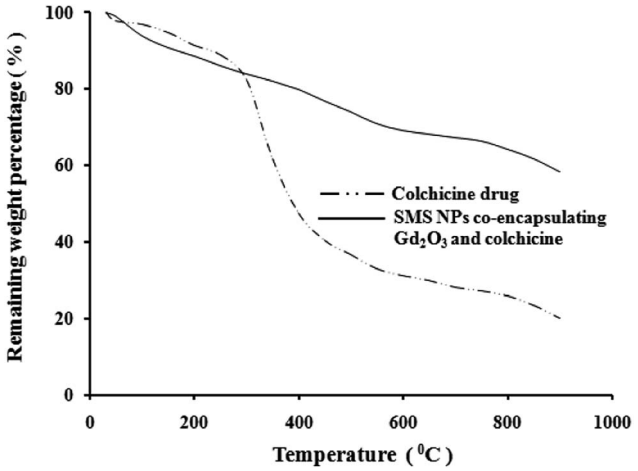


Fig. 8 Thermogravimetric analysis (TGA) of colchicine drug and SMS NPs co-encapsulating gadolinium oxide and colchicine.

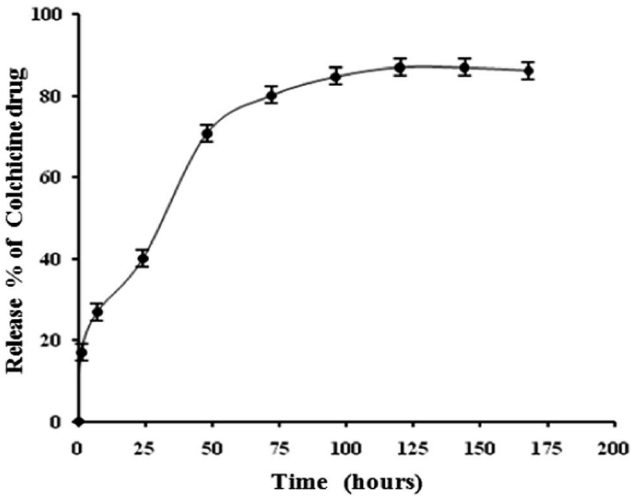


Fig. 9 Release kinetics of colchicine in phosphate buffer solution of pH 7.4 at room temperature.

components of the silica matrix.[31,32] Therefore, surface modification of silica particles not only results in entrapping higher content of drug but also plays a critical role in the release of the drug from the particle itself. To verify the feasibility of using SMS NPs co-encapsulating gadolinium oxide and colchicine for cancer therapy, MTT assay of the particles were investigated in MCF-7 breast cancer cells. The SMS NPs co-encapsulating gadolinium oxide and colchicine drug show good efficiency against the breast cancer cells, as presented in Fig. 10. Colchicine released from the silica particles is cytotoxic on MCF-7 cells. The loaded SMS NPs must have been uptaken by the cancer cells and the drug released intracellularly by diffusion process. However, a slight decrease in the activity of the colchicine loaded in SMS NPs is observed as compared to the free drug. The NPs having a void core do not exhibit any toxicity. This may be attributed to the incomplete release of the drug from the silica particles during the period of MTT assay as almost 70% of the loaded drug got released during 48 hours as we observed. An important observation during this experiment was that void SMS NPs (control 1) exhibits no toxicity on the cancer cells. A comprehensive cytotoxicity assay including a range of cancerous cell lines at different exposure conditions should be monitored to elucidate the complete anticancer behavior of these NPs. However, from this preliminary *in vitro* investigation, we can, conclude that SMS NPs are potentially safe vehicle and have the characteristics for their utilization in *in vivo* experiments for further studies.

3. Experimental

3.1. Materials

Sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate (AOT) was purchased from Acros Organics; liquid ammonia (25%) and

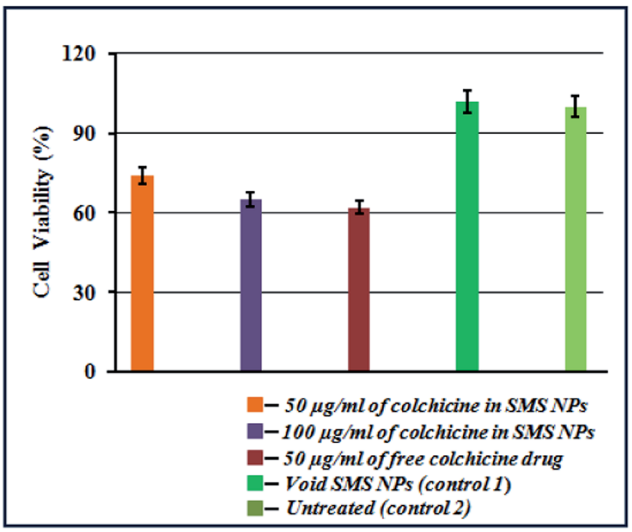


Fig. 10 MTT assay on MCF-7 cell line.

