Supplementary Information

Virucidal action against avian influenza H5N1 virus and immunomodulatory effects of nanoformulations consisting of mesoporous silica nanoparticles loaded with natural prodrugs

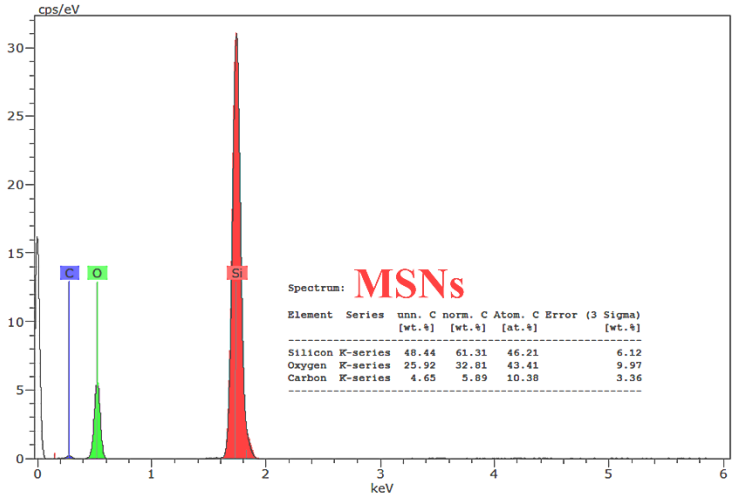
Materials

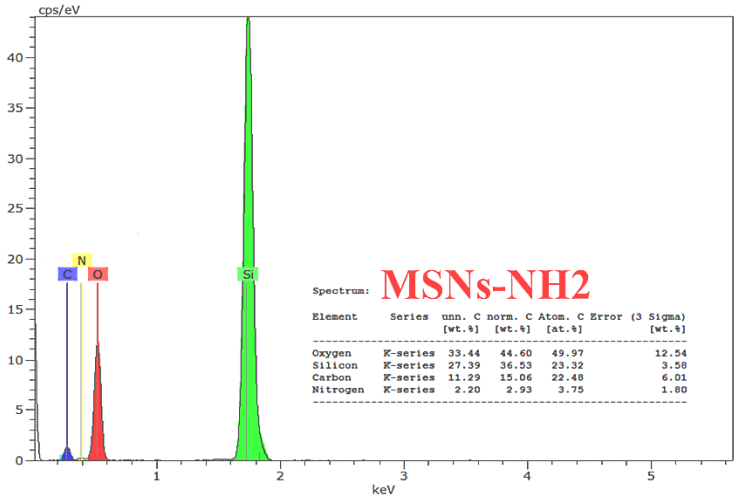
All used materials throughout the study were detailed in supplementary information. Shikimic acid, quercetin, tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), octadecene and cetyltrimethylammonium chloride solution 25% (w) in H_2O (CTAC), carrageenan, phytohaemagglutinin (PHA), lipopolysaccharide (LPS) from *E. coli* strain 0111:B4, ammonium nitrate were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Acetone absolute, methyl alcohol absolute, ethyl alcohol HPLC grade (Fisher Scientific, Loughborough, UK). Toluene anhydrous (POCH, Gliwice, Poland). Dimethyl sulfoxide (DMSO) (Tedia, Fairfield, OH, USA). N-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (Acros Organics, Geel, Belgium). Triethanolamine (TEA) (Molekula GmbH, München, Germany). Phosphate buffer saline (PBS), DMEM medium, Dulbeco's modified Eagle's medium, agarose, formalin, bovine serum abumin, penicillin G, and streptomycin were obtained from Life Technologies, Thermo Fisher Scientific, Langenselbold, Germany. 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal vieolt (Sigma-Aldrich, St. Louis, MO, USA). Tumor necrosis factor-α (TNF-α) and interleukin 1-β (IL-1β) (eBiosciences, San Diego, CA, USA). RPMI 1640 Medium (Thermo Fisher Scientific, Langenselbold,

Germany). Ultrapure water (18.2 MΩ, Milli-Q® system, Millipore, Darmstadt, Germany). All analytical and reagent grade materials were used as purchased.

