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4 Green and solvent-free supercritical CO₂-assisted
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8 production of superparamagnetic graphene oxide
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38 **Supporting Information:** EDS mapping of Fe₃O₄@GO (S1), XPS calculations for the surface
39 composition of the samples.
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50 **KEYWORDS:** Graphene Oxide, Aerogel, Supercritical CO₂, SPION, Magnetic Resonance
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ABSTRACT: Extraordinary properties are often described for 2D nanocomposites involving graphene flakes and a second component selected from particles or fibers. From this family of hybrid materials, this study is centered on composites based on graphene oxide (GO), a multifunctional oxygenated version of graphene, and superparamagnetic iron oxide nanoparticles (SPIONs), with applications as contrast agents in magnetic resonance imaging (MRI). The research focuses on the loading of SPIONs onto GO surface *via* a simple one-pot supercritical CO₂ technique. The developed all-green synthesis approach allows the preparation of hybrid materials shaped into aerogels with large mesoporosity. The use of coated and uncoated components in the aerogel composites is analyzed with emphasis in the significance of the arrangement of the NPs into clusters on the GO surface and related with the magnetic properties. The development of composites involving organic coating has been widely studied to pursue the double objective of increasing NPs/GO conjugation and improving the system biocompatibility. Extensive structural and functional characterization is carried out to understand composites behavior towards its performance as a contrast agent in MRI.

INTRODUCTION

Nanocomposites are fascinating materials that have gained ever increasing interest from the market and scientific communities, due to the additional advantageous and often synergistic properties of the components combination.¹ Among them, hybridization of 2D materials has been presented as one of the most interesting ways to prepare nanocomposites.² The best known of all 2D materials is graphene that, together with its oxidized analogue graphene oxide (GO), has been often used as a filler in the polymer industry.³ The known structures of graphene nanocomposites are englobed in four categories: 0D@2D (nanoparticles (NPs)/graphene), 1D@2D (fibers, nanotubes/graphene), 2D@2D (single-layer materials/graphene) and 2D@3D (graphene/three-dimensional macrostructures). Due to the high diversity, properties and advantages of graphene, a multitude of nanocomposite-based advanced applications have been envisioned for this material.⁴ For example, in the 2D@3D family the dispersion of exfoliated graphene flakes into crystalline metal organic frameworks (MOFs) has been extensively prospected to obtain hierarchically organized porous systems for gas separation.⁵ In the 0D@2D category, graphene provides huge surface area for the immobilization of a broad range of substances, going from linked metallic cations, molecules (biomolecules, fluorescent centers, drugs, etc.)⁶ to NPs (metallic, inorganic, polymeric, etc.)⁷ or even bacteria.⁸

Composite materials are used in many large markets, like transportation, power and energy. In the current industrial era, one of the main concerns must be the large amount of non-sustainable productions not fulfilling environmental regulations.⁹ In this regard, supercritical fluids are a class of solvents proposed for a more sustainable chemical industry. Supercritical CO₂ (scCO₂) has been considered the first real green alternative to hazardous organic solvents, and a way to design sustainable chemical engineering processes.¹⁰ In this respect, a recently patented scCO₂

method, previously described by our research group for GO processing, is here extended to 2D nanocomposites preparation.¹¹ Efforts are not only focused on using scCO₂ to form the composite, but also on simultaneously processing the material into a 3D user-friendly configuration, specifically, in the form of a highly porous aerogel. In contrast to the high-temperature (ethanol) critical point drying procedure commonly used for aerogels preparation,¹² the developed scCO₂ method uses temperatures about 100 K lower, which prevents the removal of the oxygenated functionalities of GO.¹³

NPs@GO hybrid structures are especially alluring. Particularly, the integration of superparamagnetic iron oxide NPs (SPIONs, including magnetite Fe₃O₄ and maghemite γ -Fe₂O₃) and GO into nanocomposites is currently a hot topic of research owing to the possibility of rapid magnetic SPIONs@GO displacement with a magnet. Their implementation has been proposed essentially in the areas of pollutants removal¹⁴ and biomedicine, including magnetically targeted drug delivery, immunoassays, hyperthermia and magnetic resonance imaging (MRI).¹⁵ MRI labelling with biocompatible Fe₃O₄ NPs and its composites is a powerful technique used for evaluation of the location and distribution of malignant cells.¹⁶ Due to the size-dependent magnetic properties, superparamagnetic Fe₃O₄ NPs, with sizes below 20 nm, have long been utilized as negative contrast agents for transverse relaxation time (T₂)-weighted MRI.

Fe₃O₄@GO composites can be prepared by both *in situ* and *ex situ* methods. In the *in situ* techniques, the NPs are grown in the presence of GO flakes, either by chemical deposition from solutions of Fe³⁺ and Fe²⁺ in basic media,¹⁷ or by hydrothermal¹⁸ or mechanochemical¹⁹ reduction of Fe³⁺ salts. Contrarily, in the *ex situ* protocols, pre-synthesized NPs are deposited on the GO flakes. The main drawback of the *in situ* methods is the unavoidable reduction of GO to reduced GO (rGO) during composite settling. There are only few reports describing 3D

monolithic magnetic composites, and all of them correspond to systems involving rGO as a matrix instead of GO.^{20,21}

This work focuses on the use of scCO₂ for the preparation of Fe₃O₄@GO composites for MRI and other biomedical applications. Indeed, the presence of the oxygenated functionalities in GO would allow the extra functionalization of the matrix with other molecules of interest, e.g., drugs, coating agents, fluorescent probes, and so on. The straight assembly of pre-formed Fe₃O₄ NPs was the method chosen to synthesize aerogel composites involving highly oxidized GO. The eco-friendly process designed with scCO₂ allow achieving a completely solvent (toxic or harmful to the environment)-free product, thus not compromising its use in the biomedical area. Finally, the use of the *ex situ* procedure circumvents the drawback of NPs uncontrolled aggregation during growth, since it has been proved that only the controlled clustering of the magnetic NPs results in a considerable increase of MRI efficiency.²²⁻²⁴ Eventually, the use of coating agents has also been proposed to control NPs clustering.^{25,26} Accordingly, this study first explores the use of small ligands, such as citrate,²⁷ to functionalize the magnetic NPs for the control of the aggregation during deposition on the GO surface. Next, the use of polyethylenimine (*pei*), a widely employed coating agent in the pharmaceutical industry, is investigated to modulate the behavior of the composite in water.²⁸

