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Dehydropeptide-based plasmonic magnetogels: a supramolecular composite nanosystem for multimodal cancer therapy

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Supramolecular hydrogels are highly promising for biomedical materials owing to the wide array of properties that can be tailored and modulated. The combination with plasmonic/magnetic nanoparticles into plasmonic magnetogels further improve its potential in biomedical applications, through the combination of complementary strategies, such as photothermia, magnetic hyperthermia, photodynamic therapy and magnetic-guided drug delivery. Here, a new dehydropeptide hydrogelator, Npx-L-Met-Z-ΔPhe-OH, was developed and combined with two different plasmonic/magnetic nanoparticles architectures: core/shell manganese ferrite/gold nanoparticles or gold-decorated manganese ferrite nanoparticles with *ca.* 55 nm and 45 nm size, respectively. The characterization of magnetogels were performed by HR-TEM, FTIR, circular dichroism and rheological assays. The gels were tested as nanocarriers for a model antitumor drug, the natural compound curcumin. The incorporation of the drug in magnetogel matrices was confirmed through fluorescence-based techniques (FRET, fluorescence anisotropy and quenching). The curcumin release profiles were studied with and without excitation of the gold plasmon band. The transport of curcumin from magnetogels towards biomembrane models (small unilamellar vesicles) was assessed by FRET between the fluorescent drug and the lipid probe Nile Red. The developed magnetogels showed promising results for photothermia and photo-triggered drug release. The magnetogels bearing gold-decorated nanoparticles showed the best photothermia properties, while the ones containing core/shell nanoparticles had the best photoinduced curcumin release.

Introduction

The ongoing social, economic and cultural relation changes induced by the economic transition in low-income countries and the adoption of unhealthy lifestyles in high-income countries contribute to cancer keeping as a leading cause of death worldwide, with lung, colorectal, breast and prostate exhibiting the highest incidence ratio.^{1–6} Conventional chemotherapy has only shown improved survival rates on early-stage disease in high-income countries, which can be accounted for the lack of specificity, therapeutic effectiveness and cost, besides worsening patient's quality of life.^{6,7} Supramolecular hydrogels are stimulus-responsive self-assembled intertwined fibrillar structures achieved through the cooperative effect of different non-covalent intermolecular interactions: hydrogen bonding, van der Waals, electrostatic, hydrophobic and

aromatic interactions.^{8–18} The microdomains of solvent pockets afford physical-chemical similarity to the cell matrix, but also allow the retention of nanoparticles, which tailors and modulates the hydrogel matrix structure and mechanical, electrical, optical, and thermal properties.^{10–17,19} The hydrophobic and hydrophilic microdomains allow the loading and efficient transport of a wide variety of drugs, reducing side effects and enabling higher doses in therapy.²⁰

The combination of hydrogels with magnetic nanoparticles affords magnetogels, providing control and targeting of the nanosystem to a specific location through a magnetic field gradient and a higher therapeutic efficiency, owing to the synergistic effect between magnetic hyperthermia and enhanced drug release promoted by the application of an alternating magnetic field (AMF).^{21–25} The higher magnetic susceptibility of manganese ferrite, when compared to other transition metal ferrites, makes it an appealing material for theranostic applications.^{26,27} Moreover, coating with gold might afford higher biocompatibility and lower toxicity, together with the surface plasmon resonance (SPR) phenomenon that allows the conversion of electromagnetic radiation into heat.^{28–32} Hereby, core/shell nanoparticles afford a high therapeutic potentiality owing to the combination of different strategies such as photothermia, photodynamic therapy, magnetic hyperthermia and magnetic-guided drug delivery.

In this work, gold-decorated manganese ferrite and core/shell manganese ferrite/gold nanoparticles were synthesized, characterized and incorporated into a new dehydrodiptide hydrogel (Npx-L-Met-Z-ΔPhe-OH, compound **1**) containing naproxen

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Electronic Supplementary Information (ESI) available: Detailed synthesis procedure; ¹H NMR spectra; UV-Vis absorption spectra; Critical aggregation assays; Docking in COX-1; Details of XRD analysis; Mechanical spectra; FTIR spectra; EDX spectra; Nanoparticle size histograms from TEM; FFT of TEM image; Spectral overlap for FRET assays; Deduction of eqn. (7); Data of drug release assays. See DOI: 10.1039/x0xx00000x

(Npx) and methionine (Met), to avert the leashing out of nanoparticles through the S-Au bond. The dehydroamino acid dehydrophenylalanine (Δ Phe) endows the peptide hydrogelator with proteolytic stability and adds conformational restraints to the peptide backbone, while the naproxen group affords selectivity towards cyclooxygenase.^{11,33,34} The mechanical properties of the magnetogels were assessed and the effect of the nanoparticles on the hydrogel fibrillar matrix was investigated. Moreover, the magnetogels were evaluated on the loading of a hydrophobic model drug (curcumin). The interaction with biomembrane models was studied as well as the use of photothermia as a trigger to accelerate curcumin release.

The proposed multifunctional nanosystem is promising for cancer therapy owing to the potential tunability of the mechanical properties, controlled drug delivery to biomembrane models through photothermia, besides the proteolytic stability, anti-inflammatory properties and magnetically-guided drug delivery.

Experimental

Synthesis procedure of the hydrogelator

General methods. Compound **1** was prepared by synthetic methodologies (see Supplementary Information for detailed description) developed in previous works^{35,36} and fully characterized by ¹H and ¹³C NMR spectroscopy (NMR) and High Resolution Mass Spectrometry (HRMS). ¹H and ¹³C NMR spectra were recorded on a Bruker Avance III at 400 and 100.6 MHz, respectively. DEPT θ 45° and 135°, HMQC and HMBC were used to attribute some signals. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are reported in Hertz (Hz). Petroleum ether refers to the boiling range of 40–60 °C. Acetonitrile was dried over silica and calcium hydride (CaH₂), while dichloromethane was dried over calcium hydride, and then distilled and stored over molecular sieves. Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected.

Synthesis procedure of magnetic/plasmonic nanoparticles

Preparation of manganese ferrite nanoparticles. Manganese ferrite nanoparticles were synthesized by the co-precipitation method in a solution with a total volume of 5 mL, as described by Rodrigues *et al.*³⁷ Initially, a mixture of 500 μ L of MnSO₄·H₂O 0.5 M aqueous solution and 500 μ L of FeCl₃·6H₂O 1 M was prepared and added, drop by drop, to an aqueous solution with 612 μ L of NaOH 50% at 90 °C, with constant magnetic stirring. After 2 h at 90 °C, nanoparticles were formed, and purification was carried out by repeated centrifugations, dispersion in deionized water, and drying at 100 °C.

Preparation of core-shell manganese ferrite/gold nanoparticles. The synthesis of gold-coated manganese ferrite nanoparticles was adapted from a previously reported procedure.³⁸ Thus, a 5 mL aqueous solution of MnFe₂O₄ nanoparticles (40 mg) was added to 25 mL of glycerol and left stirring until 200 °C, in a silicone oil bath. A solution of gold(III) chloride hydrate, HAuCl₄·3H₂O (2 mL, 0.04 M) was added dropwise and stirring was kept for 15 min. The mixture

was washed with ethanol through centrifugation, followed by magnetic separation and left to dry at 100 °C.

Preparation of gold nanoparticles functionalized with 3-mercaptopropionic acid. The synthesis of gold nanoparticles functionalized with 3-mercaptopropionic acid (MPA) followed a procedure adapted from a previously reported method.³⁹ 60 mg of gold(III) chloride hydrate, HAuCl₄·3H₂O, were dissolved in ethanol (50 mL), followed by the addition of 3-mercaptopropionic acid (3.5 mg) under vigorous stirring. A solution of NaBH₄ (50 mg) in NaOH 1 M (3 mL) was added dropwise and the mixture was left stirring for 30 min. A black/brown precipitate was formed, and purification was attained by centrifugation and washing with ethanol. The product was dried and stored in the freezer.

Preparation of gold-decorated manganese ferrite nanoparticles.

The MnFe₂O₄ nanoparticles (9 mg) were washed with NaOH 1M, dried and dispersed in DMSO (5 mL). Then, CDI (1,1'-carbonyldiimidazole) (63.36 mg) was added and the reaction was left for 2 h under vigorous stirring above 60 °C. The mixture was washed by repeated centrifugation and addition of DMSO. The CDI-activated magnetic nanoparticles were dispersed in DMSO and, then, MPA-functionalized gold nanoparticles (35.7 mg) were added. The reaction was left for 1 h under vigorous stirring above 60 °C. The product of the reaction was obtained by magnetic separation. The nanoparticles were purified by repeated centrifugation and washed with DMSO. The gold-decorated nanoparticles were further functionalized with 2-aminoethanol (0.2 g) using the CDI (63.33 mg) coupling procedure described above. The product was magnetically separated and washed with water to remove the reagents and DMSO.

Self-assembly of hydrogel

pH dependence. The weighted mass of hydrogelator was dissolved in the required pH buffer, previously prepared from a sodium phosphate 0.1 M solution and a mixed solution of citric acid (0.05 M) and boric acid (0.2 M), as described by Perrin and Dempsey.⁴⁰ Hydrogelator solutions at 0.2 μ M concentration were obtained in the pH range 2–10. The fluorescence emission spectra were measured for each solution to evaluate the pH influence on the hydrogelator behaviour.

Critical aggregation concentration. The critical aggregation concentration was assessed through titration of the hydrogelator in the respective pH buffer. The concentration range to consider was determined by sequentially preparing 10× diluted solutions and the range was chosen according to where a fluorescence increase, or decrease, was observed.

Critical gelation concentration. To determine the critical gelation concentration through fluorescence spectroscopy, aqueous solutions of the hydrogelator **1** in a concentration range 0.2–0.8 wt% were prepared. The pH was chosen according to the results obtained in the self-assembly study at different pH values. Self-assembly was attained by dissolving the hydrogelator in basic pH by adding NaOH 1 M and then glucono- δ -lactone (GdL) was added to decrease pH homogeneously, sonicating and left stabilizing.