Synthesis and surface modification of MSNs

The MSNs were prepared using the biphasic stratification method recently reported by Shen et al.[48] with minor modifications. In a typical preparation, 24 ml CTAC (25% solution) and 0.5 ml TEA were added to 36 ml water and stirred for 1 h at 60°C. Next, 20 ml of 20% (v/v) TEOS in octadecene was carefully added dropwise and stirred under the same conditions for another 12 h to obtain the first generation. After cooling, the upper layer containing the octadecene was removed, replaced with 20 ml (5 v/v%) TEOS in cyclohexane, and stirred for another 12 h at 80°C to obtain the second generation. Finally, the mixture was centrifuged, washed several times with ethyl alcohol, and centrifuged again, drying the resulting product in an oven at 60°C. To remove the used template, the powder material was dispersed and extracted in ethyl alcohol containing ammonium nitrate (0.6 wt.%) for 6 h at 60°C. This step was repeated twice to ensure template removal and the resulting product, the MSNs, dried at 60°C.


cps/eV
C
O
Si
keV
Spectrum: MSNs
Element Series unn. C [wt.%] norm. C [wt.%] Atom. C [at.%] Error (3 Sigma) [wt.%]
Silicon K-series 48.44 61.31 46.21 6.12
Oxygen K-series 25.92 32.81 43.41 9.97
Carbon K-series 4.65 5.89 10.38 3.36


cps/eV
N
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keV
Spectrum: MSNs-NH2
Element Series unn. C [wt.%] norm. C [wt.%] Atom. C [at.%] Error (3 Sigma) [wt.%]
Oxygen K-series 33.44 44.60 49.97 12.54
Silicon K-series 27.39 36.53 23.32 3.58
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Nitrogen K-series 2.20 2.93 3.75 1.80