MATERIALS AND METHODS

Materials

GO sheets were purchased from Graphenea Inc. (Spain), supplied as a stable dispersion in water with a concentration of 4 mg·mL⁻¹. For the preparation of Fe₃O₄ NPs, the used reagents were hydrated iron (II) chloride (FeCl₂·4H₂O) from Carlo Erba, and anhydrous iron (III) chloride

(FeCl₃), sodium hydroxide (NaOH) and hydrochloric acid (HCl) from Merck. As coating agents, citric acid (Merk) and ethylenimine monomer (aziridine, kindly donated by Menadiona S.A.) were assayed. Compressed CO₂ (99.95 wt%) and absolute ethanol (EtOH), delivered by Carburos Metálicos S.A. and Scharlau Chemie, respectively, were used as solvents.

Methods

Synthesis of Fe₃O₄ NPs

A well established precipitation method was used to synthesize the Fe₃O₄ NPs.²⁹ Briefly, 0.2 g of FeCl₂·4H₂O and 0.52 g of FeCl₃ were dissolved in 2.5 mL of deoxygenated aqueous solution acidify with 0.2 mL of HCl 6 M. The acidic solution was added dropwise into a two-neck flask containing 1.5 g of NaOH in 25 mL of water under N₂ flow and with vigorous stirring. The precipitation of a black solid occurred as a result. This product was left under stirring for 5 more minutes. After that, the black powder was flocculated at the bottom of the flask with the help of a magnet and the supernatant was decanted. The product was rinsed with 25 mL of water by consecutive centrifugation (4000 rpm) and decantation. This procedure was repeated three times. Then, the solid was treated with 50 mL of HCl 0.01 M to obtain a colloidal suspension of Fe₃O₄ NPs. Previous to aerogel composite preparation, the water in the dispersion must be exchanged by EtOH. A multi-step water-to-ethanol exchange procedure was followed to obtain a long-term stable sol of NPs in EtOH. Water replacement was performed in four successive steps of centrifugation (11000 rpm) / sonication (30 min) by using ethanol:water mixtures with increased alcohol concentration, 30:70, 50:50, 70:30 and finally 100:0. A stable colloidal suspension of Fe₃O₄ NPs in EtOH, with a concentration of *ca.* 3 mg·mL⁻¹, was thus obtained.

Preparation of the composite aerogel

For the composite aerogel, the synthetic procedure starts by exchanging the water in the commercial GO suspension by ethanol, using the same protocol than the one previously described for the Fe_3O_4 NPs. A stable colloidal suspension of GO in EtOH with a concentration of *ca.* $3 \text{ mg} \cdot \text{mL}^{-1}$ was obtained. Precursor colloidal suspensions of the aerogel were obtained by mixing the parent ethanol solutions and posterior sonication during 30 min. The studied weight ratio was 2:1 (GO: Fe_3O_4). Monolithic pieces of aerogel composites were prepared in small assay tubes of 2 mL, following a previously reported protocol.¹³ Briefly, three vials were loaded with 1 mL of the GO/NPs dispersion and placed into a non-stirred high pressure reactor of 200 mL (TharDesign). Liquid CO_2 was flushed into the vessel to pressurize the system to 6 MPa. The vessel was then gently heated up to 333 K and pressurized to 20 MPa. These conditions were kept for a period of 48 h. Pressure decrease to ambient conditions was achieved by the slow and isothermal release of CO_2 , thus avoiding the two-phase region for the fluid that could damage the aerogel structure. Aerogel samples were isolated as black cylindrical monoliths, termed as $\text{Fe}_3\text{O}_4@\text{GO}$. Note that pristine GO monoliths are usually greyish and they only became black after reduction to rGO. In this case, they appear as black due to the contribution to the color of the NPs.

Preparation of coated composites

In some experiments, the Fe_3O_4 NPs were surface modified with citrate before aerogel formation. In all the aspects, the followed protocol for coated NPs preparation was the same than the one used to prepare Fe_3O_4 NPs, but the HCl 0.01 M in the synthesis was replaced by citric acid. Hence, the black precipitate obtained after washing the NPs was treated with 50 mL of and aqueous solution containing 0.1 g of citric acid. The result was citrate coated NPs ($c\text{Fe}_3\text{O}_4$),

which were dispersed in EtOH and further processed into the monolith composite (sample $c\text{Fe}_3\text{O}_4@\text{GO}$).

In another set of experiments, the composite $\text{Fe}_3\text{O}_4@\text{GO}$ was modified with *pei* after aerogel formation by using a scCO_2 reported method.³⁰ In short, the high-pressure reactor was charged with one cylindrical monolith of either GO or $\text{Fe}_3\text{O}_4@\text{GO}$. A drop of 10 μL of liquid ethylenimine was added on the top of the monolith. To promote the polymerization of the monomer, the vessel was pressurized to 15 MPa with CO_2 at 313 K and left to react for 30 min. Dry monolith samples were recovered after depressurization, labeled as *peiGO* of *pei*($\text{Fe}_3\text{O}_4@\text{GO}$).