Development of magnetogels. The prepared nanoparticles were added to the hydrogel at a final volume of 200 μL and at the required concentration (m/m% relatively to the hydrogelator mass) from a starting solution at 2 wt%. All hydrogel/magnetogel solutions were left standing at room temperature until gel phase was attained.

Incorporation of curcumin and transport to biomembrane models. The hydrogel and magnetogels were prepared, and curcumin was added for a final concentration of 2 μM . From the hydrogelator solution, 200 μL were transferred to a fluorescence microcuvette and left standing until the hydrogel/magnetogel was formed. To study the interaction of hydrogels and magnetogels with biomembrane models, SUVs (small unilamellar vesicles) of egg phosphatidylcholine (egg-PC) and cholesterol 7:3 were prepared using the ethanolic injection technique. The lipid probe Nile Red⁴¹⁻⁴³ was incorporated into the SUVs for a final concentration of 2 μM . All studies of drug incorporation and transport into SUVs were carried out by fluorescence-based techniques (fluorescence emission, FRET and fluorescence anisotropy).

Spectroscopic measurements

General methods. Fluorescence measurements were carried out using a Fluorolog 3 spectrofluorimeter, equipped with double monochromators in both excitation and emission, Glan-Thompson polarizers and a temperature-controlled cuvette holder. Fluorescence emission spectra were corrected for the instrumental response of the system. The excitation of the hydrogelators was set at 290 nm (naproxen), and the emission spectrum was collected between 300 nm and 550 nm. Absorption spectra were recorded in a Shimadzu UV-3600 Plus UV-Vis-NIR spectrophotometer.

The fluorescence quantum yield, Φ_s , can be determined by eqn (1) (standard method),^{44,45}

$$\Phi_s = \frac{(A_r F_s n_s^2)}{(A_s F_r n_r^2)} \Phi_r \quad (1)$$

where A is the absorbance at the excitation wavelength, F is the integrated emission area and n is the refraction index of the solvents. Subscripts r and s refer to reference and sample compound, respectively. The absorbance value at excitation wavelength was always less than 0.1, in order to avoid inner filter effects. L-Tryptophan in aqueous buffer solution (pH = 7.2) was used as reference ($\Phi_r = 0.14$ at 25 $^\circ\text{C}$).⁴⁶

Fluorescence anisotropy measurements. The steady-state fluorescence anisotropy values, r , can be determined by eqn (2),⁴⁷

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (2)$$

where I_{VV} and I_{VH} are the intensities of the emission spectra obtained with vertical and horizontal polarization, respectively (for vertically polarized excitation light), I_{HV} and I_{HH} are the emission intensities obtained with vertical and horizontal polarization (for horizontally polarized excitation light) and $G = I_{HV}/I_{HH}$ is the instrumental correction factor.

FRET measurements. The drug incorporation into magnetogels network and interaction with biomembrane models was investigated by Förster Resonance Energy Transfer (FRET). FRET efficiency, Φ_{FRET} , defined as the proportion of donor molecules that

have transferred their excess energy to acceptor molecules, can be expressed by eqn (3),⁴⁷

$$\Phi_{\text{FRET}} = 1 - \frac{I_{DA}}{I_D} \quad (3)$$

where I_{DA} and I_D are the donor integrated fluorescence intensities in the presence and absence of acceptor, respectively. FRET efficiency can also be related with donor-acceptor intermolecular distance, R_{DA} , and the Förster radius (critical diameter), R_0 , through the eqn (4),⁴⁷

$$\Phi_{\text{FRET}} = \frac{1}{1 + (R_{DA}/R_0)^6} \quad (4)$$

Förster radius, R_0 , the distance at which FRET efficiency is 50%, can be determined by the spectral overlap, $J(\lambda)$, between the donor fluorescence emission and the acceptor absorption, according to eqn (5) and eqn (6) (with R_0 in $\text{\AA}$, λ in nm, $\varepsilon_A(\lambda)$ in $\text{M}^{-1} \text{cm}^{-1}$),⁴⁷

$$R_0 = 0.2108 [\kappa^2 \Phi_D n^{-4} J(\lambda)]^{1/6} \quad (5)$$

$$J(\lambda) = \int_0^\infty I_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \quad (6)$$

where $\kappa^2 = 2/3$ is the orientational factor assuming random orientation of the dyes, Φ_D is the donor fluorescence quantum yield in the absence of energy transfer, n is the refraction index of the medium, $I_D(\lambda)$ is the fluorescence spectrum of the donor normalized so that $\int_0^\infty I_D(\lambda) d\lambda = 1$, and $\varepsilon_A(\lambda)$ is the molar absorption coefficient of the acceptor.

Transmission Electron Microscopy (TEM). HR-TEM images were recorded using a Transmission Electron Microscope JEOL JEM 2010F operating at 200 kV coupled to an Electron Dispersive Spectroscopic analyzer (EDS) at C.A.C.T.I (Centro de Apoio Científico e Tecnológico á Investigación), Vigo, Spain. The samples were prepared according to the uranyl acetate staining method. After preparation of the hydrogel and magnetogel solutions, through prior dissolution at pH=10 followed by addition of GdL, a drop of each sample was placed onto a TEM 400 mesh copper grid with Formvar/Carbon (ref. S162-4 from Agar Scientific), held by tweezers and the excess solution was cleaned. A droplet of uranyl acetate was placed over the sample, the excess solution was cleaned, and the grid was left to dry. The processing of TEM images was performed using ImageJ software, which consisted in enhancing local contrast and adjusting brightness followed by selection of particles and fibres.

X-Ray Diffraction. A conventional PAN'alytical X'Pert PRO diffractometer was used for X-ray diffraction (XRD) analyses, operating with $\text{Cu K}\alpha$ radiation, in a Bragg-Brentano configuration.

FTIR measurements. Fourier Transform Infrared Spectroscopy (FTIR) measurements with Attenuated Total Reflection (ATR) were performed in a Bruker 66V Spectrometer, with a resolution of 1 cm^{-1} . The amide I region sensitivity to secondary structure was explored to assign the secondary structures. However, the variety of frequencies leads to a featureless band consisting of various component bands that are instrumentally unresolved. Thus, the second-derivative was used to identify overlapping bands and assign the corresponding bands, as it does not require any kind of arbitrary half-bandwidths and enhancement factors.⁴⁸⁻⁵² Hereby, a first approach was the direct curve fitting to the amide I after

baseline correction; the second method was curve fitting to the second derivative peaks; and the third one was the curve fitting to amide III region (1220-1330 cm^{-1}), that is mainly contributed by the in-phase combination of N-H in-plane bending and C-N stretching vibration. The amide I band is affected by the water absorption overlapping, which not only mislead the secondary structure percentages, but also worsens the ambiguity in the assignment of random coil (1642-1657 cm^{-1}) and α -helix (1648-1657 cm^{-1}) bands.⁵² Hereby, the amide I region quantitative analysis was carried out considering that α -helix does not have a significant contribution when dealing with small structures, and that only β -sheet, random coil and turns are present. The amide III region does not suffer from the water absorption signal and has a better localization of the different contributions of amide bond vibrations. However, amide III lacks its accuracy due to the overlapping of other vibrational groups and the weak distinction between random coil (1270-1255 cm^{-1}) and turn (1295-1270 cm^{-1}) frequencies.

Circular dichroism. The CD spectra were recorded at 20 °C on a Jasco model J-1500 spectropolarimeter, under a constant flow of nitrogen gas. Peptide hydrogelator 0.02 wt% solutions were loaded into 0.1 mm quartz cells. Spectra were acquired with 1 nm steps, 1 nm bandwidth and 1 second collection time per step, taking three averages. The obtained data were smoothed by an 11-point Savitsky-Golay filter to remove random noise elements from the averaged spectra.

Molecular docking studies of the hydrogelator. The crystal structure of *Mus musculus* cyclooxygenase 2 (COX-2) expressed in *Spodoptera frugiperda* complexed with naproxen (PDB code: 3NT1) and *Ovies aries* cyclooxygenase 1 (COX-1) expressed in *Spodoptera frugiperda* in complex with flurbiprofen (PDB code: 3N8Z) were used as the protein receptor models. The optimized geometries of the hydrogelator ground state were obtained from *ab initio* molecular quantum chemistry calculations with Gaussian 09 software,⁵³ and with the use of ONIOM method, setting the naproxen moiety for the high level with a 6-311+G(d,p) basis set at the DFT B3LYP level of theory and the peptide backbone for the low level at 3-21G basis set and HF B3LYP level of theory in gas phase. Docking of the receptor protein with the hydrogelator was performed using AutoDockTools-1.5.6 Software Genetic Algorithm. The calculation was set up to 50 runs, 270 000 maximum number of generations, 2 500 000 maximum number of energy evaluations, and 60×60×60 grid points for 3NT1 and 3N8Z with 0.375 Å spacing. The lowest docked energy conformation with naproxen moiety in the active site was chosen as the best conformation. Visualization of the complex protein-ligand interactions was analysed with PyMOL software.

Magnetic properties. Magnetization measurements were done in a MPMS3 SQUID magnetometer (Quantum Design). The hysteresis cycles (magnetization vs. magnetic field) of the samples were measured in the convenient field range for each sample. A specific magnetic field correction for the trapped flux in the superconducting coil was made achieving an accuracy of residual less than 2 Oe.

Rheology. The viscoelastic characterization of gels was performed with a stress-controlled rotational rheometer (MCR300, Anton

Paar). Liquid samples were loaded into the Couette geometry of the rheometer and temperature was kept at 25 °C during testing. After a three hours rest period, ensuring gel setting and structural equilibrium of samples, gels mechanical spectra were recorded using a fixed strain amplitude (values ranging from 10^{-4} to 10^{-2} % depending on the gel elasticity) and ramping the frequency from 100 Hz down to 0.01 Hz. Finally, a sweep in the strain amplitude was performed from 0.001% to 500%, to assess the linear regime of viscoelasticity and the large amplitude oscillatory strain (LAOS) regime. Experiments were repeated with softer gels in order to improve the data from the frequency sweep, using larger strain amplitudes while still belonging to the linear regimes assessed by previous strain sweeps.