sulphuric acid were obtained from Rankem. Gadolinium(III) nitrate pentahydrate ($Gd(NO_3)_3 \cdot 5H_2O$) was procured from Central drug House (P) Ltd. India and *n*-hexane from S.D. Fine Chem. Ltd. Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES) and dialysis tubing cellulose membranes (12 kD) were purchased from Sigma Aldrich. While, absolute ethanol and deutererium oxide (D_2O) were obtained from Merck and Alfa Aesar respectively. Colchicine, potassium phosphate monobasic dihydrate (KH_2PO_4) and potassium phosphate dibasic dehydrate (K_2HPO_4) were all procured from SRL India Ltd. Thiazolyl blue tetrazolinium blue (MTT), trypsin, ethylenediaminetetraacetic acid (EDTA), fetal bovine serum (FBS) culture materials were all purchased from Gibco USA. However, human breast cancer cell line MCF-7 was obtained as a gift from All India Institute of Medical Science (AIIMS), Delhi, India and maintained as per instructions. All the reagents employed were of AR grade and used without further purification. Double distilled water (DDW) was utilized for preparing all the solutions.

3.2. Synthesis of surface modified silica (SMS) NPs co-encapsulating gadolinium oxide and colchicine drug

The preparation of the particles was accomplished by preparing microemulsion A which was generated by dissolving 150 µl of 5 mg ml^{-1} of colchicine drug solution along with 200 µl of 0.1 M gadolinium nitrate solution in 25 ml of 0.1 M AOT in hexane solution. Similarly, microemulsion B was prepared by dissolving 150 µl of 5 mg ml^{-1} of colchicine drug along with 200 µl of 2 M ammonia solution in 25 ml of 0.1 M AOT in hexane solution. A calculated volume (100 µl) of water was added to both the microemulsion solutions A and B to maintain the desired W_0 value of 10. The two microemulsions were stirred till they became optically clear and then miroemulsion B was added to microemulsion A in a drop-wise manner with constant stirring at room temperature. After complete transfer, the resultant solution was stirred further for another 4 h at room temperature. The above reverse micellar solution containing colchicine loaded gadolinium oxide NPs was maintained at low temperature of 4 °C and 50 µl each of neat TEOS and APTES were added simultaneously. The solution was stirred further for 72 h at low temperature of 4 °C. The particles were then extracted and washed with hexane and ethanol four times. Finally, the ensuing particles were dispersed in 1 ml of DDW.

3.3. Characterization of the tailored nanosized modified silica particles

3.3.1. Ultraviolet-visible spectrum. All UV-vis spectra were recorded on Shimadzu-1601 UV-vis spectrophotometer fitted with a constant temperature cell holder. The temperature of the cell holder was maintained constant by circulating water around it by a water circulator from Haake instrument. The absorption spectra were recorded by taking the aqueous dispersion of the samples and scanned in the range of 190–890 nm.

3.3.2. High resolution transmission electron microscopy (HRTEM), selected area electron diffraction (SAED) and energy dispersive spectroscopy (EDAX). TEM, SAED and EDAX patterns of the tailored particles were taken with TECNAIG^2-30 U TWIN instrument operating at 300 kV. After preparation, the NPs were centrifuged at 9000 rpm for 10 min and re-dispersed in DDW *via* sonication for 3–4 min. A drop of dilute solution was put on the copper grid and the grid was dried under ambient conditions. After complete drying of the grid TEM, SAED and EDAX images of the particles were taken.

3.3.3. X-ray diffraction (XRD) analysis. XRD patterns of the synthesised SMS NPs co-encapsulating gadolinium oxide and colchicine drug were taken after drying the particles completely at room temperature. X-ray diffraction analysis of the particles was carried out on Rigaku miniflex desktop XRD instrument and scanned in the 2θ range of 10–80°. Dried lyophilized powders were employed to carry out the diffraction experiment.

3.3.4. Fourier transform infra red (FT-IR) spectroscopy. The particles were dialysed, washed thoroughly with DDW and dried. FT-IR spectra of SMS NPs were recorded on IR-Perkin Elmer, FT-IR system, Spectrum BX FTIR in the range 400 to 4000 cm^{-1}. A sufficient amount of the dried sample was mixed with dried KBr and filled in a cup. A pressure was applied to form KBr pallet which was then utilized for IR investigation at room temperature.