Characterization

The percentage of metal in the composites was determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x) after digesting the solid in hydrochloric and nitric acids. To determine the composition of the GO flakes and that of the $\text{Fe}_3\text{O}_4@\text{GO}$ composite, X-ray photoelectric spectroscopy (XPS) was recorded for C, O and Fe in a XPS Spectrometer Kratos AXIS Supra, using $\text{AlK}\alpha$ (20 eV) radiation emitted at 225 W (15 mA/ 15 kV). The binding energies of the resulting spectra were corrected employing the binding energy of adventitious carbon (284.6 eV) in the C 1s region. The backgrounds were corrected using Shirley baselines. All the analyzed regions were deconvoluted by means of mixed Gaussian–Lorentzian functions (90:10). The quantitative analyses were based on the atomic sensitivity factors stored in the CasaXPS database (v2.3.12Dev6). In the supporting information (SI), the C 1s, O 1s and Fe 2p_{3/2} core level spectra deconvolution are shown for each studied sample, as well as the criteria for band assignment. The molecular arrangement was investigated by means of Fourier transformed infrared (FTIR) spectroscopy (Jasco 4700 Spectrophotometer). The textural

properties were determined by N₂ adsorption/desorption at 77 K using an ASAP 2000 Micromeritics Inc. equipment. Samples were first degassed at 393 K for 24 h. The surface area was calculated applying the BET equation. Morphological features were examined by using a scanning electron microscope (SEM, Quanta FEI 200), coupled with an energy-dispersive X-ray spectroscope (EDS) used for metal quantification. The size and distribution of the NPs on the GO flakes were examined using a transmission electron microscope (TEM, JEOL 1210). The thermal stability of the composites was analyzed in air flow by thermogravimetric analysis (TGA, Netzsch-STA 449 F1 Jupiter) using a temperature increase step of 5 K·min⁻¹. Zeta potential measurements were carried out on a Malvern Zetasizer Nano ZS after dispersing the samples by sonication (15 min) in EtOH, the used synthetic medium in this work.

The superparamagnetic nature and saturation magnetization (M_s) of chosen composites were first studied with a superconducting quantum interference device (SQUID Quantum Design MPMS-XL magnetometer). The magnetic hysteresis curves were recorded at 300 K and up to 50 KOe. Afterwards, MRI studies were carried out in a 7T Bruker Biospec70/30 USR apparatus (Bruker Biospin BmbH) system equipped with a mini-imaging gradient set (400 mT·m⁻¹) and using a linear volume coil with 72 mm inner diameter. Phantoms containing weighted amounts of Fe₃O₄@GO or a 10 mg·mL⁻¹ agar/water solution, were prepared for relaxivity measurements. To form the gels, the composite was dispersed in the agar solution under stirring and heated to 343 K. The vials were cooled down to room temperature in a water bath under sonication. For T₂ measurements, a multi-slice multi-echo sequence was used, with a repetition time of 3 s and echo time values between 10 and 600 ms in steps of 10 ms. T₂ values were obtained as the slope of the linear regression of the relaxation rates (1/T₂) vs. iron concentration.

RESULTS AND DISCUSSION

Pristine Fe₃O₄@GO composite aerogels

scCO₂ preparation method

A simple method to efficiently decorate GO sheets with monodispersed Fe₃O₄ NPs is hereby presented. As shown in Fig. 1, this is readily achieved by first preparing monodispersed Fe₃O₄ NPs and GO suspensions in ethanol and then mixing them up. The NPs are homogeneously deposited onto the GO surface during the self-assembly of the flakes into a gel, which is induced by the addition of scCO₂, an acid gas. A dry composite, in the form of a black aerogel monolith, is obtained after isothermal depressurization. The as-made composite aerogel is magnetic and can be lifted by a magnet. The monolith is easily redispersed in contact with water, reflecting the hydrophilic character of the Fe₃O₄@GO composite. The redispersed composite can still be manipulated with an external magnetic field.

Figure 1. Schematic representation of the synthetic protocol used to prepare SPIONs@GO composites: mixing of NPs and GO ethanol dispersions and self-assembly in the high pressure reactor by scCO₂ addition to obtain a dry magnetic 3D GO monolithic aerogel decorated with NPs. The monolith is easily redispersed in water maintaining the magnetic properties.

Aerogels structure and morphology

Fig. 2 displays the powder XRD patterns recorded for the Fe₃O₄@GO composite and the parents GO flakes and Fe₃O₄ NPs. Pristine GO exhibits a single wide peak at about $2\theta = 11.1^\circ$, indicating graphene interlayer distances close to 0.8 nm,³¹ compatible with highly oxidized graphene.³² The main diffraction peaks of Fe₃O₄ NPs are observed at 2θ higher than 30° , with the most intense peak located at around 35° .³³ It is worth to mention that XRD technique does not allow discerning between Fe₃O₄ and γ -Fe₂O₃; but based on the used precipitation method in this work, the inorganic phase is regarded as Fe₃O₄. The low intensity and widening of diffraction peaks are ascribed to the small size of the NPs. In the Fe₃O₄@GO system, the XRD peak of GO is still noticeable at around 11° . The Fe₃O₄ peak at 35° , even still observable, is almost totally masked

by a wide amorphous background resulting from the composite as a whole. With the XRD data alone, it was not possible to confirm that the employed strategy had not lead to the partial reduction of GO, because rGO would present a wide peak at *ca.* 25° that could be masked by the amorphous background.

Figure 2. XRD patterns of the different studied compounds.

The morphology of the hybrid composite was characterized by SEM and TEM. The SEM images obtained for GO and $\text{Fe}_3\text{O}_4@\text{GO}$ aerogels are presented in Fig. 3. For the GO matrix, the low-magnification picture indicated the formation of a porous sponge with interconnected meso and macropores (Fig. 3a). The aerogel structure, with well-dispersed and multiple exfoliated and disordered GO sheets, could be observed at high-magnification (Fig. 3b). This morphology for the GO matrix was not lost by the NPs addition (Fig. 3c,d). Fig. 3d clearly shows that the distribution of the NPs on the GO flakes in the $\text{Fe}_3\text{O}_4@\text{GO}$ sample was homogeneous and in the

form of small aggregates. To ascertain the stability of the composite, a portion of the $\text{Fe}_3\text{O}_4@\text{GO}$ sample was re-dispersed in water, ultrasonicated for 15 min and then dried. SEM images of the recovered black powder indicated that the NPs were still forming part of the composite (Fig. 3e), with no significant separation of the inorganic and graphene phases. The stability of the $\text{Fe}_3\text{O}_4@\text{GO}$ composite against sonication indicates a rather strong binding between the NPs and GO.

