Electronic supporting Information (ESI) for:

Surface immobilization and release of sparfloxacin drug from SiO_2@Fe_2O_3 core-shell nanoparticles.

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Experimental section

Synthesis of Fe_2O_3 nanoparticles: the cube-shaped α-Fe_2O_3 nanoparticles were prepared through a facile solvothermal method in presence of PVP according to the reported procedure.[23] Briefly , 0.404 g of $Fe(NO_3)_3.9H_2O$ (Acros) and 0.600 g of PVP (Poly-N-vinyl-2-pyrrolidone, M_w = 40000) (Sigma-Aldrich) were dissolved in 36 ml DMF (99.8%, Acros). The solution was then transferred into Teflon-lined stainless autoclave at 180 °C for 30 h. After reaction, the autoclave cooled to room temperature and the red precipitates were collected by centrifugation, washed with distilled water and ethanol several times and dried in a vacuum desiccator over anhydrous calcium chloride.

Synthesis of Fe_2O_3@SiO_2 core-shell and surface functionalization: silica-coated hematite particles were prepared with the modified Stöber method by controlled hydrolysis of silane in the presence of alcohol.[24] Mixed solution of 15 ml ethanol (VWR), 5 ml of distilled water and 1 ml NH_4OH (wt 25%, VWR) were added to Fe_2O_3 nanoparticles. Afterwards 4 ml TEOS (Tetraethylorthosilicate, Acros 98%) solution (1 ml TEOS mixed with 30 ml ethanol) was added by means of a two-channel syringe pump at a rate of 0.4 ml h^{-1}, after the stirring for 24 h, the reaction mixture centrifuged and the Fe_2O_3@SiO_2 were re-dispersed in ethanol. Then, 200µl of APTMS (3-Aminopropyl trimethoxy silane, Evonic Degussa GmbH) was added to 5 ml of ethanol solution of the Fe_2O_3@SiO_2 spheres. The surface functionalization was achieved by stirring the mixture at room temperature for 24 h. The functionalized particles were centrifuged and washed many times to get rid of the excess of APTMS.

Activation of sparfloxacin using EDC·HCl/NHS and immobilization of Sparfloxacin onto functional nanoparticles: modification of the carboxylic group in sparfloxacin has been done via dissolving of 50 mM of sparfloxacin (5-amino-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid, Sigma-Aldrich) in MES buffer (Acros, 99%) (50 mM MES, 0.1 M NaOH), pH = 6.0. 100 µl of sparfloxacin was added to 200 µl of functionalized APTMS-nanoparticles in MES solution. This was followed by the addition of excess EDC·HCl (1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride, Sigma-Aldrich) (0.96 mg, 5 µmol) and NHS (N-hydroxysuccinimide, Sigma-Aldrich) (0.58 mg, 5 µmol) dissolved in 100 µl of MES. The reaction proceeded at room temperature for overnight. The orange precipitate were collected by centrifugation and dried in a vacuum desiccator over anhydrous calcium chloride.

In Vitro Cytotoxicity Tests: *(a) Pretreatment of the Nanostructures for Cytotoxicity Tests.* The derived prepared nanoparticles were sterilized by UV-Vis (ultraviolet-visible) light for 6 h. Cell culture medium, supplemented with FCS (fetal calf serum), was used to prepare the stock solutions. To prevent any agglomerates, each suspension was sonicated (Ultrasonic Cleaner) for 15 min prior to expo sure to the cells.
(b) Cell Culture and Cytotoxicity assay by MTT. Two different cell culture systems were used for the in vitro study of the nanostructure cytotoxicity: L929 mouse fibroblast cells and HEK 293 cells derived from human embryonic kidney. The viability of L929 mouse fibroblast and HEK 293 cells upon treatment with SPHX@SiO_2@Fe_2O_3 nanoparticles at different incubation periods were examined using MTT assay.[S1] MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Carl Roth) is absorbed by mitochondria, where it is transformed into formazan (purple crystals) by the enzyme succinic dehydrogenase. By assessing the activity of the mitochondrial dehydrogenases, the activity of viable cells in a cell population after treatment with the immobilized nanoparticles for different time period is measured, after that the sensitivity of the cells to the concentrations and incubations period is determined. Briefly, growing HEK 293and L929 cells are initially seeded at 150 µg/ml of cells/ml were treated with 1 mg/ml of the SPHX @SiO_2@Fe_2O_3 nanoparticles and incubated for 24 and 48 hours at 37°C, in an atmosphere supplemented with 5% CO_2. MTT was added to the final concentration of 5 mg/ml and they were further incubated for 1 h at 37°C, 5% CO_2 in order of MTT (yellow) to be transferred into formazan crystals (purple) by the viable cells. The formazan crystals were solubilized upon addition of solution containing DMSO. The results from MTT assay are expressed as the means of the absorptions at 630 nm, the measured absorbance is correlate with the number of viable cells