Photothermia in magnetogels. Hydrogels containing curcumin (2 μM) were subjected to increasing temperatures to measure the dependence of model drug fluorescence emission on temperature. Hydrogels/magnetogels loaded with curcumin were irradiated with a setup consisting on a Xenon arc lamp (200 W) and an optical fibre, using a Thorlabs FEL0600 long pass filter with cut-on wavelength at 600 nm,³⁸ to ensure no curcumin excitation. The detection was attained using a SPEX Fluorolog 2 spectrofluorimeter for 5 hours. At the fifth hour, the irradiation was stopped and fluorescence emission was measured one hour afterwards. The dependence of fluorescence emission on temperature was used to estimate the local temperature created by excitation on plasmon band of the irradiated magnetogels.

Drug release to pH=7 buffer and biomembrane models. Hydrogel and magnetogel loaded with 20 μM curcumin were prepared. A solution of egg-PC/cholesterol SUVs (model membranes) was added to the drug-loaded hydrogel or magnetogel. The curcumin accumulation into SUVs was monitored for 8 hours, with and without irradiation of the magnetogel.

The release of curcumin to pH=7 buffer followed a similar procedure as described for biomembrane models (SUVs). Hydrogels/magnetogels loaded with 20 μM curcumin were prepared and left stabilizing overnight. The hydrogel/magnetogel was washed five times with 1 mL of pH=7 buffer and water (1.5 mL) was added to the drug-loaded hydrogel or magnetogel. Aliquots of 300 μL were taken, replaced with pH=7 buffer and curcumin fluorescence emission was measured to determine the concentration at each time point.

Cytotoxicity assay. Cell viability was evaluated by the MTT reduction assay.⁵⁴ Cells were cultured in 96-well plates (25,000 cells/well) and allowed to attach for 24 h. After incubation with Npx-L-Met-Z- Δ Phe-OH for 24 h, MTT (0.5 mg/mL final concentration) was added to each well and the plate was incubated for 75 min at 37 °C. Formazan crystals were dissolved by the addition of a DMSO:isopropanol mixture (3:1) and then quantified spectrophotometrically at 570 nm using a microplate reader (Multiskan Thermo Fisher Scientific).

Results and discussion

Synthesis of the hydrogelator compound 1. The hydrogelator methionyldehydrophenylalanine *N*-protected with 2-(6-methoxy-2-naphthyl)propionic acid (naproxen, Npx) was prepared using a conventional protocol in solution (Scheme 1).

The strategy for synthesis of hydrogelator Npx-L-Met-Z-ΔPhe-OH **1** involves the coupling of *N*-*tert*-butoxycarbonyl methionine (Boc-Met-OH, **2**) with the methyl ester of β-hydroxyphenylalanine (H-D,L-Phe(β-OH)-OMe, **3**), using the *N,N'*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt) procedure. Compound **4** was obtained as diastereomeric mixture in 84% yield. The reaction of the carboxylic acid of compound **3** gives an *O*-acylurea. The latter is very reactive and can lead to the formation of several by-products such as *N*-acylureas or oxazolones.⁵⁵ Oxazolones are usually associated with epimerisation. The addition of 1-hydroxybenzotriazole to the reaction mixture reduces epimerization, through the formation of an active ester.⁵⁵ Dehydration of the β-hydroxydipeptide **4** was attained by reaction with di-*tert*-butyl dicarbonate (Boc₂O) in the presence of 4-dimethylaminopyridine (DMAP), followed by treatment with *N,N,N',N'*-tetramethylguanidine (TMG).⁵⁶

The dehydrideptide **5** was isolated in a 60% yield. The *N*-*tert*-butoxycarbonyl group of **5** was removed with trifluoroacetic acid (TFA) and the *N*-deprotected dehydrideptide **6** was coupled with 2-(6-methoxy-2-naphthyl)propionyl chloride. The hydrogelator **1** was obtained after basic cleavage of the methyl ester of compound **7** (Figure S1 in Supplementary Information).

Scheme 1. Synthesis of hydrogelator **1**. a) DCC, HOBt, Et₃N, ACN, rt; b) (1) Boc₂O, DMAP, dry ACN, rt (2) TMG; c) TFA; d) (S)-(+)-naproxen chloride, Et₃N, DCM, rt; e) (1) NaOH (1 M), 1,4-dioxane, rt, (2) KHSO₄.

Characterization of the hydrogelator Npx-L-Met-Z-ΔPhe-OH

UV-visible absorption. The UV-visible absorption spectrum of the hydrogelator Npx-L-Met-Z-ΔPhe-OH shows the characteristic bands

of naphthalene and naphthalene-based compounds³⁶ (Figure S2 in Supplementary Information). The intense band between 210 and 240 nm, with a peak at 233 nm, is associated with the naphthalene long axis π-π* transition, while the medium band at 260-290 nm (with the absorption maximum at 273 nm) is assigned to the naphthalene short axis π-π* transition.^{36,57} Moreover, the phenylalanine absorption, which has a maximum at 260 nm,⁵⁸ and the peptide backbone n-π* and π-π* transition around 210-230 nm and 180-200 nm, respectively, also contribute to the overall spectrum.³⁶ The absorption spectrum is dominated by the naproxen moiety over phenylalanine, as expected considering the low molar absorption coefficient of the latter owing to a n-π* transition.⁵⁸

Self-assembly parameters. Fluorescence spectroscopy was used to determine the pH dependence of hydrogelator self-assembly behaviour, critical gelation and aggregation concentration, by exploring the emission of intrinsic fluorophore naproxen. The hydrogelator solutions were excited at 290 nm, as there is no direct excitation of dehydrophenylalanine, besides not changing the emission spectra, as the long-axis polarized state is the emitting state.⁵⁸ The fluorescence emission spectrum of the hydrogelator shows a main band at λ_{max}=360 nm and a second fluorescence emission band with maximum around 450 nm, which are associated to the naproxen monomer and aggregate, respectively (Figure 1A).³⁶ The monomer fluorescence emission band shows little pH dependence in the range of pH values from 4 to 7, which strongly increases at higher pH, associated with the increasing deprotonated species, thus favouring the free monomer state. However, it should be noticed that, at pH=4 and 5, a large aggregate to monomer emission intensity ratio (*I*₂/*I*₁) is attained, suggesting that self-assembly into aggregates is favourable at this pH value and decreases with increasing pH. Moreover, using the MarvinSketch software, a predicted *pK*_a value of 3.7 was obtained for the terminal carboxylic acid, which is close to the pH where *I*₂/*I*₁ maximum is verified.

Figure 1. Fluorescence emission spectra of (A) the hydrogelator (2×10^{-6} M) in the pH range 2-10 ($\lambda_{\text{exc}}=290$ nm) and (B) in the concentration range 0.2-0.8 wt% at pH=6 ($\lambda_{\text{exc}}=290$ nm). Insets: Maximum fluorescence intensity (*I*_f) variation within the pH range and intensity ratio *I*₂/*I*₁ of naproxen aggregate (*I*₂, 450 nm) and monomer band (*I*₁, 360 nm).

Thus, the behaviour of the aggregate-to-monomer intensity ratio suggests that the carboxylic acid pK_a must be close to this pH, which has been previously observed for other dehydropeptide-based hydrogelators containing naproxen.³⁶

The hydrogelator critical gelation concentration (CGC) assay was carried out in pH=6 buffer, considering that it is close to the physiological pH, while ensuring the hydrogelator presents more Npx aggregates than at pH=7. This is an advantage, considering changes in aggregation when the hydrogel gets in contact with more acidic environments, such as the tumour region. The increased concentration used in this assay (comparatively to the pH assay) shows no wavelength shifts of the monomer band, while the aggregate band is redshifted to 490 nm (Figure 1B). Moreover, in this concentration range, it is observed an incremental quenching of the monomer fluorescence emission associated with a rising aggregate band, where the minimum is attained at 0.7 wt%. The aggregate/monomer intensity ratio notably increases from 0.2 wt% to 0.3 wt%, with no changes on the monomer emission wavelength, suggesting that gelation is attained close to this concentration, where the presence of aggregates is higher. Nevertheless, a stable hydrogel could be attained at 0.1 wt%, which was gelated by dilution in a NaOH (1 M) aqueous solution (2 v/v%) followed by pH decrease with glucono- δ -lactone (GdL, 0.4 wt%).

The critical aggregation concentration was studied in the nanomolar range (Figure S3B in Supplementary Information). An enhancement of monomer fluorescence emission band is observed until a maximum at 0.025 nM. Hereby, the maximum fluorescence emission is associated with the concentration at which self-assembly/aggregation takes place at room temperature, without applying any stimulus. The low aggregation concentration might be associated with the high predicted $\log P$ value of 4.12.

Molecular docking studies of hydrogelator Npx-L-Met-Z- Δ Phe-OH

Considering the anti-inflammatory property of the naproxen moiety, that might inhibit unwanted side effects by the immune system upon administration of the gel, the specificity and selectivity of the hydrogelator **1** towards cyclooxygenase 2 (COX-2) and cyclooxygenase 1 (COX-1) was computationally evaluated. Direct docking of the crystallographic naproxen to evaluate the reliability of the defined protocol was previously reported and a root mean square deviation of 0.61 Å was calculated.⁵⁹ The direct docking of the crystallographic flurbiprofen in COX-1 (Figure S4 in Supplementary Information) attained a RMSD of 0.765 Å, thus making the defined protocol suitable.

Cross docking between the hydrogelator and cyclooxygenases followed the same procedure applied to naproxen and flurbiprofen direct docking, to evaluate the binding affinity for the active site and which parameters influence the hydrogelator stabilization in the binding pocket. The stabilizing hydrogen bonding interactions and distances to the Tyr³⁵⁵ hydroxyl group and Arg¹²⁰ guanidinium moiety⁶⁰ are represented in Figure 2. Moreover, the common hydrophobic residues Leu³⁵², Val³⁴⁹, Ala⁵²⁷, and cyclooxygenase 2 (Val⁵²³) and 1 (Trp³⁸⁷, Leu⁵³¹, Ser⁵³⁰) specific ones are also represented, due to their role in the stabilization of the crystallographic ligands (naproxen and flurbiprofen) through hydrophobic interactions.⁶⁰

The obtained lowest estimated free energies of binding, final intermolecular energy contribution in the binding pocket and the internal energy (both in bound and unbound state) that result from the AutoDock calculations are presented in Table 1.