Figure 3. SEM images of the different prepared samples: (a,b) pristine GO aerogel; (c,d) $\text{Fe}_3\text{O}_4@\text{GO}$ composite aerogel; (e) $\text{Fe}_3\text{O}_4@\text{GO}$ composite after ultrasounds treatment in water causing aerogel destruction and flakes compaction, but not NPs detachment; and (f) $c\text{Fe}_3\text{O}_4@\text{GO}$ composite aerogel.

By analyzing TEM micrographs of the as-synthesized Fe_3O_4 sample (about 100 NPs), the diameter was estimated to be in the range 7-12 nm (Fig. 4a), which is in the superparamagnetic size range. The GO flakes in the composite can be identified from the background by the fringes and wrinkles (Fig. 4b). The NPs are dispersed on the GO surface forming small clusters of less than 100 nm.

Figure 4. TEM micrographs of NPs and composites: (a) Fe_3O_4 ; (b) $\text{Fe}_3\text{O}_4@\text{GO}$; (c) $c\text{Fe}_3\text{O}_4$; and (d) $c\text{Fe}_3\text{O}_4@\text{GO}$.

A positive zeta potential value (31 mV) was measured for pristine Fe_3O_4 NPs synthesized in acid media, since at low pH positive ions of Fe^{2+} and Fe^{3+} were expected to be driven into the Stern layer.³⁴ Moreover, the dispersed GO platelets had inherent negative charge (-30 mV) as a result of the ionization of the oxygen functionalities. The different sign in the magnitude of the charges of NPs and GO would result in strong electrostatic interactions between both components in the composite. The measured zeta potential for the $\text{Fe}_3\text{O}_4@\text{GO}$ composite was -30 mV, similar to

the value found for net GO. This result is rationalized on the basis of the considerably higher contribution of GO to the surface area with respect to the NPs.

The differences in the structure and textural characteristics of pristine GO aerogel and the composite were further analyzed by low temperature adsorption/desorption of N₂. Recorded isotherms are plotted in Fig. 5. GO and Fe₃O₄@GO exhibited both a type IV isotherm according to the IUPAC classification, characteristic of mesoporous materials. Moreover, these isotherms had a H3-type hysteresis loop related to slit-shaped pores and aggregates of plate-like particles,³⁵ representative of the studied GO system. Measurements yielded similar BET surface areas for GO and Fe₃O₄@GO with values of 231 and 228 m²·g⁻¹, respectively. The microporosity, observed at very low relative pressures in the isotherm, slightly increased in the Fe₃O₄@GO composite, in which small pores are generated by the aggregation of the Fe₃O₄ NPs into clusters on GO surface.

Figure 5. Low temperature adsorption/desorption N₂ isotherms recorded for the Fe₃O₄@GO and cFe₃O₄@GO aerogel composites. The inset displays the isotherm of pristine GO aerogel.

Aerogels composition

FTIR spectroscopy of samples dispersed in KBr was used to qualitatively ascertain the composition of the aerogels (Fig. 6). A wide absorption band at 3430 cm^{-1} (not shown) and a peak at around 1620 cm^{-1} were observed in all spectra and assigned to vibrations of structural OH groups (C-OH in GO and Fe-OH on the Fe_3O_4 surface), as well as to adsorbed molecular water. The spectrum of Fe_3O_4 is characterized by an intense band at *ca.* 580 cm^{-1} , generated by the torsional vibration mode of Fe-O.³⁶ In the region of $1800\text{-}600\text{ cm}^{-1}$, most of the bands in the spectra of the aerogels were originated by vibrations of the oxygenated functionalities of GO and the carbon skeletal.³⁷ Thus, the C=O group vibration was identified at 1725 cm^{-1} ; while the shoulder at 1580 cm^{-1} was assigned to aromatic C=C from non-oxidized domains. Finally, peaks at 1380 , 1235 and 1054 cm^{-1} corresponded to carbonyl, epoxy and alkoxy functionalities. For the composite material, the band at 580 cm^{-1} confirms the presence of the Fe_3O_4 NPs.

Figure 6. FTIR spectra of the different studied compounds.

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Calculated from the content of iron measured by atomic absorption, the Fe_3O_4 loaded on $\text{Fe}_3\text{O}_4@\text{GO}$ was quantified as 36 wt%. TGA in air atmosphere was also used for the mass quantification of the composite, as well as for the scrutiny of the thermal stability of the aerogels. The results are shown in Fig. 7. For samples involving GO, the mass loss below 400 K (*ca.* 5 wt%) was attributed to the evaporation of weakly adsorbed water. The decomposition of GO occurred in two steps, in which the first gradual decay (*ca.* 430-500 K) was assigned to the loss of oxygenated groups. The values of weight loss in this area were estimated as 31 and 20 wt% for GO and $\text{Fe}_3\text{O}_4@\text{GO}$, respectively. Considering for the weight loss calculation that this decay was only due to the GO component in the hybrid composite, a similar percentage of *ca.* 30 wt% of oxygenated functionalities can be allocated to GO in both the pristine and composite aerogels. Hence, the decoration of GO surface with Fe_3O_4 NPs did not significantly reduce (or deoxygenate) the GO in the resulting composite. A second abrupt weight loss was noticeable between 650 and 700 K, attributed to the pyrolysis of graphene in air. For pristine GO aerogels, the thermal decomposition was completed at 700 K. Contrarily, the TGA curve for the composite indicated a significant percentage (*ca.* 36 wt%) of non-pyrolized material at this temperature, which was assigned to the inorganic fraction. It should be taken into account that at this temperature the inorganic component must be Fe_2O_3 , instead of Fe_3O_4 . After recalculation, the estimated weight of Fe_3O_4 in the aerogel composite was estimated to be around 34 wt%, in line with ICP-MS results. EDS coupled with SEM was used to estimate the atomic percentage of C, Fe and O elements in the composite aerogel. Pieces of $\text{Fe}_3\text{O}_4@\text{GO}$ monolith with enough density to avoid X-ray excitation of the background (graphite tape) were used for the measurements (Fig. S1 in the SI). The atomic ratio Fe/O was estimated as 0.27, which is much lower than that corresponding to Fe_3O_4 (0.75), indicating oxygen excess. This low value reflects the contribution

to atomic O of GO. The weight percentage of atomic Fe in the sample was estimated as 23 wt%, which corresponded to a percentage of *ca.* 32 wt% of Fe_3O_4 in the composite, again in line with the values found by TGA and ICP-MS.