Characterization: Fourier transform infrared (FTIR) spectroscopy measurements recorded on PerkinElmer FTIR spectrophotometer in the range of 400-4000 cm^{-1}. The crystallographic study of the prepared nanoparticles was performed using powder XRD STOE-STADI MP X-Ray diffractometer operating in a transmission mode using Cu-Kα (λ = 1.5406 Å) radiation. UV-Vis absorption spectrum was recorded on a Lambda 950 UV/Vis spectrophotometer Perkin Elmer. The structural morphology and the particles size of the nanoparticles were investigated by Transmittance Electron Microscope (TEM) using Phillips CM 300 microscope, operating at 600 KV. The zeta potential was evaluated by a dynamic light scattering technique with a Zetasizer 3000HSA (Malvern-Herrenberg Instrument). Zeta potential

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determinations were based on electrophoretic mobility of the nanoparticles in diluted suspensions (5 mg in 25 ml). These measurements were performed at least in triplicate with independent particle batches.

Detailed discussion on FTIR

After addition of APTMS, the –OH functional groups on the surface of ironoxide nanoparticles reacted with APTMS forming a complex, then the sparfloxacin drug was activated using EDC/NHS and successful covalent immobilization are occurred on the surface of the iron oxide nanoparticles. Detailed characterization of the surface modification of the nanoparticles by adding core-shell of silicon oxide following by immobilization of the sparfloxacin on the functionalized nanoparticles was verified with FTIR measurement as shown in Fig. S1

Fig. S1 a, b and C shows the FTIR spectra for uncoated Fe_2O_3, core-shell sphere and SPHX @SiO_2@Fe_2O_3 nanoparticles, respectively. Fig. S1 a represents absorption peaks at 465 and 516 cm^{-1} which are characteristic for hematite.[S2] Fig. S1b shows sharp bands around 802, 955 and at 1045 cm^{-1} which are attributed to symmetric stretching vibration of Si-O-Si, stretching vibration of Si-O-H and antisymmetric vibration of Si-O-Si vibration of Si-O stretching as seen in the spectrum of Fe_2O_3@SiO_2. In the case of SPHX @SiO_2@Fe_2O_3 nanoparticles, (Fig. S1 C), the disappearance of the bands at 802 and 955 and shifting the third band from 1045 to higher frequency at 1072 cm^{-1} with decreasing in its intensity are characteristic of Si-O-C in APTMS. The pyridone stretch $\nu(C=O)_p$ in sparfloxacin appears at 1631 cm^{-1} and the asymmetric and symmetric stretching $\nu(COO)_{carb}$ appeared at 1716 and 1371 cm^{-1}, respectively. The participation of the carboxylate O atom with APTMS is confirmed by low shift in position of these bands to 1415 and 1072 cm^{-1}, respectively. A broad band between 3400 and 3150 cm^{-1} which could be attributed to the N–H stretching vibration bands of the piperazinyl moiety [S3] and amino group in sparfloxacin drug or in the SPHX @SiO_2@Fe_2O_3 nanoparticles. Small peaks around 2940 and 2850 cm^{-1}, attributed to asymmetric and symmetric CH_2 stretching, respectively; whereas disappearance of peaks at 2960 and 2885 cm^{-1} which assigned for the asymmetric and symmetric CH_3 stretching; confirms that the silane coupling reaction progressed at the surface of the nanoparticles leading to the chemical binding of APTMS moiety to the surface of ironoxide nanoparticles.

