

Zirconia-Based Magnetoplasmonic Nanocomposites: A New Nanotool for Magnetic-Guided Separations with SERS Identification

Anna Del Tedesco, Valentina Piotto, Gabriele Sponchia, Khohinur Hossain, Lucio Litti, Davide Peddis, Alessandro Scarso, Moreno Meneghetti, Alvis Benedetti, and Pietro Riello*



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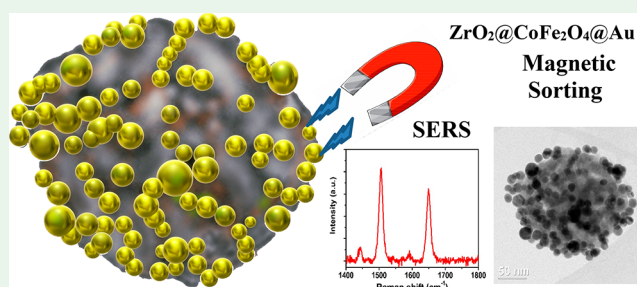
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ABSTRACT: In the field of nanomaterials, multifunctional nanosystems play a prominent role in many applications as new magnetically recoverable catalysts, information processing, fuel cells, efficient bio/nanosensors, and nanomedicine. Aiming at the obtainment of new nanomaterials for biotechnological applications such as biosensors or theragnostic systems, we present a multifunctional system able to merge different properties in only one nanotool. The system is prepared by loading the pores of mesoporous zirconia nanoparticles with CoFe_2O_4 , by a wet impregnation method, further modifying the surface of the material with bis(phosphonic acid) to load gold nanoparticles, produced by laser ablation. The obtained nanocomposite functionalized with a SERS probe represents a specific example of a magnetoplasmonic nanosystem. The results show the efficacy of the strategy of exploiting mesoporous zirconia nanoparticles for obtaining magnetoplasmonic nanotools.

KEYWORDS: zirconia nanoparticles, CoFe_2O_4 nanoparticles, bis(phosphonic acid), gold nanoparticles, magnetic sorting, SERS



INTRODUCTION

A recent achievement in the field of nanomaterials is represented by the development of multifunctional nanocomposites that, by combining the properties of different materials, allow to one obtain multiple functionalities for specific applications. The combination of different physical–chemical properties in a single nanostructure allows one to greatly expand the fields of application, with just one nanotool. The potential field of application of these multifunctional particles is large, ranging from the realization of multimodal contrast agents for nanomedicine,^{1–5} the selective loading, delivery,⁵ and detection⁶ of therapeutic drugs, and theranostic particles^{7–9} to devices for magnetoprocessing of optical information,¹⁰ new and magnetically recoverable catalysts,^{11–14} highly efficient nanosensors,^{8,15} fuel-cell materials,¹⁶ or Raman dispersion substrates (SERS) with improved surface properties.^{2,7,17,18} Furthermore, it is worth underlining that the properties of magnetoplasmonic nanosystems and surfaces can be exploited for their magneto-optical activity.^{19,20}

For applications in nanomedicine, a system that could exploit different properties may represent the right answer to the urgent demand for complete theranostic systems and excellent biosensors. Coupling together magnetic and plasmonic properties is an interesting approach to achieving this goal. In this way, a system that acts as a biosensor as well as a theranostic agent can be produced: magnetic properties can be exploited for the separation of analytes from a complex

matrix for magnetic resonance imaging measurements or magnetic hyperthermia and the plasmonic ones for the detection of biomolecules, for different imaging techniques, or for plasmonic hyperthermia.^{7,21–25} Moreover, SERS signals associated with magnetic nanoparticles are useful for the identification of different analytes, enabling their separation by magnetic sorting.^{7,21,26,27} SERS signals show intensities similar to those of fluorescence signals but with some advantages such as narrower peaks with characteristic fingerprints and the absence of bleaching. For this reason, the SERS signal can be easily identified also in complex matrixes typical of in vivo and/or ex vivo experiments.^{28,29} High enhancement of the Raman signal, up to 10–14 orders of magnitude,³⁰ can be achieved with metal nanostructures or nanoparticle arrangements, showing the so-called “hot-spots effect”^{31,32} in which the local electromagnetic field can be increased by several orders of magnitude.