Figure 7. TGA curves under flowing air recorded for the different studied compounds.

Table 1 shows the relative abundance of the main components in the XPS spectra recorded for GO and $\text{Fe}_3\text{O}_4@\text{GO}$ aerogels (see the SI for the deconvolution procedure). For pristine GO aerogel, the C/O atomic ratio was around 2, indicating a high degree of oxidation for the flakes. Deconvolution of the spectra obtained for the pristine matrix revealed peaks at binding energy values of 284.6, 286.6 and 532.5 eV corresponding, respectively, to the typical C 1s (C=C and C-O) and O 1s (C-O) binding environment of GO, with only a small contribution of ketone and carbonyl groups (less than 5 at%). Carboxylic acid functionality was not detected (absence of

signals at 288.8 and 534.4 eV in C 1s and O 1s regions, respectively). The GO flakes description inferred from XPS matches the widely accepted GO model developed by A. Lerf *et al.*,³⁸ in which GO is described as constituted by nonstoichiometric platelets, wherein both the carbon plane and the edges are mainly decorated with hydroxyl and epoxy functional groups. Carbonyl groups are also present in low quantities, mostly as carbonyl and keto groups at the periphery of the flakes, but also as organic carbonyl defects within the sheets.³⁹ The addition of Fe₃O₄ NPs did not produce any noticeable reduction on the GO sheets, as can be observed by comparing the C/O ratios in GO and Fe₃O₄@GO samples. On the contrary, the total percentage of oxygen increased for the Fe₃O₄@GO sample with respect to pristine GO, but this is due to the extra contribution to O given by Fe₃O₄. XPS analysis indicates that the surface of the NPs in the composite was composed mainly of bohemite (FeOOH at 711.7 eV) and hematite (Fe₂O₃ at 710.8 eV). The presence of bohemite bands in the spectrum, along with those of hematite, can be explained by the metastability of small magnetite nanocrystals with respect to oxidation by X-ray irradiation during measurement. The percentage of surface iron was measured as 1.8 at%, which corresponded to 9.7 wt% of Fe₃O₄. On the decorated surface of GO, the dispersion of the NPs is defined as the ratio of the surface atoms to the total number of atoms in the particle.⁴⁰ The high dispersion values found in the Fe₃O₄@GO system (9.7 over 36 wt%) reflects the small size of the NPs of only few nanometers. These NPs are in some way associated to the hydroxyl and epoxy groups formerly present on the GO surface, which change towards >C=O functionalities.

Table 1. XPS derived surface composition of the different samples in atomic percentage.

	GO	Fe ₃ O ₄ @GO	peiGO	pei(Fe ₃ O ₄ @GO)
C/O	1.90	1.70	6.05	5.96
N/C	---	----	0.30	0.29
%O	33.5	36.0	11.3	11.4
%N	1.4	1.2	20.2	20.0
% Aminic/amidic N	0.67	0.62	19.90	16.70
% Pyridinic N	0.00	0.00	0.00	1.45
% Graphitic N	0.69	0.58	0.00	1.85
%C	65.2	61.0	68.4	68.0
%C in C=C	22.31	16.92	10.09	5.35
%C in sp ³ C	2.56	8.93	24.89	31.31
%C in C-N	0.67	0.62	19.90	16.70
%C in C=N	0.69	0.58	0.00	3.30
%C in C-O-C	19.24	10.79	4.45	3.23
%C in C-OH	15.20	11.73	1.46	0.47
%C in >C=O	0.00	6.66	7.65	6.86
%C in R-O-C=O	3.72	3.68	0.00	0.63
%C in O=C-O-C=O	0.79	1.10	0.00	0.14
%C in -COOH	0.00	0.00	0.00	0.00
%Fe	-	1.8	-	0.6
%Fe in FeO	-	0.10	-	0.33
%Fe in Fe ₂ O ₃	-	0.63	-	0.01
%Fe in FeOOH	-	1.07	-	0.30

Magnetic properties

The magnetic properties were studied with a SQUID apparatus. Magnetic hysteresis curves obtained from Fe_3O_4 NPs and $\text{Fe}_3\text{O}_4@\text{GO}$ sample are shown in Fig. 8a,b. Hysteresis loops were recorded at room temperature (300 K) using an applied magnetic field that was swept from -600 to 600 $\text{A} \cdot \text{m}^{-1}$ and fitted to the Langevin function.⁴¹

Figure 8. Field dependent magnetization curves obtained for: (a) Fe_3O_4 ; (b) $\text{Fe}_3\text{O}_4@\text{GO}$; (c) $c\text{Fe}_3\text{O}_4$; and (d) $c\text{Fe}_3\text{O}_4@\text{GO}$. Colored continuous lines represent the M values with respect to percentage of Fe_3O_4 , i.e., subtracting the weight of GO in the composite. Dashed lines correspond to M values weighted for the composite. Black lines corresponded to the Langevin fit.

The magnetic curves revealed negligible coercivity and magnetic remanence, which indicates superparamagnetic behavior, also for the composite. The M_s values obtained from the magnetic hysteresis curves were $48 \text{ emu} \cdot \text{g}^{-1}$ for Fe_3O_4 NP and $26 \text{ emu} \cdot \text{g}^{-1}$ for $\text{Fe}_3\text{O}_4@\text{GO}$ ($75 \text{ emu} \cdot \text{g}^{-1}$ when re-calculated by subtracting the mass of GO in the composite). As usual, the M_s value arising from the bare Fe_3O_4 NPs was smaller than the one of the bulk material ($M_s(\text{bulk Fe}_3\text{O}_4) = 92 \text{ emu} \cdot \text{g}^{-1}$).⁴² The Langevin function, fitted in each case for the curves recalculated for the exclusive weight of Fe_3O_4 , was used to estimate the size of the NPs. The obtained values were 9.5 and 9.9 nm for the NPs in pristine Fe_3O_4 and in the composite $\text{Fe}_3\text{O}_4@\text{GO}$, respectively. These values are in accordance with the sizes previously observed by TEM analysis.