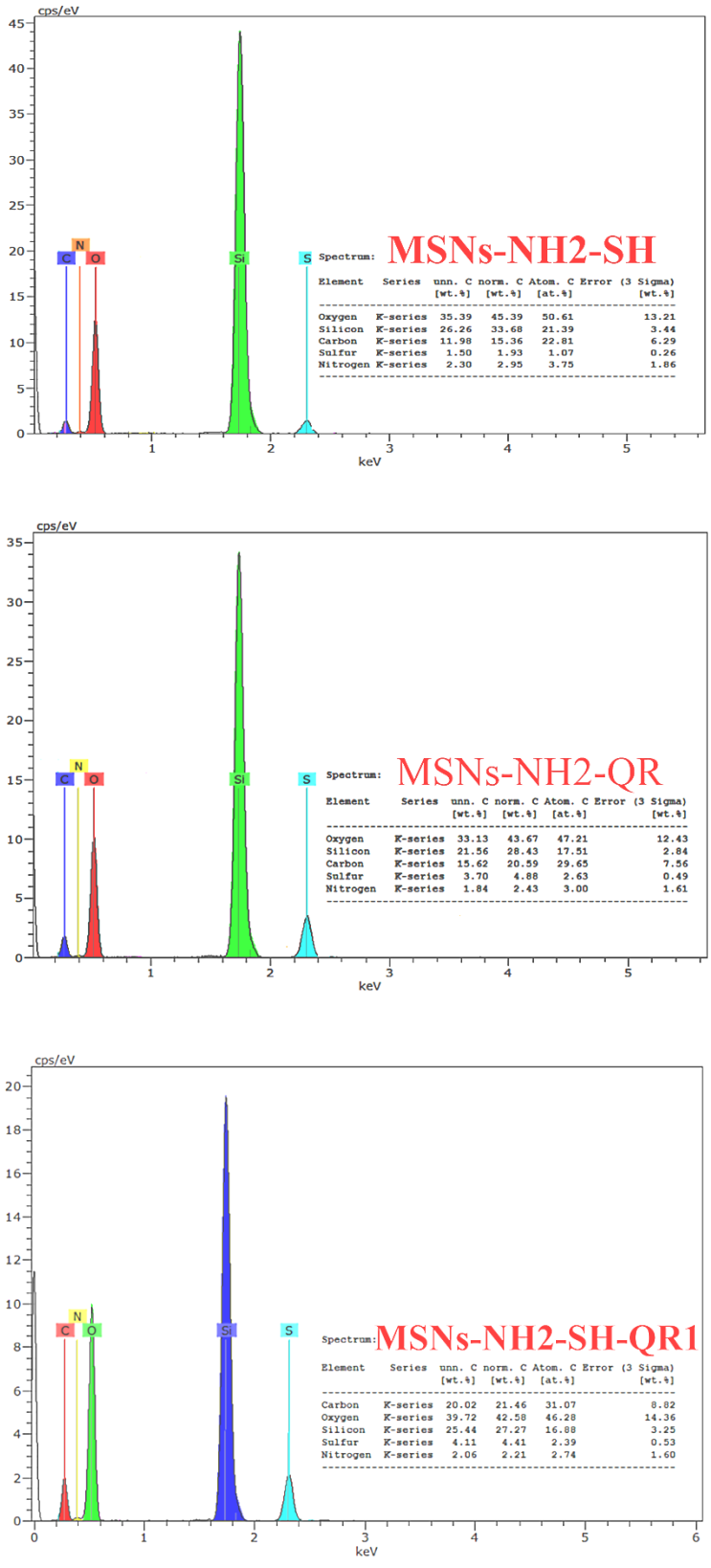


Fig. S1. EDS analysis of mesoporous silica nanoparticles: nanoparticles, modified nanoparticles, nanoformulation and combined nanoformulation. Sulfur detected in the spectrum starting when the antiviral compound(s) loaded nanoparticles, this detectable content may be coming from the solvent used especially DMSO during the loading process.