On the other hand, when the magnetic properties of such materials are focused on, it is well-known from the literature that Fe ions can interact with a ZrO_2 lattice, stabilizing in this way the tetragonal phase.^{33–35} The so-formed solid solution of

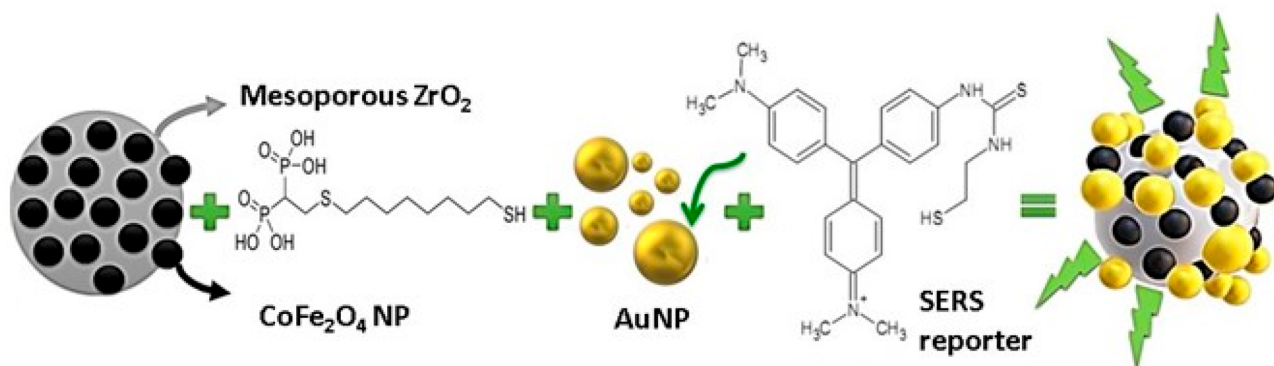
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Scheme 1. Schematic Representation of the Work Done from a Chemical Point of View



iron and zirconium exhibits peculiar magnetic behavior.^{34,36} The high magnetic anisotropy and saturation magnetization of cobalt ferrite [CoFe_2O_4] and its behavior at room temperature make this oxide particularly interesting for biomedical applications, despite the presence of cobalt making it potentially toxic if not sufficiently screened.^{37–39} However, both functionalization of the surface and inclusion in other more biologically safe matrixes open an interesting prospective for this material in the biomedical field.^{40–42}

From the latter considerations, we decided to couple together zirconia and cobalt ferrite following a previously described synthetic route used for the preparation of CoFe_2O_4 -doped silica,⁴³ together with gold nanoparticles (AuNPs) obtained by laser ablation.⁴⁴

Furthermore, the resulting magnetoplasmonic nanostructures could be functionalized with organic molecules to target cells or other kinds of structures. As far as cell targeting is concerned, it can be used, for example, for enriching samples of rare cells like circulating tumor cells (CTCs).^{7,45}

In the present article, first we describe the preparation methods (see Scheme 1) and the characterization of a system that couples magnetic and plasmonic properties. Subsequently, we define the magnetic behaviors of the nanocomposite, and at the end, we show that SERS can be exploited for controlling the magnetic separation. In the present study, in fact, the magnetic property of the synthesized CoFe_2O_4 -doped zirconia nanoparticles allows the recovery of a target system, in few minutes, using a common magnet. This property could be very useful in the field of biological sensing; in fact, this approach enables the purification of a very complex matrix, allowing the separation of different analytes. From a biological point of view, it is crucial to avoid interference and to increase the analytical method sensitivity in biological analyses, in particular for biomedical applications, which need to detect analytes at nano- and picomolar concentrations. To increase the sensitivity, we coupled magnetic nanoparticles with SERS-active AuNPs obtained by laser ablation synthesis in solution (LASiS).⁴⁴ It is shown that the nanoparticles can be attracted by a laboratory small magnet and that the motion in the presence of a magnetic gradient can be explained with a magnetophoretic model. A proof-of-concept experiment is also reported to show that a mixture of magnetic and nonmagnetic zirconia particles, functionalized with AuNPs, can be efficiently separated and that the sorting can be easily monitored with the SERS signals.

