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Supporting Information

Improving the Genistein oral bioavailability via its formulation into the Metal-Organic Framework MIL-100(Fe)

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S1. Materials and Methods

Materials and reagents

General reagents and solvents were commercially available and used as received. High performance liquid chromatography (HPLC) and quality methanol (MeOH) were purchased from Merck (Germany). Ethanol (96%, EtOH), acetone and *N,N'*-dimethylformamide (DMF) were purchased from Carlo Erba (Italy). Dichloromethane (DCM) was acquired from Acros Organics (Belgium). Formic acid, monopotassium phosphate (KH_2PO_4), and sodium chloride (NaCl) were purchased from Sigma-Aldrich (France). Genistein (GEN) was purchased from LC Laboratories (USA). Hydrochloric acid (HCl) and acetic acid (HAc) were purchased from Scharlau (Spain). Disodium phosphate (Na_2HPO_4) was purchased from Prolabo (Spain). 1,3,5-benzenetricarboxylic acid or trimesic acid (H_3BTC; 95%), and the metal precursor iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$; 97%) were purchased from Sigma-Aldrich (France).

Phosphate buffer solution (PBS, 0.04 M, pH = 2.5) for HPLC determination was prepared by solving 2.4 g (0.02 mol) of NaH_2PO_4 and 2.84 g (0.02 mol) of Na_2HPO_4 in 1 L of MilliQ water. The pH was then adjusted to 2.5 with H_3PO4 (≥ 85%).

Phosphate buffer solution (PBS, 0.08 M pH = 7.2) for GEN release was prepared by solving 1.02 g (0.008 mol) of KH_2PO_4, 1.92 g (0.014 mol) of Na_2HPO_4, and 45.32 g (0.8 mol) of NaCl in 500 mL of MilliQ water. The pH was then adjusted to 7.2 with HCl.

Experimental techniques

MIL-100 nanoparticles (NPs) were prepared by microwave-assisted synthesis using a Mars-5 instrument (CEM, US). Fourier transform infrared spectroscopy studies (FTIR) were recorded using a Thermo Nicolet 6700 spectrometer (Thermo, USA) in the 400-4000 cm^{-1} region. PXRD patterns were measured using a high-throughput Bruker D8 Advance diffractometer working on transmission mode and equipped with a focusing Göbel mirror producing CuKα radiation (λ = 1.5418 Å) and a LYNXEYE detector. Data were collected at room temperature (RT), in the 2Θ range 2–30˚, with a 0.02˚ step width. Thermogravimetric analyses (TGA) were carried out in a Perkin Elmer Diamond TGA/DTA STA 6000 running from RT to 600 °C using a heating rate with 2 ºC·min^{-1} scan rate. The particle size and ζ-

potential measurements were carried out with a Zetasizer Nano Series Nano-ZS® from Malvern Instruments (UK). The particles were dispersed in ultrapure water by using a Microson™ XL 2000 ultrasonic liquid processor (Qsonica, USA) for 30 seconds at 20 watts with amplitude of 30%. The N_2 isotherms were obtained at 77 K using a Belsorp Mini (Bel, Japan). Prior to the analysis, approximately 40-60 mg of the samples were evacuated at 130 °C under vacuum for 3 hours.

HPLC analyses were performed with a RP-C_{18} 3 µm Gemini NX 150 x 2.0 mm column from Phenomenex (USA), protected by a KJO-4282 Security Guard Cartridge from the same package from Phenomenex (USA), using an Agilent Technologies Series 1200 equipped with an Infinity Diode Array Detector HPLC system, and controlled by ChemStation for LC 3D systems from Agilent Technologies (USA). The analyses were performed with a mobile phase containing MeOH and formic acid (0.1%) at a ratio of 60:40 (v/v), at a flow rate of 0.160 mL·min^{-1}, an injection volume of 5 µL, followed by needle wash, monitoring the GEN peaks at 260 nm (retention time (rt) = 8.0 min). The quantification of trimesic acid was performed using a mobile phase consisting of 50:50 solution (v/v) of phosphate buffer saline (PBS, 0.02 M, pH = 2.5) and MeOH, as previously described.[1] The injection volume was set at 5 µL with a flow rate of 1 mL·min^{-1} and the column temperature fixed at 25 ºC, monitoring the H_3BTC peaks at 215 nm and a rt of 3.3 min.