Table 1. AutoDock results in COX-2 and COX-1 for the hydrogelator compared to naproxen in COX-2 and flurbiprofen in COX-1.

Energy (kcal/mol)	Naproxen (COX-2) ⁵⁹	Flurbiprofen (COX-1)	COX-2	COX-1
Estimated free energy of binding	-8.25	-9.10	-7.43	-6.52
Final intermolecular energy	-9.15	-10.00	-11.01	-10.10
Final total internal energy	-0.31	-0.38	-3.26	-1.24
Loss of torsional free energy	+0.89	+0.89	+3.58	+3.58
Unbound system energy	-0.31	-0.38	-3.26	-1.24

Figure 2. Representation of the best docking poses and polar interactions distances (Å) for the hydrogelator in (A) COX-2 and (B) COX-1 binding pocket obtained from PyMOL software.

The hydrogelator shows a lower free energy of binding towards the cyclooxygenases than the crystallographic ligands (naproxen and flurbiprofen). The greatest contribution is associated with the favourable intermolecular interactions established between the binding pocket and the hydrogelator.

The methionine side chain affects the orientation of naproxen moiety in the binding pocket. Thus, the hydrogelator lower affinity than the crystallographic ligands can be accounted for the higher loss of torsional free energy parameters.

The peptide backbone seems to have introduced a slight selectivity towards COX-2. Nevertheless, the results suggest that an anti-inflammatory activity through interaction with cyclooxygenases is possible, though at a lesser extent than naproxen and flurbiprofen.

Cytotoxicity assay. The effect of Npx-L-Met-Z- Δ Phe-OH hydrogelator (**1**) on the viability of rat macrophages (RAW 264.7

cells) was tested. The results show that the hydrogelator did not show any statistically significant cytotoxic effects on RAW cells up to 100 μ M (Figure 3).

Figure 3. Cell viability in the presence of Npx-L-Met-Z- Δ Phe-OH for 24 hours (three independent experiments performed in triplicate).

Synthesis of the magnetic/plasmonic nanoparticles

The core/shell magnetic/plasmonic nanoparticles were synthesised based on the direct deposition of gold onto the manganese ferrite.⁶¹ The gold-decorated nanoparticles were synthesised through CDI coupling of the nanoparticle surface OH⁻ groups to the MPA-functionalized gold nanoparticles carboxylic moiety. The obtained UV-visible absorption spectra are represented in Figure 4. From the Tauc plot (inset of Figure 4A), the optical band gap (E_g) of the manganese ferrite nanoparticles was determined and a linear relation was obtained for an indirect semiconductor with a band gap of 1.06 eV, which is similar to the previous reported values of 1.08 eV³⁷ and 0.98 eV.⁶² The gold shell resulted in the appearance of a wide plasmon band with maximum between 542 and 560 nm (Figure 4B), with a small shoulder near 850 nm.

Figure 4. Absorption spectra of (A) MnFe₂O₄ nanoparticles and (B) Core/shell MnFe₂O₄/gold and gold-decorated MnFe₂O₄ nanoparticles. Inset: (left) Tauc plot of MnFe₂O₄ nanoparticles and (right) Absorption spectrum of gold nanoparticles.

An universal scaling exponential decay of $\Delta\lambda/\lambda_0$ with the ratio between shell thickness and magnetite (Fe₃O₄) core radius was proposed,^{61,63} where $\Delta\lambda = \lambda - \lambda_0$, λ being the LSPR wavelength of the core-shell nanoparticle and λ_0 the LSPR position of the corresponding gold core. Considering cores of 9 nm, 21 nm and 48 nm, the corresponding gold nanoparticles in water exhibit LSPR bands at 517 nm, 521 nm and 533 nm, respectively.⁶⁴ Taking into account that the medium at the surface of prepared core-shell nanoparticles is expected to be composed by glycerol, for which the refractive index (1.47) is higher than that of water, a ~ 20 nm red shift in the LSPR band is expected for spherical gold nanoparticles with 40 nm size,⁶⁵ with the magnitude of the shift decreasing with lowering particle size. Therefore, the LSPR of the considered gold cores are predicted to be at < 537 nm, < 541 nm and > 553 nm. The LSPR of core-shell nanoparticles would then be, respectively, < 758 nm, < 853 nm and > 916 nm, for a 1 nm shell and < 538 nm, < 579 nm and > 691 nm for a 10 nm shell. However, the red-shifted plasmon band arising from coupling of the gold shell with the core medium was found to be damped when the core imaginary refractive index increases,⁶³ remaining an anti-coupled mode slightly deviated to the blue. MnFe₂O₄ is expected to have a higher imaginary component of the refractive index than magnetite, as the indirect band gap is much lower than that of Fe₃O₄, which has been reported to be 2.12 eV.⁶⁶ Thus, the obtained absorption spectrum of the Au@MnFe₂O₄ nanoparticles is compatible with near 50 nm core and 1 nm gold shell, with the anti-coupled mode appearing below 553 nm, or, considering that the coupled mode is not damped, a core near 20 nm and gold shell above 10 nm.

The prepared spherical gold nanoparticles have its characteristic surface plasmon resonance band centred around 530 nm, which can be associated to nanoparticles with a size in the range 5-10 nm, comparing to gold nanoparticles capped with thiol ligands published in other works.^{39,67} Moreover, the colour shown upon nanoparticle precipitation is not associated with aggregation of nanoparticles, as no absorption band around 700 nm is observed.³⁹ This means that the capping layer is sufficiently long to avoid coupling between gold nanoparticles. After coupling with manganese ferrite nanoparticles, the contribution from the gold nanoparticles plasmon band is observed around 550 nm. This shift indicates that gold nanoparticles have been effectively bonded to manganese ferrite nanoparticles.

X-Ray Diffraction. The X-ray diffraction (XRD) pattern of manganese ferrite nanoparticles presents well-defined peaks (Figure 5), suggesting a structure of crystalline nature, even without calcination and after addition of the gold shell, as previously reported.^{37,38} The gold-decorated manganese ferrite nanoparticles show a neat amorphous contribution associated with the organic layer of the MPA-capped gold nanoparticles (Figure 5C). Nevertheless, diffraction peaks of gold (Figure 5B,C) are observed at $2\theta = 38.1^\circ$ (1 1 1), 44.4° (2 0 0), 64.6° (2 2 0), 77.6° (3 1 1) and 81.8° (2 2 2), corresponding to CIF 9013035 (space group Fm-3m). Diffraction peaks of the MnFe₂O₄ crystalline structure (Figure 5A) are observed at $2\theta = 29.7^\circ$ (2 2 0), 34.9° (3 1 1), 36.5° (2 2 2), 42.5° (4 0 0), 52.7° (4 2 2), 56.2° (3 3 3) and (5 1 1), 61.8° (4 4 0), 65.0° (5 3 1), 70.1° (6 2 0), 73.1° (5 3 3), 74.0° (6 2 2), 78° (4 4 4), 85.6°

(6 4 2), 88.5° (7 3 1) and (5 5 3), corresponding to CIF file 23000618 (space group Fd-3m:2).

Figure 5. X-ray diffraction pattern of (A) manganese ferrite nanoparticles; (B) core/shell manganese ferrite/gold nanoparticles; (C) manganese ferrite decorated with gold nanoparticles. Gray lines: experimental patterns; black lines: fitted patterns. Background (A,B) and amorphous background (C): short-dashed lines. Miller indices: Black: manganese ferrite; Red: gold.

Rietveld analysis was performed using FullProf software suite⁶⁸ (details in Supplementary Information). The calculated parameters and phase sizes are presented in Table 2.

The manganese ferrite phases have an average size of approximately 34.8 nm, that is found to increase to 48.8 nm upon the thermal treatment in glycerol that leads to formation of the gold shell.³⁸ The gold phase in the core/shell nanoparticles is estimated to be 22.6 nm, resulting in 94 nm overall particle size. But several studies report the absence of core diffraction peaks when the gold shell has a thickness higher than 2 nm,^{69,70} or a huge intensity decrease with gold shell up to 8.5 nm.⁷¹ In the latter case, a 13 nm core is observed to have a broader diffraction peak than a 2.5 nm shell. On the other hand, gold shell diffraction peaks seem to have distinct broadening effects, as for 10 nm magnetite core coated with a 2 nm gold shell the diffraction peak widths were identical (where its dimensions were obtained from TEM measurements).⁶⁹ Thus, in the obtained core/shell nanoparticles, either the gold shell is incomplete, or a thin shell is present. The

obtained gold weight percentage is only 7.1%. If the sizes obtained from the Rietveld analysis were the correct ones, and even considering that the size of gold phase corresponds to the double of the thickness (a given X-Ray when interacting with a core-shell nanoparticle encounters gold phase two times), a 90% value would be expected (71.4 nm overall size). This means that only 8% of the MnFe₂O₄ nanoparticles would have been coated. Considering a 1 nm shell thickness (50.8 nm overall size), then 33% weight percentage is expected, with a corresponding more reasonable 21% value for the coating efficiency.

For the gold-decorated nanoparticles and considering again that the gold phase size given by the Rietveld analysis corresponds to the double of the gold nanoparticles size, the obtained gold weight percentage indicates that the average number of gold nanoparticles per MnFe₂O₄ is 16.8. This value would be 2.1 if the gold phase size corresponds to the gold nanoparticles size.

Table 2. X-ray diffraction Rietveld refinement calculated parameters R_f and χ^2 , phase sizes and percentages. MnFe₂O₄/Au: core/shell nanoparticles; Au@MnFe₂O₄: gold-decorated MnFe₂O₄ nanoparticles.