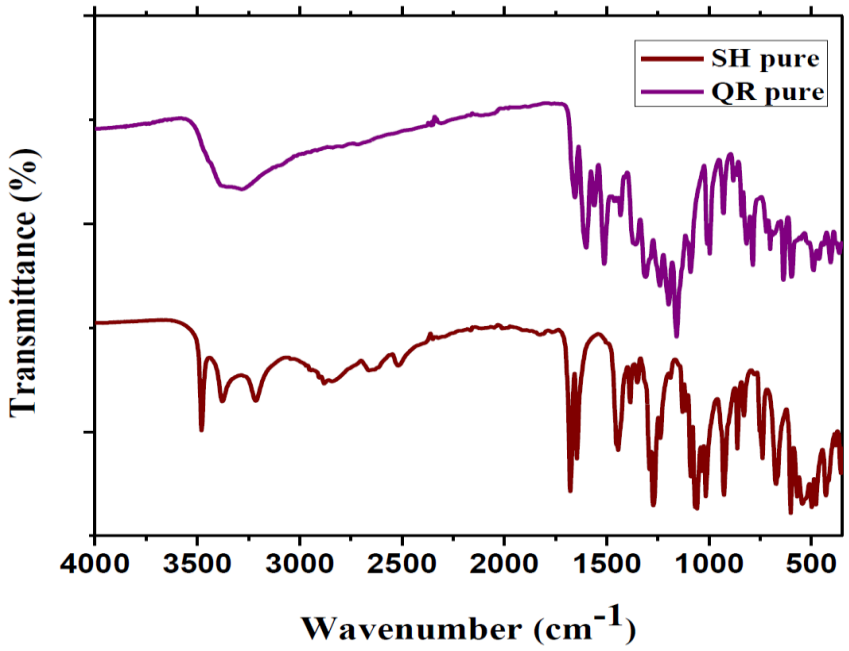
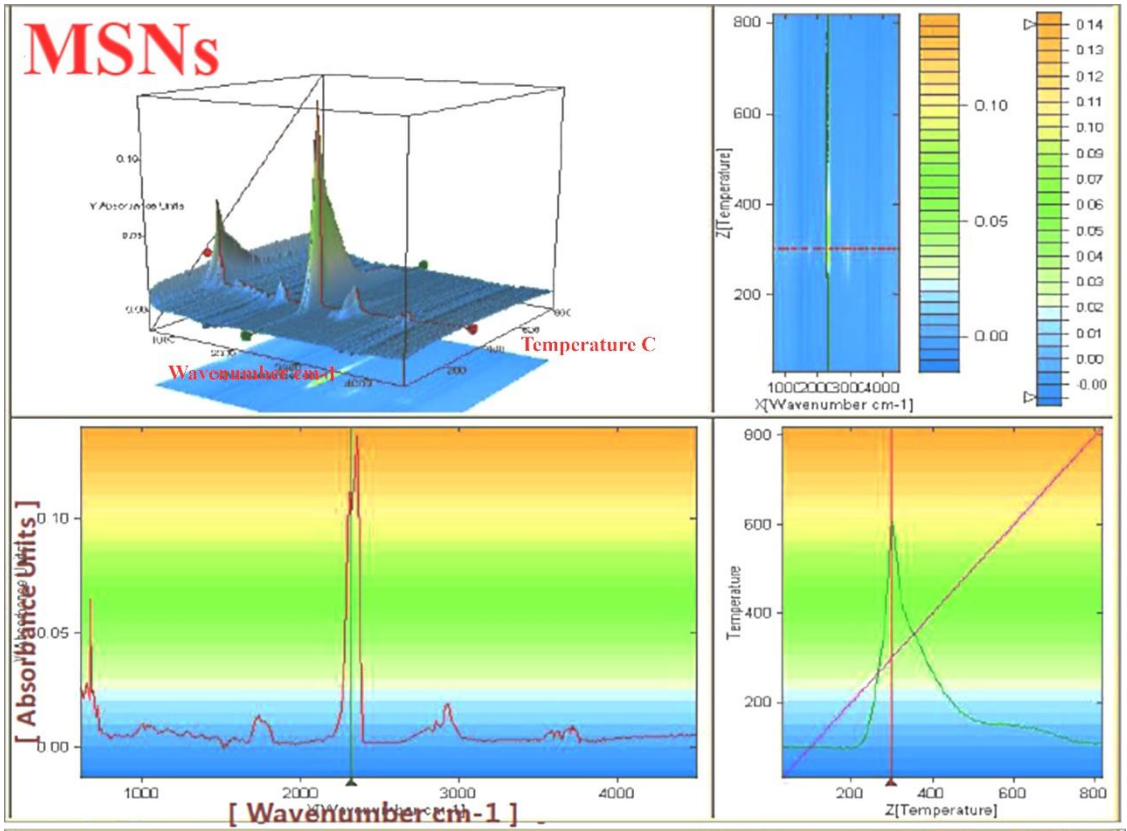