Synthesis of MIL-100(Fe) NPs [$Fe_3O(H_2O)_2OH(BTC)_2$] *n*H_2O

MIL-100 NPs were obtained using a microwave procedure previously described.[2] $FeCl_3 \cdot 6H_2O$ (2.43 g, 6.01 mmol) and H_3BTC (0.84 g, 4.00 mmol) were dissolved in distilled water (30 mL). The reaction was heated to 130 °C over 30 s, then maintained at this temperature for 5 min 30 s (1600 W). The reacting mixture was cooled down by putting them in an ice bath to room temperature and centrifuged at 10500 rpm for 25 min. The solid (*ca.* 500 mg) was then cleaned with 20 mL of water (6-fold) and ethanol (1-fold), and stored in ethanol (reaction yield ~ 86.8%).

S2. Genistein encapsulation

Considering GEN solubility, three different solvents were tested in GEN adsorption. Finally, the formation of a calcium genistein salt (denoted GCa) was selected as the ultimate adsorption method.

Table S1. Solubility of GEN at room temperature (RT), boiling point, and median lethal dose 50 (LD_{50}) after oral administration in rats in the studied solvents.

Solvent	Solubility ($mg \cdot mL^{-1}$)	Boiling point (ºC)	LD_{50} ($mg \cdot Kg^{-1}$)
DCM	2.0*	40	2100
EtOH	1.3*	78	7060
H_2O	0.0008 1.4**	100	-

* Solubility values at RT

** Solubility value for GCa (GEN calcium salt at 37 ºC)

Calcium genistein salt formation: To improve the GEN aqueous solubility, a calcium genistein salt was synthesized. 54 mg (0.2 mmol) of GEN, 15 mg (0.2 mmol) of $Ca(OH)_2$ and 2 mL of distilled water were added to a glass vial and maintained at RT under magnetic stirring away from light exposure for 24 h. Then, the grey colored solution formed was centrifuged at 7000 rpm for 10 min, obtaining a light-grey pellet which was further dried at 100 ºC. Considering the basic pH value of the obtained solution and the GEN pK_a values (7.2, 10.0, and 13.1) reported by Zielonka et al.,[3] the obtained solid corresponds to the monocalcium salt of GEN. The experimental aqueous solubility at 37 ºC of GCa is much higher than that of the commercial GEN (1.4 $mg \cdot mL^{-1}$ *vs.* 0.8 $\mu g \cdot mL^{-1}$).

GCa adsorption: Prior to the encapsulation of MIL-100 NPs, the solid was exchanged twice in pure water. 300 mg of MIL-100 NPs were suspended in an aqueous solution of GCa (250 mg in 250 mL of water) at 37 ºC under stirring for 24 h (1:2 MOF:GCa molar ratio). Then the GCa loaded material (named GEN@MIL-100 NPs) was collected by filtration with a 0.2 µm filter. The amount of GEN incorporated in MIL-100 NPs was calculated by HPLC and TGA.