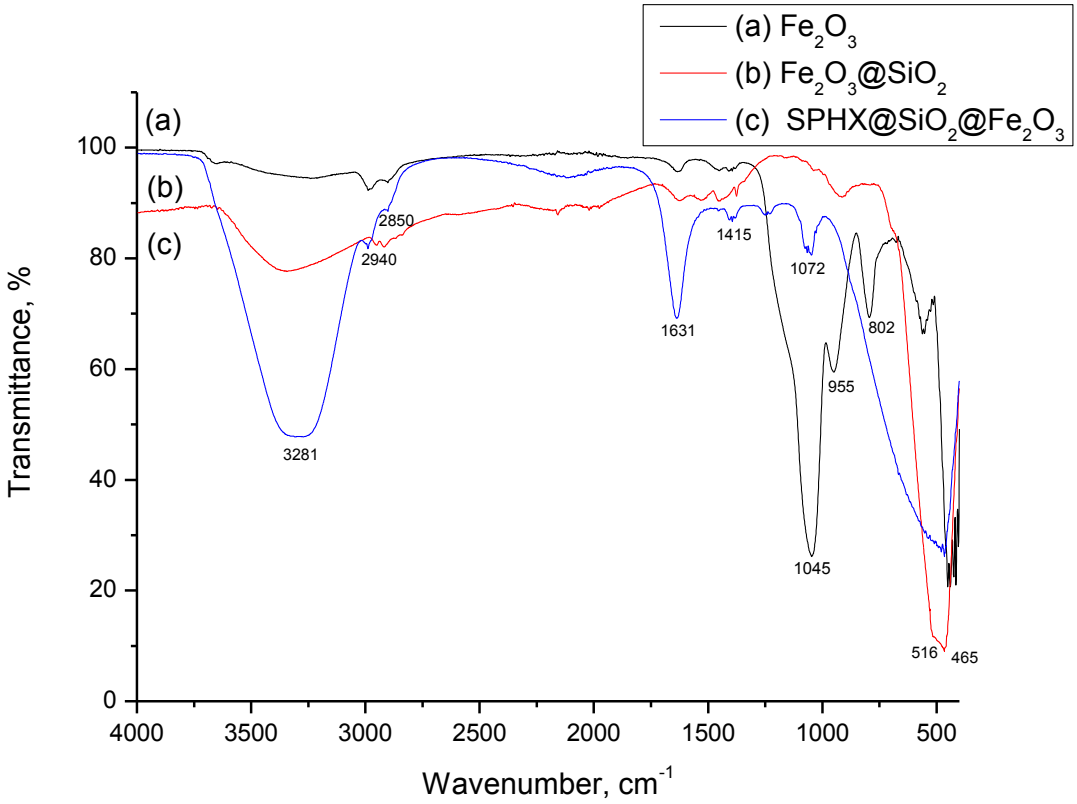

Fig. S1 FT-IR spectra of (a) Fe_2O_3, (b) Fe_2O_3@SiO_2 and (3) SPHX@SiO_2@Fe_2O_3 nanoparticles.

X-ray diffraction study

X-ray diffraction pattern of the studied Fe_2O_3 nanoparticles (a) and core-shell sphere (b) are recorded to identify their crystal structure as shown in Fig. S2. The peak positions and their relative intensity are in good agreement with those found in the JCPDS (card No. 89-2810), confirming that the nanoparticles are α-Fe_2O_3. By comparing the coating pattern, it observed the decrease of the peak intensities due to thickness of the silica shell, which suggest that silica shell could be either amorphous or nanocrystalline.[S4]

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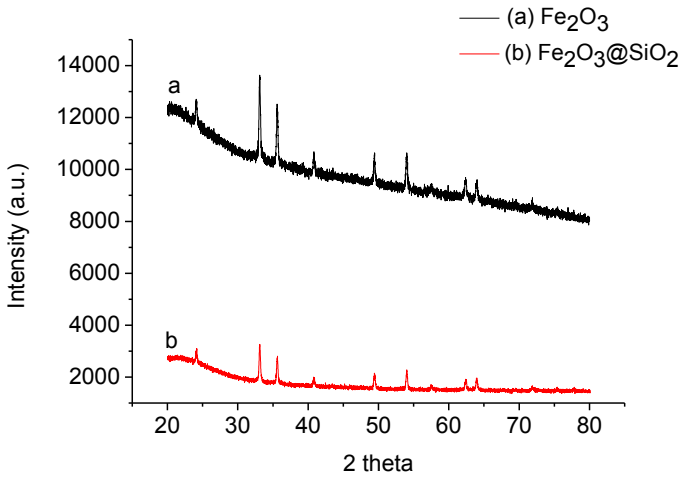

Fig. S2 XRD patterns of (a) Fe_2O_3 nanoparticles and (b) Fe_2O_3@SiO_2 sphere.

Zeta potential measurement

Dynamic light scattering (DLS) was used to determine the zeta potential of the prepared nanoparticles and after the modification. All zeta potentials measurements were performed on a Zetasizer Nano ZS (Malvern-Herrenberg Instrument). All zeta potentials were recorded in an aqueous solution with a pH = 7. The pH of the solution was adjusted with HCl and NaOH.

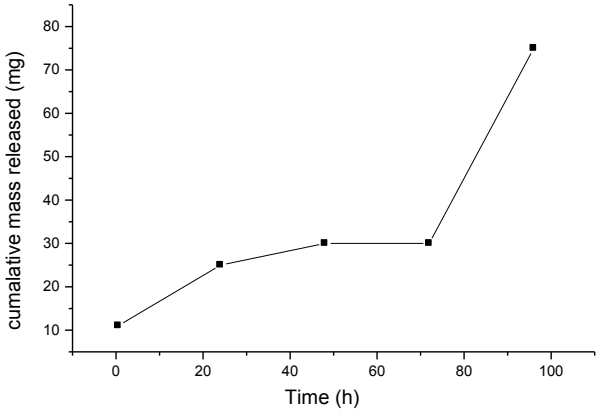

Fig. S3. In vitro profile of sparfloxacin-release from nanoparticles at different time intervals.

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