Surface functionalized composites

Citrate as a coating agent for NPs

The surface of SPIONs can be stabilized by the adsorption of citric acid being coordinated *via* one or two of the carboxylate functionalities. Carboxylate adsorption has important effects on the growth of the SPIONs, the particle size and the magnetic properties.⁴³ Zeta potential measurements were used to monitor coating efficiency and its influence in NPs stability. By coating, the zeta potential was shifted from positive in net Fe_3O_4 (30 mV) to negative in citric-coated $c\text{Fe}_3\text{O}_4$ (-33 mV). This sign is assigned to the presence of negatively charged carboxylate groups on the surface of $c\text{Fe}_3\text{O}_4$. TEM micrographs indicated the precipitation of a highly monodisperse system of $c\text{Fe}_3\text{O}_4$ NPs, with sizes between 5-9 nm (Fig. 4c). Hence, the average size of $c\text{Fe}_3\text{O}_4$ was slightly smaller than for uncoated Fe_3O_4 (Fig. 4a) due to the effect in the growth of the NPs of the citrate capping agent. This observation was corroborated by data on particle size obtained from the Langevin function applied to the magnetic curves recorded for $c\text{Fe}_3\text{O}_4$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ (Fig. 8c,d), giving size values of 8 and 8.1 nm, respectively. FTIR was

used to study the pre-functionalization of the Fe_3O_4 NPs with citrate. The shoulder in the band at 1620 cm^{-1} , observed at *ca.* 1560 cm^{-1} in the $c\text{Fe}_3\text{O}_4$ spectrum, together with the neighbor band at 1400 cm^{-1} , can be assigned to C=O vibration,⁴⁴ demonstrating the presence of adsorbed citrate on the NPs surface. TGA analysis of the synthesized NPs showed that below 400 K both samples, Fe_3O_4 and $c\text{Fe}_3\text{O}_4$, lost adsorbed water, but, afterwards, a weight decay was only observed for $c\text{Fe}_3\text{O}_4$ and assigned to citrate elimination (Fig. 7). A percentage of *ca.* 8 wt% was estimated for the citrate coating the NPs.

The addition of citrate onto the surface of the NPs changed the sign of the zeta potential from positive in Fe_3O_4 (30 mV) to negative in $c\text{Fe}_3\text{O}_4$ (-33 mV). Hence, in this case both GO and the NPs have the same sign, which would reduce the possibility of electronic interactions. However, in the pre-made $c\text{Fe}_3\text{O}_4$ NPs, the active carboxylate groups, not grafted on the NPs surface, are amenable for binding to other hydroxylated surfaces, like GO platelets (Fig. 1), thus expecting a strong chemical interaction between both components. As occurs for the $\text{Fe}_3\text{O}_4@\text{GO}$ sample, the $c\text{Fe}_3\text{O}_4@\text{GO}$ nanocomposite was negatively charged, with a zeta potential value of -38 mV.

XRD analysis of the $c\text{Fe}_3\text{O}_4@\text{GO}$ aerogel displayed the same structure than for $\text{Fe}_3\text{O}_4@\text{GO}$ (Fig. 2), with the GO peak being clearly visible along with an amorphous background. The presence of the NPs in the composite was investigated by FTIR spectroscopy (Fig. 6), characterized by the Fe-O vibration at *ca.* 580 cm^{-1} . TEM (Fig. 4d) and SEM (Fig. 3f) images showed that the distribution of the NPs on the GO flakes was homogeneous and in the form of small aggregates of less than 100 nm. The BET surface area for the $c\text{Fe}_3\text{O}_4@\text{GO}$ composite was $206\text{ m}^2\cdot\text{g}^{-1}$ (Fig. 5), similar to that found for $\text{Fe}_3\text{O}_4@\text{GO}$ ($228\text{ m}^2\cdot\text{g}^{-1}$). The Fe_3O_4 loaded on $c\text{Fe}_3\text{O}_4@\text{GO}$ was quantified as 38 wt.% by ICP-MS. TGA of $\text{Fe}_3\text{O}_4@\text{GO}$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ presented the same thermal events (Fig. 7). From the first abrupt mass loss in $c\text{Fe}_3\text{O}_4@\text{GO}$ (23 wt%), a percentage

of *ca.* 30 wt% of oxygenated functionalities can be assigned to the GO matrix in the composite. From the second decay, the weight of Fe_3O_4 in the $c\text{Fe}_3\text{O}_4@\text{GO}$ aerogel composite was estimated to be around 34 wt%. Comparing weight loss differences for $\text{Fe}_3\text{O}_4@\text{GO}$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ samples at 550 K, the mass fraction of citrate in $c\text{Fe}_3\text{O}_4@\text{GO}$ was estimated as 3 wt%, which match the initial value of 8 wt% for net $c\text{Fe}_3\text{O}_4$ when recalculated subtracting the weight of GO. The saturation magnetization values obtained from the magnetic hysteresis curves were $50 \text{ emu}\cdot\text{g}^{-1}$ for $c\text{Fe}_3\text{O}_4$ NPs and $26 \text{ emu}\cdot\text{g}^{-1}$ for $c\text{Fe}_3\text{O}_4@\text{GO}$ ($81 \text{ emu}\cdot\text{g}^{-1}$ when recalculated by subtracting the mass of GO in the composite) (Fig. 8c,d).