Nanoparticles	Intensity percentages	Phase size (nm)		Quality Parameters	
		Lattice Constant (Å)		MnFe ₂ O ₄ Au	
		MnFe ₂ O ₄	Au	R_f	χ^2
MnFe ₂ O ₄	100	---	---	7.88	1.03
MnFe ₂ O ₄ /Au	92.9	7.1	34.8	7.01 3.52	0.93
			48.8		
Au@MnFe ₂ O ₄	88.9	11.1	22.6	9.32 6.00	1.53
			48.83		
			4.076		
			34.8 (*)		
			8.64		
			8.486		
			4.085		

(*) fixed value

Development of magnetogels

The structure of the hydrogel is emphasized in Figure 6A and 6B, where it can be observed that the hydrogel matrix comprises a fibrous structure, with fibres exhibiting average cross-section of 16.6±2.4 nm, the thinnest being 7 nm and the thickest 21 nm. The fibres attain diameters larger than 2 µm.

Magnetogels were developed by mixing magnetic nanoparticles with the hydrogelator solution at pH=10 and left incubating at room temperature for 4 hours (figure 6C). During the self-assembly process trigger, several phenomena will be competing, mainly the sedimentation and aggregation of nanoparticles, the formation of fibrils and coating of the nanoparticles. Hereby, the magnetogels were previously evaluated at 10, 20 and 30 m/m% concentration of nanoparticles for 0.3 wt% of hydrogelator, as no magnetogel was possible to be formed at a lower hydrogelator concentration. A homogenous dispersed magnetogel was obtained for 10 m/m% (Figure 6D, 6E and 6F) owing to the rapid gelation, while for 20 m/m% core/shell nanoparticles a gradient was obtained, and at 30 m/m% both nanoparticles sedimented.

Figure 6. (A) STEM image of hydrogel in bright field (scale bar: 1 μm). (B) STEM image of hydrogel in dark field (scale bar: 2 μm). (C) Scheme of the gelation strategy of hydrogel and magnetogel. (D-F) Images of the glass vial inversion test of the magnetogel containing (D) core/shell nanoparticles 10 m/m%; (E) gold-decorated nanoparticles at 10 m/m% and (F) at 20 m/m%. The magnetogels show a homogeneous dispersion and no visible deposition.

Rheological properties. The mechanical spectra of equilibrated Npx-L-Met-Z- $\Delta\text{Phe-OH}$ hydrogel and magnetogels (10, 20 and 30 m/m%) are shown in Figure 7. The rheological data measured for magnetogels formulated with 10 m/m% nanoparticles underline the effects of the nanoparticles chemical modification on hydrogel elasticity. The addition of gold-decorated manganese ferrite nanoparticles results in the reinforcement of the hydrogel elasticity, as G' is five times larger (Figure 7A). In contrast to this, the addition of 10 m/m% core/shell nanoparticles leads to a significant reduction (10 times) of the gel elasticity (Figure 7B).

Depending on the function and location of the tissue, the elastic modulus of native tissues ranges from 0.1 kPa (brain) to 100 kPa (cartilage).⁷² The developed hydrogel and magnetogels elastic modulus fall in the range 1 kPa to 100 kPa, which covers soft tissues such as skin, pancreas, spleen, glands and muscles.⁷² Therefore, from a mechanical perspective, the hydrogel and magnetogels elastic properties seem to be promising for biomedical applications.

Figure 7. Frequency dependence of the shear elastic modulus G' for hydrogels formulated with 0 m/m% (squares), 10 m/m% (circles), 20 m/m% (triangles) and 30 m/m% (diamonds) of gold-decorated nanoparticles (A) or core/shell nanoparticles (B). Insets: mechanical spectra (G' , empty symbols; G'' , solid symbols) of the hydrogel (squares) and of the magnetogels formulated with 30 m/m% nanoparticles (diamonds).

Relatively to previous reported hydrogels with similar chemical nature and prepared at similar concentration, the methionine residue hydrophobic collapse seems to have reinforced the elasticity of the hydrogel (4 kPa at 0.3 wt%) compared to hydrogels containing phenylalanine (Npx-L-Phe-Z- $\Delta\text{Phe-OH}$, 1.7 kPa at 0.4 wt%), valine (Npx-L-Val-Z- $\Delta\text{Phe-OH}$, 0.7 kPa at 0.6 wt%) or alanine (Npx-L-Ala-Z- $\Delta\text{Phe-OH}$, 0.8 kPa at 0.8 wt%), being similar to the tryptophan containing hydrogel (Npx-L-Trp-Z- $\Delta\text{Phe-OH}$, 4 kPa at 0.4 wt%).^{34,36} Thus, a long hydrophobic residue close to the N -terminal aromatic moiety is expected to favour a higher elasticity. The addition of more nanoparticles changes the picture, since the viscoelastic features of the hydrogel are progressively recovered. As a result, adding 30 m/m% nanoparticles does not bring any significant viscoelastic change to the hydrogel (mechanical spectra of gels at 20 m/m% are presented in Supplementary Information). Nonlinear viscoelastic data were recently shown to be efficient in revealing the hierarchical structures of gelatin gels.⁷³ Therefore, large amplitude oscillatory shear strain sweeps (LAOSS) were performed in an attempt to assess possible structural differences in gels prepared with the two types of particles. The strain dependence of the ratio G'/G_0 (G_0 being the value of the shear modulus G' measured at 1 Hz in the linear viscoelastic regime) is presented in Figure 8.

Overall, the strain dependence of the ratio G'/G_0 indicates a strain hardening behavior for all samples but the gel formulated with 20 m/m% gold-decorated manganese ferrite nanoparticles. Strain hardening, characterized by an increase in G' with the strain, is the nonlinear mechanical signature of a wide range of gels with structures ranging from networks of semi-flexible filaments to networks of strongly linked fractal flocs of colloidal particles.⁷⁴⁻⁷⁶ Magnetogels formulated with the gold-decorated manganese ferrite nanoparticles are more sensitive to shear deformation, since the critical strain beyond which the strain hardening kicks in is smaller (0.2% against 0.4%). In addition, these magnetogels exhibit a more intense hardening, as the maxima in G'/G_0 are larger than those measured for hydrogels filled with bare core/shell nanoparticles.

Figure 8. Strain dependence of the shear elastic modulus G' scaled by its value (G_0) measured in the linear regime for hydrogels formulated with gold-decorated nanoparticles (A) and with core/shell nanoparticles (B): 0 m/m% (lines), 10 m/m% (circles), 20 m/m% (triangles) and 30 m/m% (diamonds). Inset: Strain dependence of the elastic (G' , squares) and loss (G'' , circles) moduli for hydrogels formulated with 0 m/m% (lines) and 30 m/m% (symbols) of nanoparticles.

All gels showed the Payne effect, as can be seen in the inset of Figure 8, for the hydrogel and magnetogels doped with 30 m/m% nanoparticles. The Payne effect has been reported for a large variety of viscoelastic matrices reinforced by solid fillers and is characterized by a local maximum in G'' , which is concomitant with a significant decrease of G' with the increasing strain. Such effect can be associated with the breakage and recovery of weak interaction bonds linking adjacent filler clusters, the aggregation/disaggregation of nanoparticles, or the molecular disentanglement.⁷⁷ Nevertheless, data displayed in Figure 8 indicate that the nonlinear viscoelastic response of samples is sensitive to differences in particle-particle and particle-hydrogelator interactions.

Magnetogels characterization. The addition of core/shell nanoparticles prior to solution pH increase with NaOH resulted in the formation of irreversible aggregates, which was confirmed by UV-visible absorption spectroscopy (Figure S6 in Supplementary Information). Therefore, core/shell nanoparticles could only be added to the solution after pH increase, while the gold-decorated ones did not required this specific procedure.

The intrinsic fluorescence of hydrogelator naproxen moiety allows following the incorporation of magnetic nanoparticles into the hydrogel through changes on the photophysical behaviour. The fluorescence spectra show a maximum emission at 360 nm, associated with the monomer state of the naproxen moiety, and the aggregates band around 450 nm³⁶ (Figure 9A). The magnetic nanoparticles introduce several photophysical effects, mainly the fluorescence quenching. Such effect can be a result from the electronic energy transfer to the nanoparticles owing to its wide absorption spectrum, but also due to the heavy atom effect, where the presence of heavy atoms enhances the spin-orbit interaction that favours the intersystem crossing.⁴⁷

However, more effects are included due to the localized surface plasmon of the gold shell. The near-field enhancement close to the nanoparticles will increase absorption and fluorescence, where the

latter is enhanced owing to the coupling of the lowest order plasmonic modes (electric dipolar mode) to the far-field.⁷⁸⁻⁸⁰

Figure 9. (A) Fluorescence emission spectra of hydrogel (H) and magnetogels with core/shell (CS) and gold-decorated (D) nanoparticles, 10 m/m%. (B) Hydrogel and magnetogels amide I infrared absorption spectra. (C) Hydrogel and magnetogels circular dichroism spectra of the hydrogelator solution at 0.02 wt%.

Nevertheless, the obtained fluorescence quenching evidences the predomination of the non-radiative mechanisms on the magnetogels. On the other hand, the introduction of gold-decorated manganese ferrite nanoparticles surface functional groups might hinder the proximity of the fibres or modify its structure, as denoted by the higher monomer-to-aggregate intensity ratio and the blueshift in monomer emission (Figure 9A). Moreover, the naproxen moiety fluorescence anisotropy values suggest that the hydrogel ($r = 0.113$) fibrils have suffered a structural change with the introduction of the core/shell ($r = 0.043$) and gold-decorated ($r = 0.035$) nanoparticles.