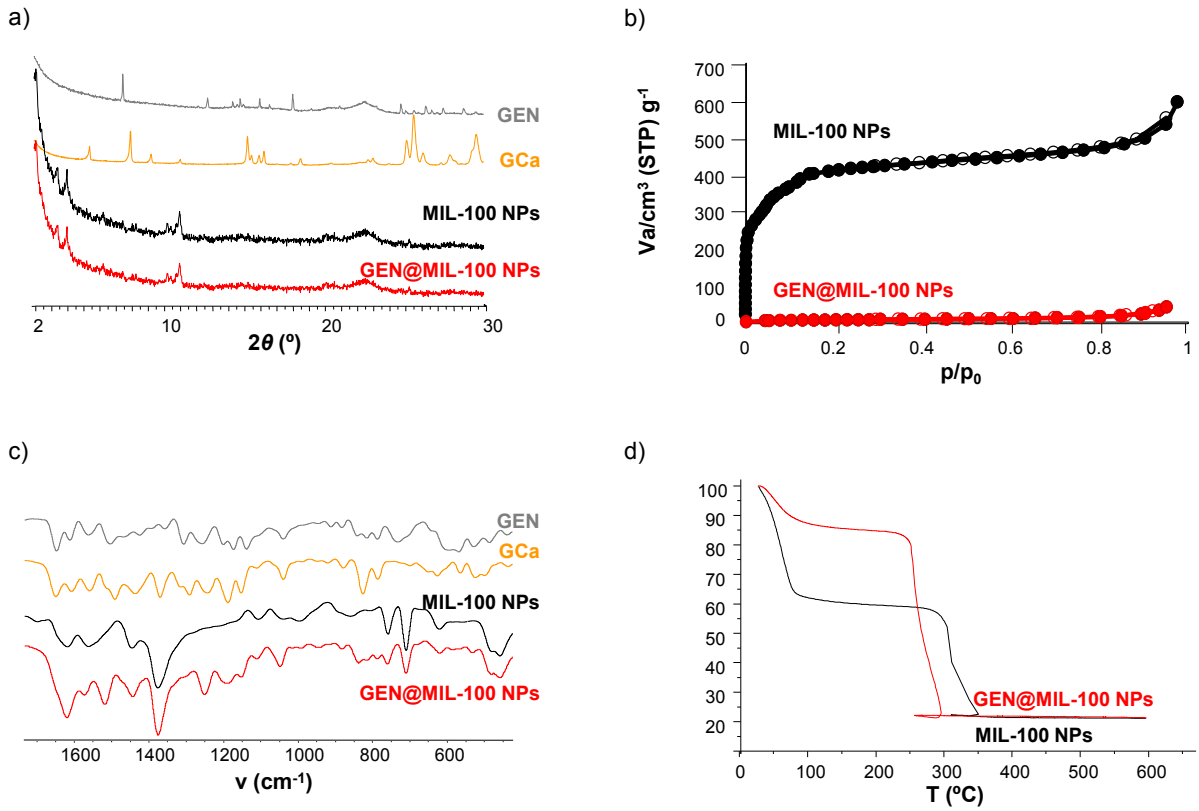

Figure S1. a) PXRD patterns, b) N_2 sorption isotherms at 77 K, c) FTIR spectra, and d) TGA of pristine MIL-100 NPs and the drug loaded GEN@MIL-100 NPs. PXRD patterns and FTIR of the free drug are included for purposes of comparison. In addition, the structural integrity after the incorporation of GEN is maintained, with no Bragg reflections corresponding to the free recrystallized GEN, being consistent with the GEN entrapping inside the nanoMOF porosity. Note that empty symbols correspond to the desorption branch.

Table S2. Quantification of the GEN loading ($mg \cdot g^{-1}$) by TGA and HPLC.

	HPLC	**TGA**
GEN@MIL-100	271 ± 34	298 ± 61

Table S3. Particle size (nm) and -potential (mV) in MilliQ water of MIL-100 NPs before and after GEN encapsulation.

Size (nm) (PDI)		**ζ-potential (mV)**	
MIL-100	GEN@MIL-100	MIL-100	GEN@MIL-100
129 ± 26 (0.29)	161 ± 46 (0.09)	-25 ± 2	-27 ± 9

S3. Computing simulation

The saturation of GEN in MIL-100 was estimated using Grand Canonical Monte Carlo (GCMC) calculations. By means of GCMC calculations, it is possible to estimate the number of molecules present in the pores as a function of the chemical potential. It is also possible to elucidate the preferential adsorption sites for guest molecules on the solid framework. Partial charges and force field parameters were implemented to run GCMC calculations. We performed all the simulations at 300 K with a simulation box large enough to use a cut-off equal to 12.5 Å with typically 5 x 10^6 Monte Carlo steps for both equilibration and prediction steps. Saturation was estimated using P = 10000 kPa (such a high value allows us to assure the saturation to be accessed).

The framework was kept rigid, with atoms at the positions previously derived for the empty material during the whole adsorption process. Short range interactions were estimated using a cut-off distance of 12.5 Å. The differential adsorption enthalpy at low coverage was calculated through the fluctuation over the number of particles in the system and from the internal energy. The parameters are given in the previous section.

In the GCMC calculations, the solid was supposed to be rigid and the unit cell parameters were therefore fixed at the experimental values. For this study, we considered the largest unit cell structure.

The adsorbed GEN molecule was initially optimized by Density Functional Theory (DFT) calculations and considered as rigid during the GCMC calculations. It follows that no rearrangement of the conformation for the confined molecules was possible in the MIL-100 pores.

Regarding the solid, we used the Universal Force Field (UFF) for Lennard Jones interatomic potentials, which is usually used to describe solid interactions,[4] and Equalization Electronegativity Method implemented in Material Studio to determine the partial charge distribution for the solid.[5] In contrast, the UFF parameters and DFT partial charges (calculated with a geometry optimization performed with DMol^3 using PW91 functional and DNP basis set) were used for the adsorbate molecules.