Coating with *pei*

Some reports on the cytotoxicity of graphene and its derivatives have shown that these materials exhibit a certain degree of toxicity to cells and animals.⁴⁵ In biomedicine, this problem is typically solved by coating the system with a biocompatible polymer, such as polyethylene glycol, dextran or polyethylenimine.^{46,47} The latter, *pei*, is a water soluble polymer widely used in its branched form to coat materials for bioapplications.⁴⁸ Indeed, a facile scCO_2 strategy has been previously developed in our laboratory to functionalize aerogel magnetic NPs with *pei* polymer, with the objective of increasing biocompatibility and, simultaneously, controlling drug release.²⁸ The *pei* random branched structure is produced by the CO_2 -catalyzed exothermic polymerization of aziridine monomer in the supercritical medium. This method was applied in this work to covalently conjugate the polymer to the aerogels surface (Fig. 9). Low molecular weight *pei* results from the scCO_2 process, which, in the presence of the composite, is expected to be grafted onto the surface. For easy understanding and characterization, the coating of pristine GO aerogels with *pei* was first analyzed. Certainly, GO, with the main contribution to the surface area in the $\text{Fe}_3\text{O}_4@\text{GO}$ composite, would be the primarily functionalized component.

Figure 9. Schematic representation of the process occurring during *pei* coating of the aerogel.

The polymerization is initiated by compressed CO_2 addition, then the exothermic reaction is propagated to give low molecular branched *pei*, which is grafted to the GO surface. The monolith (partially damaged by the liquid aziridine) is recovered dry.

Functionalization of GO with aminopolymers by amidation and/or amination are widely studied processes.⁴⁹ The amidation reaction between amine and carboxyl groups at the periphery of GO requires a previous chemical treatment (acylation reaction) for carboxylic acid activation. Contrarily, amination is a more straightforward process, since alkylamines are highly reactive to both epoxy (nucleophilic substitution) and hydroxyl (condensation) groups on the basal plane of GO flakes. XPS analysis of the as-synthesized *pei*GO sample showed a significant reduction in the percentage of both C-OH and C-O-C groups with respect to pristine GO (Table 1), hence indicating the prevalence of the amination reaction.^{50,51}

C-OH amination did not cause any modification in the aromatic GO network, although hydroxyl groups can be eliminated together with hydrogen in the β -carbons of the alkylamine to form aliphatic C=C groups.⁵² Contrarily, nucleophilic attack at the epoxy functionalities causes

simultaneous aromatic C=C bond formation and deoxygenation. The concurrent slight increase of >C=O functionalities, already observed in other low temperature (423-473 K) treatments, occurred by the rearrangement of epoxy and hydroxyl groups closely placed.⁵³ As a result, GO in the *pei*GO sample was significantly reduced, as typically observed for GO flakes treated with amine-containing molecules.⁵⁴ The formation of reduced GO was aided by the increase in temperature caused by the exothermic ring opening polymerization of aziridine in scCO₂. The quick heat release vaporized the monomer resulting in a visually observed vapor cloud of, likely, small *pei* oligomers. The aerogel macro and mesoporosity is expected to enable the internal diffusion of the polymer and, thus, the homogeneous distribution of the coating. The amount of sp³ C detected by XPS greatly augmented after coating due to the contribution of *pei* (two CH_x per each NH_x). The result of the deconvolution treatment of the N 1s spectrum revealed an intense peak at 399.8 eV, which was attributed to N-C bond. The percentage of this functionality in the material is of almost 20 at% (Table 1), which indicates a high loading of polymer in the *pei*GO sample. The surface covalent attachment of *pei* was supported by the analysis of the FTIR spectrum of the *pei*GO sample (Fig. 6).⁵⁵ The band at 1657 cm⁻¹ indicated the presence of NH₂ groups. Bands at 1564 and 1455 cm⁻¹ corresponded to NH vibration, even the former also had a contribution of C=C stretching. The band at 1384 cm⁻¹, owing to the N-C aromatic vibration, is representative of the covalent bond established between GO and the polymer. The region from 1250 to 1050 cm⁻¹ showed a wide band, with contribution of -CH₂ (1245, 1179 and 1083 cm⁻¹), N-C aliphatic (1140 cm⁻¹) and, in small percentage, remaining -OH and C-O-C groups. TGA characterization of this sample showed an insignificant mass loss below 500 K, region corresponding to the elimination of the oxygenated functionalities of GO, indicating again its reduced character of the *pei*GO sample (Fig. 7). After this temperature, the weight loss can be

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related to *pei* decomposition and rGO pyrolysis. For aerogels, the main drawback of the reported supercritical method is related to the liquid character of the aziridine monomer, which partially destroys the delicate structure of the aerogel due to capillary forces (Fig. 9).

Regarding the *pei*(Fe₃O₄@GO) sample, XPS results matched those previously described for *pei*GO, indicating again a high degree of reduction for the GO matrix in the composite (Table 1). This composite incorporates N in large quantity, thus demonstrating successful *pei* coating without significant Fe₃O₄ detachment. Indeed, the presence of aggregates of nanoparticles on the GO surface was observed in the SEM pictures taken from the *pei*(Fe₃O₄@GO) sample (Fig. 10a). The *pei*(Fe₃O₄@GO) monolith is attracted by a magnet, in both air and water media (Fig. 10b), also indicating the presence of the magnetic nanoparticles on the GO aerogel structure.

Contrarily to the behavior of Fe₃O₄@GO, *pei*(Fe₃O₄@GO) composite was not easily dispersed in water just by dipping. Indeed, the *pei*(Fe₃O₄@GO) sample adsorbed water when immersed in this liquid indicating its hydrophilic character, but it is stable as a monolith when stored under ambient conditions at least for six months (Fig. 10b). This behavior can be explained by the intercalation of the branched polymer among the flakes. On one side, it should be taken into account that C-O-C and C-OH groups on GO are located above and below each layer; on the other side, branched polymers act as multifunctional molecules that can be chemically anchored through several termination points (-NH₂). Hence, the functionalization of GO aerogels using aziridine monomer can produce stitched graphene, as previously demonstrated for other bifunctional amines and branched alkyl polymers.⁵⁶⁻⁵⁸

Figure 10. Characterization of *pei*(Fe₃O₄@GO): (a) SEM image, and (b) behavior in water, medium in which the magnetic monolith was stable in the absence of stirring, although it can be dispersed by sonication.