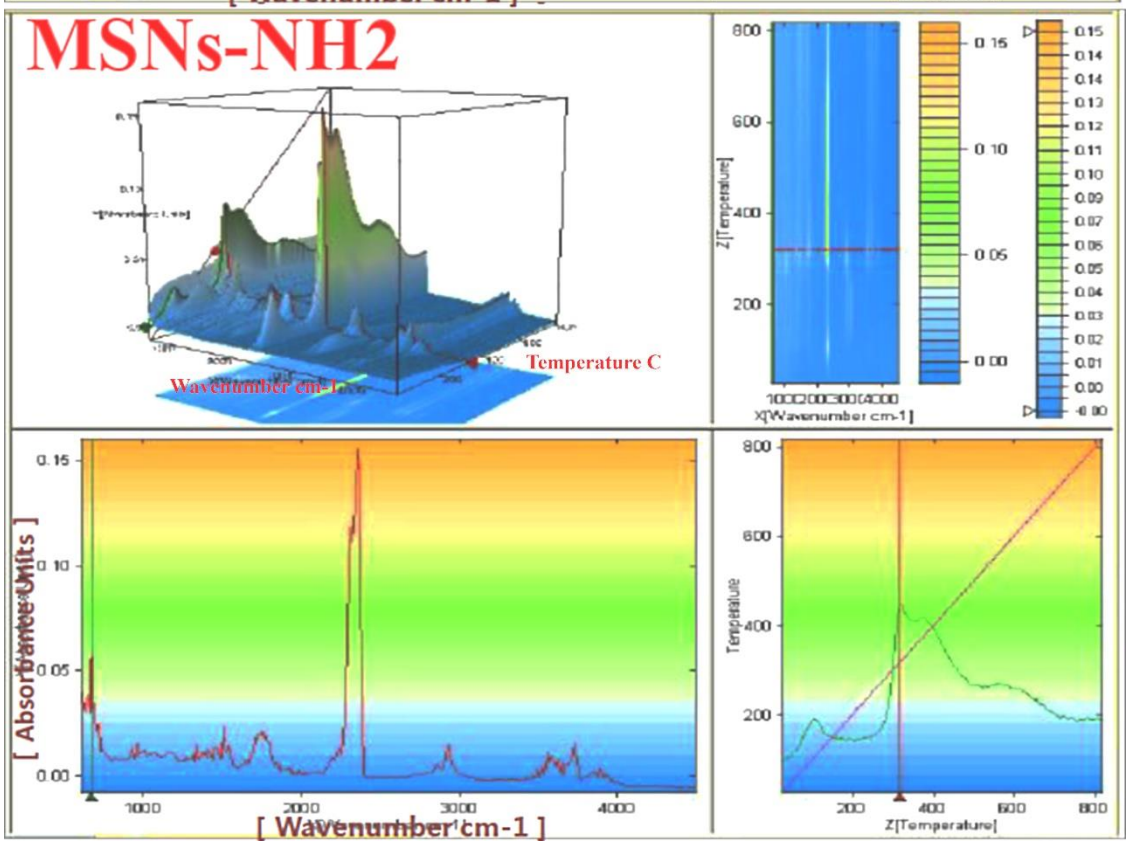
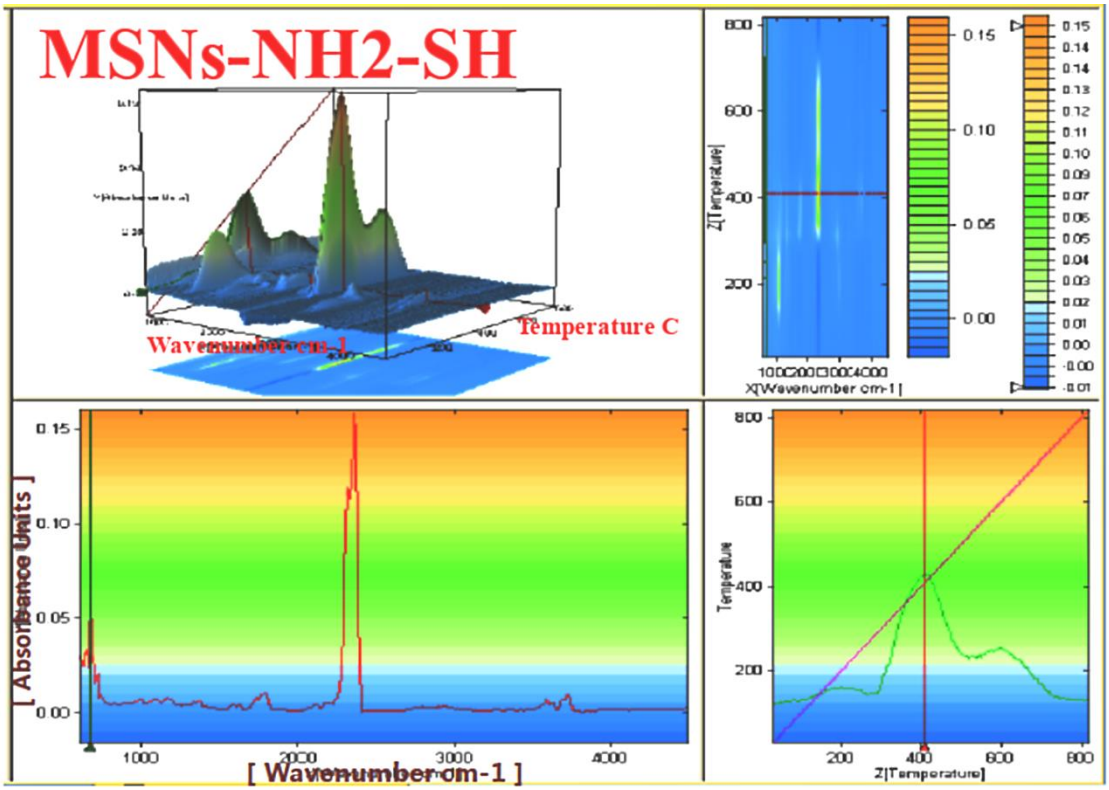