S4. Genistein release

The release assays were fulfilled under simulated intestinal conditions at sink conditions. 1 mg of GEN@MIL-100 NPs was suspended in 15 mL of PBS (pH = 7.2) at 37 ºC and kept under rotational stirring. After different incubation times, an aliquot of 7.5 mL of supernatant (half of the total volume) was recovered by centrifugation (11500 rpm for 15 min) and replaced with the same volume of fresh medium at 37 °C. The 1 mL of the collected aliquots of GEN supernatants was added together with 1 mL of NaOH 0.50 M. Then, each sample was maintained for 2 h with the NaOH solution under rotational stirring, followed by 5 min vortex homogenisation and sample centrifugation at 11500 rpm for 3 min. Thus, the supernatant was filtered through a 0.22 µm pore diameter filter and analysed by HPLC.

Quantification of non-released GEN: In order to quantify the possible non-released GEN in the MIL-100 NPs, the pellet recovered after 3 days of GEN release in PBS (pH = 7.2) was suspended in 1 mL of NaOH 0.5 M and maintained under rotational stirring for 7 h. A low detection of GEN agrees with a complete release of the GEN into the medium.

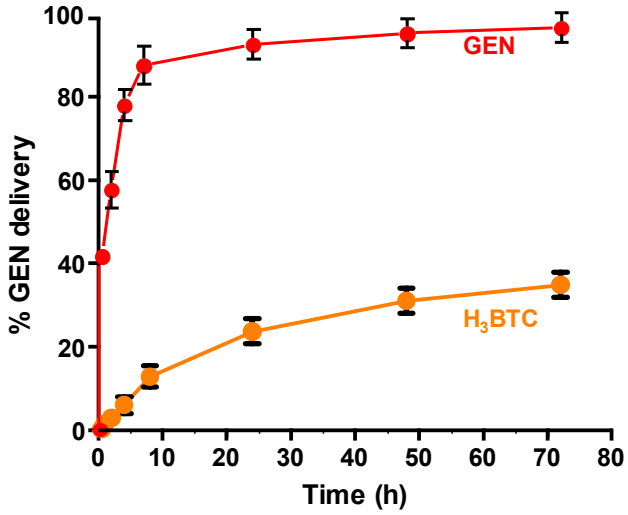

Figure S2. GEN release during 3 days from MIL-100 NPs under simulated intestinal conditions (red). Moreover, the MOF degradation (monitored *via* the release of the organic linker H_3BTC) is also depicted (orange). Note that lines are only visual guides.

S5. *In vivo* experiments

All experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the University of Navarra (Spain), project number: 14013361. The studies of biodistribution were performed in BALB mice (6 - 8 weeks old, 20 ± 1 g body weight) purchased at Harlan (Harlan Ibérica, Spain). Taking into account previous MOF biodistribution studies and the quite important previously evidenced inter-individual variability, the groups were composed by 6 animals.[6–8] After fasting overnight, mice were randomly divided into groups of 6 animals according to the treatment. All animals were maintained between 22-25 ºC with a 20% relative humidity environment in a 12 h light/dark cycle.

Two different groups denoted as GEN@MIL-100 and GEN (positive controls) were studied. A single dose of 100 µL of an homogeneous GEN@MIL-100 NPs suspension or GEN solution of 30 $mg \cdot Kg^{-1}$ containing 10% of DMSO, 25% of poly(ethylene glycol) (PEG) 400, and 65% of water for injection was administered. This highly stable formulation permitted a suitable dispersion of the NPs together with a very low GEN release (only 10% of the total cargo is released after 6 h).

To study the pharmacokinetics (PK) profile of GEN, 200 µL of blood was extracted after 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 h using EDTA-coated tubes to avoid blood coagulation. After blood extraction, samples were centrifuged (7000 rpm, 10 min) at 4 ºC and plasma was kept at -80 ºC until sample analysis. All the PK parameters (maximal concentration (C_{max}), time to reach the maximum concentration (T_{max}), biological half-life ($t_{1/2}$), mean residence time (MRT), and bioavailability (F)) resulting from the comparison between the free and encapsulated GEN, were obtained with WinNonLin® software (Certara, USA). Mice were sacrificed at 8 and 48 h post-administration and their kidneys, spleens and livers were removed, homogenized with 1 mL of PBS (pH = 7.4) and kept at -80 ºC until sample analysis. GEN molecule was analyzed by HPLC following a similar procedure as previously described.[9]

S6. References

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