Influence of Fe₃O₄ coating in MRI contrast agent efficiency

As previously discussed in the introduction, SPIONs have proven to be excellent MRI contrast agents, offering sufficient sensitivity for T₂-weighted imaging. These materials enhance image contrast by decreasing the T₂ time of nearby water protons. The contrast agent efficiency in MRI is given by its relaxivity, which describes how much the transversal relaxation rate (1/T₂) increases due to the contrast agent concentration. Several strategies have been developed to increase contrast offered by SPIONs. For instance, enhanced MRI contrast could be achieved *via* the interaction of NPs sitting on neighboring nanocarriers.⁵⁹ Alternatively, aggregation of NPs can generate very high magnetic field gradients from the collective magnetic behavior of individual units.⁶⁰ However, aggregation also causes precipitation and shortening of circulation time in blood. To achieve enhanced MRI contrast and better biocompatibility and physiological stability, the magnetic materials can be constructed by the controlled clustering of the NPs on a surface, e.g., that of GO sheets.^{61,62} To prove the rational of this idea in the prepared composites, Fe₃O₄@GO or *c*Fe₃O₄@GO samples were tested as MRI contrast agents. A six-well plate

containing $\text{Fe}_3\text{O}_4@\text{GO}$ or $c\text{Fe}_3\text{O}_4@\text{GO}$ suspended in agar with different iron concentration were imaged using a 3T MRI instrument (Fig. 11a). The obtained phantom images were used to plot $1/T_2$ maps as a function of the added iron concentration in each formulation (Fig. 11b). The iron concentration was chosen in a range that results in a linear increase of $1/T_2$. The proton relaxivity values (r_2) were obtained from the slope of these lines. The r_2 values obtained for $\text{Fe}_3\text{O}_4@\text{GO}$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ composites were 69 and 131 $\text{mM}^{-1}\cdot\text{s}^{-1}$, considered in line with reported values for the commercially approved MRI contrast agent Endorem (*ca.* 100 $\text{mM}^{-1}\cdot\text{s}^{-1}$).⁶³ It is worth mentioning that mainly lower values of r_2 than those measured for $c\text{Fe}_3\text{O}_4@\text{GO}$ are found in the literature for other SPIONs@graphene composites.

Figure 11. MRI data for the $\text{Fe}_3\text{O}_4@\text{GO}$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ composites: (a) phantoms MRI of the $\text{Fe}_3\text{O}_4@\text{GO}$ and $c\text{Fe}_3\text{O}_4@\text{GO}$ composite aerogels at different iron concentrations and (b) inverse transverse relaxation time ($1/T_2$) as a function of the iron molar concentration.

The main advantage of the supercritical procedure used in this work is the uninterrupted exfoliation of GO flakes during the different steps of composite formation, including the drying step; thus facilitating the homogeneous distribution of the NPs on the surface and reducing the number of NPs encapsulated between layers. Moreover, in almost all the published examples, the composite involves rGO instead of GO. Values of r_2 from 10 to 120 $\text{mM}^{-1}\cdot\text{s}^{-1}$ have been reported for SPIONs@rGO composites.^{47,60-62} Values of $r_2 > 250 \text{ mM}^{-1}\cdot\text{s}^{-1}$ are claimed for systems in which the NPs are neatly clustered on the surface of rGO.⁶⁰ Only few publications report r_2 data for SPIONs@GO materials, with values of in the range of 75-145 $\text{mM}^{-1}\cdot\text{s}^{-1}$.^{19,64,65} In this study, the reason behind $c\text{Fe}_3\text{O}_4$ @GO exhibiting a higher relaxivity value than Fe_3O_4 @GO (even though both composites have similar M_s values) is related with the characteristics of the clusters formed by NPs aggregation on the surface of the GO matrix. The spatial arrangement of the of the citrate coated NPs in clusters, in a way, would facilitate the diffusion of water molecules within the composite and the interaction of the trapped water with the small $c\text{Fe}_3\text{O}_4$ NPs, which lead to high proton relaxivity values.

CONCLUSIONS

A fast, simple and eco-friendly manufacturing method was developed in this work for the formation of SPIONs@GO composite aerogels. The procedure is based on the exclusive use of scCO_2 technology, thus, resulting in an organic solvent-free, one-pot process, which can be easily scaled up. Aside from sustainability, an additional advantage of the method is related to the use of low-temperature that do not trigger the reduction of GO and, as a consequence, the lasting oxygenated groups would favor a further functionalization of the composite, as well as improve the biocompatibility and physiological stability. Moreover, the formation of composites

shaped into mesoporous aerogels is easily achieved. This investigation paid a special attention on how the addition of GO to SPIONs improves the resulting device in regard of MRI applications by controlling NPs clustering, which is easily achieved through the formation of superparamagnetic 2D nanocomposites. The produced hybrid aerogels were water dispersible and showed dose-dependent MRI properties. The proton relaxivity values obtained for the citrate-coated $c\text{Fe}_3\text{O}_4@\text{GO}$ composite were in the order of *ca.* $130 \text{ mM}^{-1}\cdot\text{s}^{-1}$, which are superior to the reported values for commercially approved MRI contrast agents involving net SPIONs (*ca.* $100 \text{ mM}^{-1}\cdot\text{s}^{-1}$).

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Author Contributions

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ABBREVIATIONS

$1/T_2$ transversal relaxation rate; EDS energy-dispersive X-ray spectroscopy; Fe_3O_4 magnetite; γ - Fe_2O_3 maghemite; $FeCl_2 \cdot 4H_2O$ hydrated iron (II) chloride; $FeCl_3$ anhydrous iron (III) chloride; FTIR Fourier transformed infrared; GO graphene oxide; HCl hydrochloric acid; ICP-MS inductively coupled plasma mass spectrometry; MRI magnetic resonance imaging; M_s saturation magnetization; NaOH sodium hydroxide; NPs nanoparticles; *pei* polyethylenimine; r_2 proton relaxivity; rGO reduced GO; $scCO_2$ supercritical CO_2 ; SEM scanning electron microscope; SPIONs superparamagnetic iron oxide NPs; SQUID superconducting quantum interference device; T_2 transverse relaxation time; TEM transmission electron microscope; TGA thermogravimetric analysis; XPS X-ray photoelectric spectroscopy

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