Fig. S2. FTIR spectra of pure prodrugs: SH and QR.

We employed simultaneous thermal analysis (STA)-coupled with FTIR (STA-FTIR), as a powerful method to investigate gases evolved during desorption or pyrolysis of the MSNs. The FTIR absorbance spectra were identified for a selected temperature (reddish brown line), where the highest intensity of evolved gases was observed, according to TG results. These findings show that STA-FTIR is an useful approach for qualitative analysis of the evolved gases. The results are shown in Fig. S3 below.


MSNs
Temperature C
Wavenumber cm-1
Z[Temperature]
X[Wavenumber cm-1]
[Absorbance Units]
[Wavenumber cm-1]
Temperature
Z[Temperature]


MSNs-NH2
Temperature C
Wavenumber cm-1
Z[Temperature]
X[Wavenumber cm-1]
[Absorbance Units]
[Wavenumber cm-1]
Temperature
Z[Temperature]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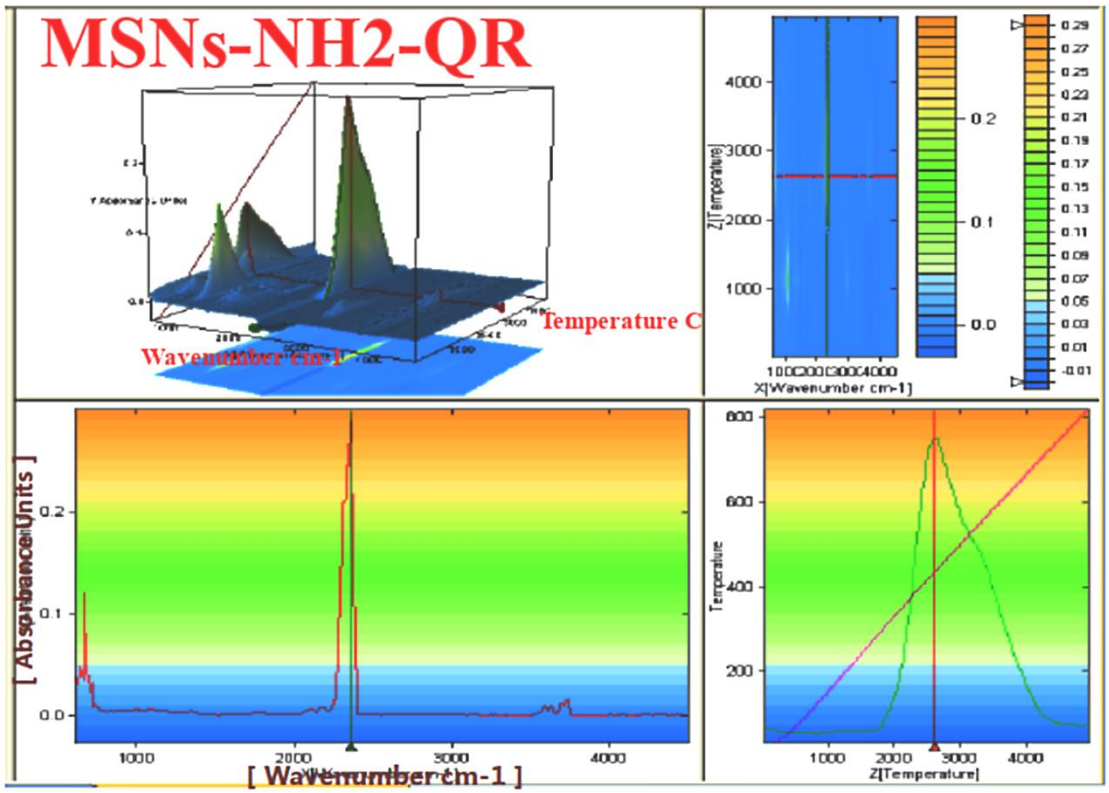
MSNs-NH2-QR
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[Wavenumber cm-1]
Z[Temperature]
X[Wavenumber cm-1]