Figure 9B displays the FTIR absorption spectra in the amide I region ($1600-1700\text{ cm}^{-1}$). In this region, the main contribution for infrared absorption is the C=O stretching, which is sensible to the protein secondary structure owing to the hydrogen bonding pattern and molecular geometry. The second derivative shows a peak around 1625 cm^{-1} and $1639-1641\text{ cm}^{-1}$ that can be assigned to β -sheet and random coil, respectively (Figure S7 in Supplementary Information). Other peaks were identified at 1668 cm^{-1} and 1685 cm^{-1} , being associated with the presence of turns and loops, while a peak at 1699 cm^{-1} might be a result of strong hydrogen bonds between β -sheets or β -strands aggregates.⁴⁹ The major difference is that magnetogels have a decrease in intensity of the bands of ordered structures, suggesting that, in the presence of nanoparticles, the hydrogelators are more randomly oriented. Table 3 displays the percentages of secondary structures obtained for the hydrogel and magnetogels. The results show a close agreement between the amide III and second derivative curve fitting method, mainly when the percentages of ordered (α -helix and β -sheet) and unordered (random coil and turns) structures are compared. Overall, a reduction of ordered secondary structures was obtained on the magnetogels when compared to the hydrogels, which is accompanied by an increase of unordered structures. Hereby, a higher percentage of unordered structures might reduce the compactness of the well-ordered self-assembled structures, owing to the lack of ordered hydrogen bonding, which not only might induce thicker fibres than in the hydrogel, but also a less stable matrix.

Table 3. Percentage of secondary structures according to the ranges reported in literature,^{48–52} obtained through curve-fitting of the amide I band (AI), amide I second derivative (ASD) and amide III band (AIII). Legend: OC: ordered structures (α -helix and β -sheet); UC: unordered structures (turn and random coil).

System	H			CS			D		
Method	AI	ASD	AIII	AI	ASD	AIII	AI	ASD	AIII
α -helix	0	0	9.8	0	0	13.3	0	0	12.8
β -sheet	45.8	28.1	19.7	41.6	24.3	13.0	34.4	25.2	13.0
Turn	19.9	17.0	23.0	9.4	24.5	33.9	41.2	16.3	32.0
Random coil	34.3	54.9	47.4	49.0	44.2	39.8	24.4	58.5	42.2
OC	45.8	28.1	29.6	41.6	26.1	26.3	34.4	25.2	25.8
UC	54.2	71.9	70.4	58.4	73.9	73.7	65.6	74.8	74.2
χ^2	4×10^{-7}	1×10^{-11}	3×10^{-8}	4×10^{-8}	3×10^{-11}	7×10^{-10}	2×10^{-7}	6×10^{-12}	1×10^{-10}

A molecule is optically active in circular dichroism (CD) if the chromophore contains an asymmetric (chiral) environment or display an induced chirality owing to three-dimensional bonding structures.^{81–83} Hereby, the spectrum corresponds to the sum of its conformational elements.⁸³ The β -sheet structures are characterized by a sharp positive band in the region 195–210 nm and a broad negative band in the region 215–230 nm, while α -helices display negative ellipticity at 222 nm and 208 nm, and positive ellipticity at 193 nm.⁸³ The random coil structures have a weak positive band at 217 nm and a large negative near 200 nm.⁸³ These bands are associated with the peptide bond transitions π - π^* and n - π^* , which are centred around 190 nm and 220 nm, respectively.^{36,84} The obtained CD spectra of diluted hydrogelator solutions, with and without nanoparticles, are represented in Figure 9C. The solutions show similar spectral shape, where an increase of ellipticity is observed in the presence of core/shell nanoparticles and a red-shift for the magnetogel containing gold-decorated nanoparticles. Overall, the exciton couplets are evidenced by a negative cotton effect with a broad negative ellipticity centred at 220 nm and a slow ellipticity decrease for lower wavelengths, which suggest a predominance of β -sheets and that hydrogelator molecules establish similar intermolecular interactions in hydrogel and magnetogels. Nevertheless, the naphthalene moiety commonly shows exciton couplets between 200 and 230 nm, besides being stronger in magnitude than the peptide transitions.⁸⁵ Thus, the broad negative ellipticity arises from the sum of contributions of secondary structure and naphthalene exciton couplets.

Transmission Electron Microscopy. The transmission electron microscopy (TEM) images of the magnetic nanoparticles and magnetogels are presented in Figure 10. Core/shell manganese ferrite/gold nanoparticles show an average size of 55 ± 14 nm, while the gold-decorated manganese ferrite nanoparticles show diameters of 45.7 ± 8 nm containing gold nanoparticles with 3.8 ± 0.9 nm (size histograms of the nanoparticles in Figure S8 of Supplementary Information). The electron dispersive X-ray (EDX) spectra evidence the presence of gold in both types of nanoparticles (Figures S9 and S10 in Supplementary Information). For the core-shell nanoparticles, subtracting (from the obtained average size) the value of MnFe_2O_4 core diameter that resulted through XRD Rietveld analysis, the shell thickness can be estimated as 3 nm. But the defect on Au coating seen in the darker zone of Figure 10-A3 seems to indicate a much thinner shell layer. Fast

Fourier Transform (FFT) of this figure shows bright spots that prove the existence of both MnFe_2O_4 and Au phases (Figure 10-A4). Specifically, these spots appear at 2.98, 2.66, 1.70, 1.58, 1.45 and 1.34 Å, corresponding to d-spacing values of the MnFe_2O_4 spinel crystal structure with 8.483 Å lattice constant of 3.00, 2.56, 1.73, 1.63, 1.43 and 1.34 Å, where the (h k l) Miller indices are indicated in the figure. The Au phase originates spots at 1.45, 1.08 and 0.9 Å, that are assignable to d-spacing values of the Au fcc crystal structure with 4.076 Å lattice constant of 1.44, 1.02 and 0.91 Å. The bright spots at low spacing values (~ 0.4 Å and 0.8 Å) and marked with a dark arrow, are found to be instrumental artifacts, as they appear in the FFT of images with different resolutions at exactly the same pixel positions (data not shown). FFT of circular spots in the lighter and darker regions of Figure 10-A3 (Figure S11 in Supplementary Information) shows that the gold spots mainly originate from the darker region.

For the gold decorated MnFe_2O_4 nanoparticles, the estimation of overall particle size was very difficult due to extended agglomeration in the TEM grid. That explains why the obtained average value of 45.7 nm is higher than the sum of the size of MnFe_2O_4 phase obtained from XRD Rietveld analysis (34.8 nm) with that of the Au nanoparticles obtained from TEM (3.8 nm). Nevertheless, the size of gold nanoparticles from TEM imaging is similar to half the value of the size determined from XRD for the gold phase ($7.88/2 = 3.94$ nm), confirming the hypothesis that, in average, the X-rays at a given diffraction angle, detected two gold nanoparticles per MnFe_2O_4 core. The FFT of image in Figure 10-B3 also shows bright spots that confirm the presence of both Au and MnFe_2O_4 phases. Specifically, these spots appear at 4.72, 2.98, 2.54, 1.89, 1.61, 1.51 and 1.42 Å, corresponding to d-spacing values of the MnFe_2O_4 spinel crystal structure with 8.486 Å lattice constant of 4.90, 3.00, 2.56, 1.94, 1.63, 1.50 and 1.41 Å. For Au, the bright spots are observed at 2.38, 1.98 and 1.42 Å and are attributable to d-spacing values of the Au fcc crystal structure with 4.085 Å lattice constant of 2.35, 2.04 and 1.44 Å.

In Figures 10-C1 and 10-C2, it can be observed that the nanoparticles work as template surfaces for the growth of the hydrogel fibres, which results in a porous structure with an average pore size of 82.2 ± 44.6 nm, where the smallest pore is 20 nm and the largest is 223 nm. The fibres have an average cross section of 11.2 ± 3.6 nm, the thinnest being 5.6 nm and the thickest 21 nm. The high density of nanoparticles and fibre crosslinks suggest that the gels are highly saturated at 10 m/m% of nanoparticles.

Figure 10. Transmission electron microscopy images of (A) Core/shell manganese ferrite/gold nanoparticles; (B) gold-decorated manganese ferrite nanoparticles. A4 and B4 are the Fast Fourier Transform of the respective nanoparticles, where some gold planes (yellow) and MnFe_2O_4 planes (white) were indexed. (C) TEM image of Npx-L-Met-Z- Δ Phe-OH (0.08 wt%) magnetogel containing 10 m/m% core/shell manganese ferrite/gold nanoparticles (C1) and gold-decorated manganese ferrite nanoparticles (C2).

Magnetic properties. The magnetic properties can be determined from the magnetic hysteresis loop, which relates the induced magnetic moment with the applied magnetic field (H). It can be observed that the maximum magnetization (emu/g) is higher for the core/shell nanoparticles than for the decorated ones (Figure 11). This behavior can be explained considering the high amorphous content of the decorated nanoparticles due to the organic component, as these nanoparticles are functionalized with MPA. On the other hand, the result for core/shell nanostructures is consistent with a thin gold shell, as the saturation magnetization is much higher than the previously reported for MnFe_2O_4 nanoparticles with a ~ 5 nm gold shell³⁸ (Table 4).

Figure 11. Magnetization hysteresis loops of manganese ferrite/gold nanoparticles measured at room temperature ($T=300$ K). Inset: Enlargement of the loops in the low field region.

Table 4. Coercive field (H_c), saturation magnetization (M_s), remnant magnetization (M_r), and ratio M_r/M_s for core/shell manganese ferrite/gold nanoparticles and gold-decorated manganese ferrite nanoparticles, at room temperature ($T=300$ K).

	H_c (Oe)	M_s (emu/g)	M_r (emu/g)	M_r/M_s
Core/shell	63.2	17.1	2.04	0.12
Decorated	39.1	6.15	0.52	0.08

As evidenced from the M_r/M_s ratio, which is around 0.1 for both types of nanoparticles, both nanoparticles present roughly a superparamagnetic behaviour.⁸⁶

Therefore, both nanoparticles design shows promising results for magnetic targeting and hyperthermia, though the gold-shell manganese ferrite nanoparticles design represents an improvement due to the reduction of the diamagnetic contribution of gold. The presence of both plasmonic and magnetic materials with promising magnetic properties is expected to synergistically enhance both photodynamic therapy and hyperthermia (through the combined use of magnetic hyperthermia and phototherapy), to allow the use of the unique modality magnetomotive photoacoustic imaging,⁶¹ and to guarantee the retention of nanoparticles in the target site (through a magnetic field gradient) to avert the reoccurrence of cancer even when the hydrogel has been degraded/absorbed.