3.3.5. Thermo gravimetric analysis (TGA). The sample was monitored with Perkin Elmer DTA/TGA/DSC instrument by taking an adequate amount of nanoscale SMS particles on the sample holder of the instrument in nitrogen atmosphere. Dried lyophilized powder sample was utilized for the analysis. The change in weight of the subjected materials with respect to temperature in the range 0–1000 °C was carried out.

3.3.6. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR). The ^{1}H-NMR of SMS NPs with and without gadolinium oxide doped were recorded using 400 MHz spectrometer (JNM-ECX-400P, JEOL, Tokyo, Japan) in D_2O as solvent. The particles after preparation were washed properly with hexane and ethanol; dialysed for 24 hours in DDW to remove unwanted materials and then finally lypholized to obtain their dried powder form.

3.4. Estimation of entrapment efficiency

The entrapment efficiency of SMS NPs co-encapsulating gadolinium oxide and colchicines drug was assayed indirectly by employing UV-vis spectrophotometer. The particles after the preparation in reverse micelles were separated and collected through centrifugation at 9000 rpm for 10 minutes. Amount of the colchicines drug present in the supernatant liquid after separation *via* centrifugation was determined spectrophotometrically at wavelength, $\lambda = 353$ nm, a prominent peak of colchicine drug.

3.5. Drug release kinetics

For *in vitro* drug release, 2 ml of phosphate buffer solution (PBS) of pH 7.4 containing 9 mg of the drug loaded SMS NPs doped with gadolinium oxide was transferred into a dialysis bag with and then placed into 70 ml of PBS with gentle stirring at room temperature. At predetermined time intervals, 3 ml aliquot of solution outside the dialysis bag was withdrawn and the amount of colchicine was estimated by measuring the absorption at 353 nm. After the measurement, whole of the solution was transferred back in the assembly gingerly. The percentage of the drug release was plotted against the duration taken.

3.6. *In vitro* cytotoxicity through MTT assay

The cytotoxicity of free colchicine, as an anticancer drug, and SMS NPs co-encapsulating gadolinium oxide and colchicine drug were studied *via* MTT assay on MCF-7 cell line. MCF-7 cells were incubated in Eagle's MEM, supplemented with 10% FBS, 1% penicillin. When the cells were 90% confluent they were detached using trypsin–EDTA solution. The trypsinised cells were added to complete media in 15 ml falcon tubes and centrifuged at 500 rpm for 5 minutes. The supernatant was discarded and the cells re-suspended in 1 ml of fresh complete media. 1×10^4 cells were added to each well and incubated overnight at 37 °C and 5% CO_2. Following optimum cell growth, 20 μl of dialysed samples and void NPs (control 1), as well as free colchicine were added to 12 test wells and incubated for 6 hours. Control wells without NPs (control 2) were maintained alongside. After a complete incubation time of 6 hours, 50 μl of media with the NPs was removed, leaving behind only cells that were adherent to the plate surface. 50 μl of fresh media and 10 μl of MTT solution was added to each well and the plates incubated at 37 °C and 5% CO_2 for 24 hours. Absorbance of the media was measured at 550 nm and the cell viability calculated in comparison to the absorbance of the control cells which was considered to be 100% viable.

4. Conclusions

We have developed a simple, less sophisticated and versatile approach for the fabrication of amino SMS NPs co-encapsulating gadolinium oxide and an anticancer drug, colchicine, for synchronous magnetic resonance imaging and as drug delivery vehicles. The SMS particles were spherical in shape and have a narrow size distribution of 60 nm. Moreover, the so-synthesized particles have good stability and have exhibited high drug storage capacity as well as sustained drug release behaviour as a consequence of probable interaction between the amine functional group and the functional group present in the drug molecules. The peak broadening pattern observed in proton NMR associated with the SMS NPs doped with gadolinium oxide NPs indicates their significant edge to the purpose of magnetic resonance imaging. Our preliminary *in vitro* experimental study reveals that the ultra-small silica based NPs have significant potential to perform as drug delivery vehicle and imaging probe. Thus, drug delivery systems based on SMS nanospheres may efficiently contribute to controlled release strategies in the future. The inculcation of organic and inorganic moieties at the molecular level will promote these silica particles to be tuned for extensively diverse biomedical applications. We perceive that continuous development of such novel hybrid nanomaterials and their potential applications in living beings will open a new dimension for diagnosis and therapy.

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