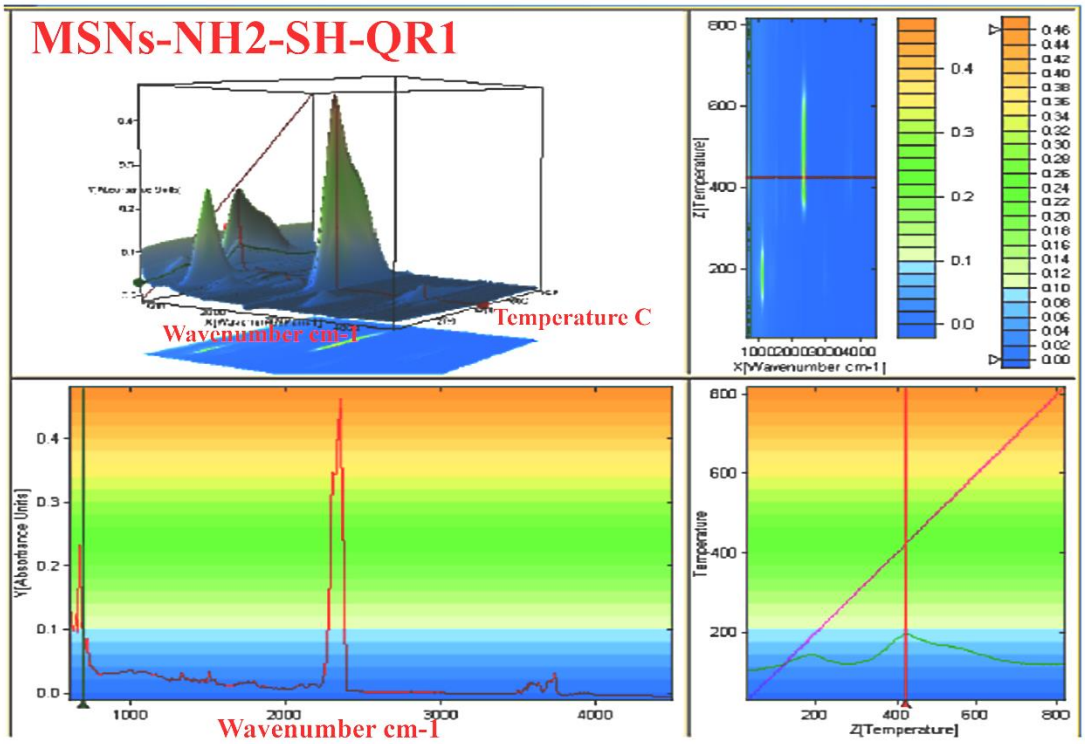

Fig. S3. Three dimensional graphs of FTIR absorption as a function of wave number and temperature for pristine, modified and loaded nanoparticles.

Table S1. The calculated molar concentrations (µM) regarding to all used microgram concentrations (µg.ml) for anti-inflammatory studies

Concentration at µg/ml	Calculated molar concentrations (µM)			
	SH	QR	SH-QRmix1	SH-QRmix2
12.5	71.7	41.3	52.5	105
25	143.5	82.7	104.9	209.8
50	287.1	165.43	209.9	419.8
100	574.2	330.8	419.9	839.8

Table S2. The calculated molar concentrations (µM) regarding to all used microgram concentrations (µg.ml) for antiviral studies

Concentration at µg/ml	Calculated molar concentrations (µM)			
	SH	QR	SH-QRmix1	SH-QRmix2
1	5.7	3.3	4.1	8.2
10	57.4	33	41.9	83.8
25	143.5	82.7	104.9	209.8
50	287.1	165.43	209.9	419.8
75	430.6	248.1	314.9	629.8
100	574.2	330.8	419.9	839.8
150	861.3	496.3	629.9	1259.8

** In case on combined nanoformulations contained SH-QR, we calculated the molar concentration based on the following equation SH-QR M.W = molecular weight of SH + molecular weight of QR/2.