Drug encapsulation. Curcumin, here used as model drug, is barely soluble in water,⁸⁷ and its fluorescence emission will be an indicator of its presence in the hydrophobic cavities or near the fibres. The spectral overlap between the hydrogelator aromatic groups fluorescence and curcumin absorption shows that Förster resonance energy transfer (FRET) is possible, which allows following the incorporation of curcumin into the hydrogel/magnetogels (Figure S12 in Supplementary Information).

The fluorescence emission (around 500 nm) of curcumin due to FRET from the hydrogelator is overlapped by the aggregates emission band (Figure 12). However, direct excitation at 420 nm results in a strong emission from curcumin in all gels (insets of Figure 12), indicating that curcumin is in hydrophobic domains of the systems network (near the gel fibres), as it does not emit fluorescence in aqueous environments. Highly similar quenching effect of the monomer band was obtained, and similar curcumin to monomer emission ratio was also verified. This demonstrates that the nanoparticles did not significantly affect the distance of curcumin relatively to the fibrils, as the quenching effect on the gels due to FRET from hydrogelator aromatic moieties to curcumin remains similar. However, it is observed that, for the three systems, curcumin emits fluorescence with different bandwidth, shape and wavelength maximum (Figure 12, insets). The magnetogel containing gold-decorated nanoparticles seems to have the stricter variety of environments for curcumin location, owing to its sharper emission band and blue-shifted fluorescence maximum. Thus, in this system, curcumin is restricted to more hydrophobic environments than in the magnetogel based on core/shell nanoparticles and in the hydrogel. Such hydrophobic environments might be associated with the long chain functional groups at the surface of the nanoparticles or changes on the fibres organization due to nanoparticle-fibre interactions.

Figure 12. Fluorescence emission spectra of (A) Hydrogel, (B) Magnetogel with core/shell manganese ferrite/gold nanoparticles (10 m/m%) and (C) Magnetogel with gold-decorated manganese ferrite nanoparticles (10 m/m%), with (w/) and without (w/o) curcumin ($\lambda_{exc} = 290$ nm). Insets: Fluorescence emission spectra of directly excited curcumin ($\lambda_{exc} = 420$ nm).

Fluorescence anisotropy values of curcumin in the hydrogel ($r_H = 0.29$) and magnetogels ($r_{core/shell} = 0.11$ and $r_{gold-decorated} = 0.25$) demonstrate that the introduction of nanoparticles produced an increase on the fluidity of curcumin microenvironment, which can be associated with a different arrangement in the fibres promoted by the nanoparticles, especially in the system containing the core-shell ones. Moreover, the high anisotropy values evidence a high affinity of curcumin towards the matrix, even though it can also be related to more compact fibres and a denser network. The hydrogelator fluorescence quantum yield was determined through eqn. (1), where a value of 0.041 was obtained. The calculated FRET efficiencies (Φ_{FRET}), Förster radius (R_0), and donor-acceptor distances (R_{DA}) are presented in Table 5. The

introduction of the nanoparticles did not produce a significant change on the distance between fibre aromatic groups and curcumin in all the nanosystems.

Table 5. FRET efficiency (Φ_{FRET}), Förster radius (R_0) and donor-acceptor distances (R_{DA}) from the interaction of curcumin with the hydrogel (H), with magnetogel based on core/shell nanoparticles (CS) and with magnetogel containing gold-decorated nanoparticles (D).

Nanosystem	Φ_{FRET}	R_0 (nm)	R_{DA} (nm)
H	0.72	2.9	2.6
CS	0.73	2.9	2.4
D	0.73	2.8	2.4

Drug delivery to biomembrane models. The delivery of curcumin into small unilamellar vesicles (SUVs) used as models of biomembranes was investigated by FRET, from curcumin (energy donor) to Nile Red (energy acceptor). Nile Red is a solvatochromic probe that intensely emits fluorescence in non-polar environments, but has almost negligible emission in water, the emission being accompanied by a blueshift with reduction of polarity.^{41-43,88} The high hydrophobic character of Nile Red leads to its preferential location in the lipid membranes, which can be explored to follow up the interaction between hydrogels/magnetogels and membrane models. Hereby, curcumin was loaded into the hydrogel or magnetogel and Nile Red into the SUVs. FRET can occur as evidenced by the overlap of curcumin fluorescence emission and Nile Red absorption (Figure S13 in Supplementary Information). Although Nile Red can be directly excited at 420 nm (curcumin excitation) such is highly inefficient, thus the increased fluorescence will be a result of the FRET process (Figure 13).

The interaction of magnetogel/hydrogel with membrane models can occur either by SUVs penetration into the hydrogel/magnetogel matrix, or through drug delivery. In the latter, the drug incorporated in the gel diffuses into the biomembranes. The gels might have small cavities, as no FRET was obtained in the hydrogel/magnetogel matrix, i.e. the biomembrane models did not penetrate the matrix. Nevertheless, the aqueous phase containing the added SUVs showed curcumin fluorescence emission and FRET to Nile Red about one hour after its addition. The curcumin emission after interaction is compared to the one of curcumin in SUVs, for the same final concentration (Figure 13).

The curcumin emission decrease (compared to SUVs containing only curcumin) and the enhanced Nile Red fluorescence indicate that drug delivery has occurred upon interaction between the gel and SUVs. Moreover, comparison with curcumin inside the hydrogel/magnetogel reveals that curcumin has localized to a more hydrophobic environment (biomembranes). Also, as the maximum emission wavelength of Nile Red after interaction is similar in SUVs, such suggests that the gel did not interfere on the organization of the biomembranes.

Donor-acceptor (curcumin-Nile Red) distances in SUVs were calculated from FRET data, using equations (3)-(6), being similar in all cases (Table 6).

Figure 13. Fluorescence emission spectra ($\lambda_{\text{exc}}=420$ nm) of SUVs of egg-PC/cholesterol 7:3 containing Nile Red, before and after interaction with curcumin-loaded hydrogel (A), magnetogel containing core/shell nanoparticles (B) and magnetogel containing gold-decorated manganese ferrite nanoparticles (C). Emission of curcumin in SUVs is also shown for comparison.

Table 6. FRET efficiency (Φ_{FRET}), Förster radius (R_0) and donor-acceptor distances (R_{DA}) between curcumin and Nile Red in SUVs, after drug delivery from the hydrogel (H), the magnetogel based on core/shell nanoparticles (CS) and the magnetogel containing gold-decorated nanoparticles (D).

Nanosystem	Φ_{FRET}	R_0 (nm)	R_{DA} (nm)
H	0.46	2.6	2.7
CS	0.41	2.6	2.8
D	0.49	2.8	2.6

Photothermia assays. For the study of photothermal ability, the emission of curcumin loaded in magnetogels was measured under irradiation (at $\lambda > 600$ nm, to ensure the excitation of only the gold) along time. Upon irradiation, the absorbed light is converted into heat through non-radiative processes that are associated with the fast phase loss of the coherently excited electrons.^{31,32} The heat generated by the nanoparticles will be transferred to the medium, which is accompanied by the formation of vapor nanobubbles, where the expansion occurs until cooling and the collapse leads to a stress wave that propagates in the surrounding medium.⁸⁹ Hereby, curcumin was used as a fluorescence thermometer to determine the mean temperature in the vicinity of the nanoparticles surface when embedded inside the hydrogel matrix. If the fluorescence quantum yield of curcumin close to the nanoparticles is negligible, then the dependence of the fluorescence intensity (I_F) on temperature can be expressed as eqn. (7) (see detailed explanation in Supplementary Information):

$$\frac{I_F^0}{I_F} \propto A e^{-(E_a/RT)} \quad (7)$$

where I_F^0 is the fluorescence intensity without irradiation, A is a pre-exponential constant and E_a is the activation energy.⁹⁰ Accordingly, for the curcumin-loaded hydrogel, the plot of logarithm of (I_F^0/I_F) as a function of $1/T$ is linear, with a slope of -61 K (Figure S14 in Supplementary Information), when temperature is changed from 20°C to 60°C . The obtained logarithm of fluorescence intensity ratio for curcumin in the magnetogels subjected to continuous irradiation ($\lambda > 600$ nm) allowed extrapolation of the nanoparticles surface temperature through the above determined parameter. The results qualitatively demonstrate

that a heating effect is being produced upon continuous irradiation of the magnetogels (Figure 14). When the irradiation is stopped, a temperature decrease (fluorescence increase) is observed. Therefore, the heating effect is reversible, as demonstrated in a two cycle photothermia experiment in Figure 14B, even though the maximum temperature is slightly decreased in the second cycle. Hereby, it is expected that irradiation will enhance drug release from the magnetogel to the medium.

Figure 14. Temperature variation through irradiation of curcumin-loaded magnetogels containing core/shell manganese ferrite/gold nanoparticles (CS) and gold-decorated manganese ferrite nanoparticles (D) at 10 m/m% of nanoparticles. (A) First irradiation cycle. (B) Subsequent cycles of 5 hours.

Although quantitative information cannot be inferred owing to the several processes involved, the gold-decorated nanoparticles exhibit a high potential for photothermia. Such might be associated with the high gold nanoparticle concentration and the surface functionalization that favours a larger accumulation of curcumin around the nanoparticle cluster and higher temperatures, as heat will have a higher difficulty to dissipate than in the non-functionalized core/shell nanoparticles.

However, thermal degradation of curcumin must not be excluded (which further reduces fluorescence), besides the increased non-radiative relaxation rate by the heating of gold nanoparticles. The thermal degradation of curcumin results in the formation of vanillin, ferulic acid and 4-vinyl guaiacol that are reported to have biological activity (antioxidant and anti-inflammatory).⁹¹

Drug release assays. Curcumin release from hydrogel and magnetogels was evaluated with and without excitation of the nanoparticles plasmon band. The nanosystems exhibit an initial burst release, followed by a slower release phase, where a similar behaviour was observed either in hydrogel and irradiated magnetogels on the first eight hours. The obtained results for plasmonic magnetogels were also compared to magnetogels containing non-coated manganese ferrite nanoparticles, to assess the influence of the gold coating on drug release. An interaction between curcumin and gold-containing nanoparticles is evidenced by the drug release profiles (Figure 15), as a lower curcumin concentration and slower drug release kinetics are observed for the non-irradiated magnetogels, while a higher release was obtained for the manganese ferrite containing magnetogels and hydrogel.

Figure 15. Cumulative drug release profiles (3 replicates) of curcumin (20 μ M) in hydrogel (H), magnetogel containing 10% core/shell $\text{MnFe}_2\text{O}_4/\text{Au}$ nanoparticles (CS), magnetogel with 10% gold-decorated MnFe_2O_4 nanoparticles (D), and magnetogel containing 10% non-coated MnFe_2O_4 nanoparticles (Mn), with (IR) and without incidence of radiation, for a contact area of 3.8 cm^2 .

Therefore, the results obtained with non-coated manganese ferrite nanoparticles suggest that the incorporation of nanoparticles opens the hydrogel pores and facilitates drug release, while nanoparticles capable of interacting with the loaded drug (core/shell and gold-decorated nanoparticles) will hinder its release. The photothermia effect of core/shell nanoparticles on curcumin release is reflected by the enhanced release compared with the non-irradiated magnetogel. Hydrogel incubation in water at room temperature resulted in a 2% swelling increase, while incubation at 40°C reduced the hydrogel swelling in 11%. Thus, the drug release may be associated with the heating-induced gel contraction. However, the difference in drug release is small relatively to both the hydrogel and the magnetogel containing non-coated manganese ferrite nanoparticles. This profile is useful as the release of plasmonic magnetogels, even being lower when compared to the other systems (hydrogels and magnetogels containing magnetic nanoparticles), can be enhanced, making possible to control curcumin release. The interaction of the loaded drug with nanoparticles is more pronounced for the gold-decorated nanoparticles, where surface functionalization might have hindered drug release even with the use of photothermia.

To quantitatively evaluate the effect of nanoparticles and irradiation on the magnetogel, several mathematical models were fitted to the cumulative drug release profiles and the coefficients of determination were calculated (Table S1 in Supplementary Information). The first-order model is usually applied to the drug dissolution of water-soluble drugs in porous matrices.^{92,93} The Hixson-Crowell model describes the remaining concentration at time t from a system that changes in surface area and diameter over time, but the initial geometrical form is kept constant.⁹² The Korsmeyer-Peppas model is applicable to polymeric systems that include both diffusion and erosion of polymer chains, which is described by a rate constant K_s that includes the structural and geometrical characteristics of the nanosystem and a coefficient n that is related with the diffusion mechanism.⁹²⁻⁹⁵ The Higuchi model assumes that the drug concentration in the nanosystem is much

higher than its solubility, drug diffusion occurs in a single dimension and diffusivity is constant, the drug particle size is much smaller than the nanosystem thickness, effects due to matrix swelling and dissolution are negligible, and that the release environment provides perfect sink conditions, i.e. the solvent volume is much larger than the saturated nanosystem solution.^{92,94} The Gompertz model adequately describes the release profiles where the drug has good solubility and intermediate release rates.⁹² Overall, the R^2 values (Table S1 in Supplementary Information) demonstrate that the results are not modelled by the first-order and the Hixson-Crowell model, i.e. the released drug (curcumin) is not water soluble and the hydrogel does not change in diameter overtime. The results are best fitted by a Gompertz model (eqn. 8),

$$X_t = X_{max} e^{-ae^{b \log_{10} t}} \quad (8)$$

where X_t and X_{max} are the dissolved fraction percentages at time t and its maximum, a is a shape parameter and b is the dissolution rate per unit of time.⁹²

However, the Korsmeyer-Peppas model (eqn. 9) also suggests that the release can be associated to a diffusion-controlled mechanism,

$$C_t = C_0 K_s t^n \quad (9)$$

where C_0 and C_t are the concentrations at time 0 and t , and K_s is the rate constant. When $n < 0.45$, the release mechanism is diffusion-controlled (Fickian release), $0.45 < n < 0.89$ indicates a combination of diffusion and erosion drug release (non-Fickian release), $0.89 < n < 1$ indicates a relaxation-controlled release, and if $n > 1$, the release is controlled by swelling and polymer chain relaxation.⁹²⁻⁹⁵

The determined kinetic dissolution constants and parameters of these two models (eqn. 8 and 9) are presented in Table 7.

Table 7. Release coefficients of the Korsmeyer-Peppas and Gompertz models obtained for the curcumin release profiles (20 μ M) in hydrogel, magnetogel containing 10% core/shell manganese ferrite/gold nanoparticles (CS), magnetogels containing 10% gold-decorated nanoparticles (D), and magnetogels containing 10% non-coated manganese ferrite nanoparticles (MnFe_2O_4), with (IR) and without incidence of radiation.

	Korsmeyer-Peppas		Gompertz		
	K_s	n	X_{max}	a	b
Hydrogel	0.87	0.32	10.48	36.97	1.67
CS	0.51	0.37	18.89	8.11	0.69
CS_IR	1.99	0.21	18.89	7.25	0.76
D	3.93	0.21	9.25	11.23	1.30
D_IR	3.72	0.23	9.99	13.27	1.34
MnFe_2O_4	1.18	0.29	18.96	6.54	0.73

The Gompertz model parameters demonstrate that the nanoparticles in magnetogels hampered the release of curcumin comparatively to the hydrogel. This effect may be associated with either an interaction between curcumin and the nanoparticles, or the nanoparticles introducing inhomogeneities in the hydrogel matrix that difficult the drug diffusion through it. Moreover, the irradiated magnetogels demonstrated faster drug release kinetics, where the highest increase in b coefficient is observed for the core/shell or the control with manganese ferrite nanoparticles. On

the other hand, the lower increase in b coefficient in the case of gold-decorated nanoparticles can be associated with the surface functionalization not favouring the release of curcumin.

The nanoparticles do show an impact on the predicted maximum curcumin dissolution that can be associated with a perturbation of free energy difference between the magnetogel matrix and the solution. The core/shell nanoparticles might induce a reduced affinity of curcumin towards the matrix, while the gold-decorated nanoparticles, due to the functionalized surface, might lead to a higher affinity of the hydrophobic drug towards the matrix.

Considering the potential anti-inflammatory activity of these nanosystems, release profiles of the hydrogelator compound were also assessed, with and without irradiation (Figure S15 and Table S2 in Supplementary Information). The nanoparticles did not severely affect the release of the hydrogelator. After gelation, a certain amount of free hydrogelator is in equilibrium with the aggregate state in the hydrogel matrix, which is released to the buffer. The release profiles were fitted with the previous described drug release models (Table S3 in Supplementary Information), showing that they cannot be described by the dissolution of a water-soluble drug or geometrical changes of the gel. The good fitting obtained with the other models suggests that the release mechanism can be described by a diffusion-controlled mechanism. The lack of agreement of Korsmeyer-Peppas model with the Gompertz model indicates that nanoparticles and irradiation do not significantly affect the hydrogelator release rate.

The irradiation influence on the release of curcumin towards biomembrane models (SUVs) was assessed by measuring the interaction during 8 hours. The hydrogel and magnetogels without irradiation showed a similar sigmoidal behaviour (Figure S16 in Supplementary Information), while the magnetogels subjected to irradiation exhibited an accelerated accumulation of curcumin in biomembrane models in the first two hours. Hereby, considering that saturation is due to curcumin attaining a partitioning equilibrium between the hydrogel/magnetogel and the limited number of available liposomes, the initial release acceleration (that was also observed in release to buffer) further demonstrates that photothermia might be used as a trigger to burst the release of the encapsulated drug in supramolecular magnetogels.

Conclusions

Considering the sulphur affinity towards gold, a hydrogelator comprising naproxen and a thioether, Npx-L-Met-Z- Δ Phe-OH, was synthesized and physicochemically characterized. The hydrogelator was computationally evaluated as a potential anti-inflammatory agent.

Magnetogels were successfully obtained for a limit of 10 m/m% of core/shell manganese ferrite/gold or gold-decorated manganese ferrite nanoparticles, above which sedimentation was faster than gelation. The nanoparticles induced an increase of random coil and a decrease of ordered structures, and no major effect over the chiral stacking of naproxen moieties.

The nanosystems showed to be suitable for encapsulation of the model drug curcumin. The presence of nanoparticles improved the encapsulation, as it induced less polar regions in the hydrogel matrix. Moreover, interaction of the gels with biomembrane

models (SUVs) showed that curcumin can be delivered, but no penetration of vesicles into the gel matrix occur owing to the high density of fibres and small aqueous cavity size.

Gold-decorated manganese ferrite nanoparticles were more efficient in the generation of heat compared to the core/shell manganese ferrite/gold nanoparticles, according to the obtained results using curcumin as a fluorescence thermometer. However, drug release by irradiation was only enhanced in the core/shell nanoparticle-containing magnetogels, though delivery was higher in both irradiated gels compared to non-irradiated ones.

The superparamagnetic properties of the nanoparticles are suitable for biomedical applications and the gels elastic properties fall in the range of soft tissues. Therefore, the magnetogels based on Npx-L-Met-Z- Δ Phe-OH are promising for multimodal cancer therapy by combining controlled drug delivery and photothermia.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT) in the framework of the Strategic Funding of CF-UM-UP (UID/FIS/04650/2019) and CQUM (UID/QUI/00686/2019). FCT, FEDER, PORTUGAL2020 and COMPETE2020 are also acknowledged for funding under research projects PTDC/QUI-QFI/28020/2017 (POCI-01-0145-FEDER-028020) and PTDC/QUI-QOR/29015/2017 (POCI-01-0145-FEDER-029015).

The magnetic measurements were supported by projects UTAP-EXPL/NTec/0046/2017, NORTE-01-0145-FEDER-028538 and PTDC/FIS-MAC/29454/2017. Authors gratefully acknowledge Dr L. M. Vieira for carrying out the FTIR measurements at the Infrared Spectroscopy Lab. of the Centre of Physics, Univ. Minho.

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