The constructed nanocomposites are new tools that show a new strategic approach based on mesoporous zirconia

nanoparticles (MZNs) for obtaining magnetoplasmonic nanosystems that can be profitably exploited in the biotechnology field.

EXPERIMENTAL SECTION

Materials. Zirconium propoxide (Aldrich, 70 wt % in propanol), hexadecylamine (Alfa Aesar, 90%), sodium fluoride (NaF; Eurobio 99%), cobalt nitrate hexahydrate [$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; Janssen Chimica, 99.99%], iron nitrate nonahydrate [$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$; Aldrich, 99.99%], 1,8-octanedithiol (Tokyo Chemical Industry, >97%), bromotrimethylsilane (Sigma-Aldrich, 97%), ethanol (EtOH; Aldrich, 99.8%), and ultrapure water.

Reagents and solvents of high purity were purchased by the providers and used as received. Otherwise, they were purified following the procedures reported in the literature.⁴⁶ Anhydrous solvents were prepared by adding activated 3 Å molecular sieves to the specific solvent under an inert atmosphere. Molecular sieves were activated shortly before use by continuous heating under vacuum. Flash chromatography was performed with silica gel Merck 60, 230–400 mesh, following procedures reported in the literature.⁴⁷

Synthesis of MZNs. The synthesis of MZNs has been reported elsewhere.⁴⁸ Briefly, a first solution was prepared by dissolving the surfactant in EtOH and adding a second aqueous solution with the correct concentration of dissolved NaF. A third solution of the EtOH precursor was finally added dropwise under vigorous stirring. The precipitate was recovered by centrifugation and washed thoroughly. Finally, the recovered powder was treated under vacuum at 120 °C to remove the residual surfactant, avoiding any modification of the structure of the nanoparticles.

Zirconia Nanoparticles/Iron–Cobalt Oxide [MZN@ CoFe_2O_4 , a Magnetic Mesoporous Zirconia Nanoparticle (mMZN)]. The synthesis of metal oxide doped nanoparticles was adapted from the procedure developed by Parma et al.⁴³ MZNs (10 mg mL⁻¹) were dispersed in ultrapure water and impregnated using nitrate salt of the desired metal (cobalt or iron) in the proper amount. After stirring overnight, the impregnated materials were dried under vacuum and then annealed at 700 °C for 12 h in air. The synthesized samples are described in Table 1.

Synthesis of AuNPs. AuNPs were synthesized using the LASiS method.⁴⁴ LASiS was obtained by focusing (10 cm focal length) 9 ns at 1064 nm of a Nd:YAG (Quantel YG981E, 10 Hz) laser on a pure gold target under a bidistilled water solution of 10⁻⁵ M NaCl. AuNPs

Table 1. Nominal Compositions of the mMZNs Loaded with Magnetic CoFe_2O_4

$(\text{CoFe}_2\text{O}_4 \text{ moles}/\text{ZrO}_2 \text{ moles}) \times 100$	sample name	CoFe_2O_4 , mol %
4	mMZN-4	3.8
8	mMZN-8	7.4
12.5	mMZN-12.5	11.1

were obtained with an average size of 25 nm and a ζ potential of -30 mV. Nanoparticles were easily functionalized thanks to their naked surface.

Synthesis of [2-[(8-Mercaptooctyl)thio]ethane-1,1-diy]bis-(phosphonic acid) (BPODE). The bis(phosphonic acid) bearing a terminal thiol moiety useful for nanoparticle surface functionalization was prepared following a modified procedure reported in the literature.⁴⁹ Details can be found in the [Supporting Information](#).

The synthesis of the bis(phosphonic acid) was followed with a TLC Polygram Sil G/UV254 (0.25 mm thickness). ¹H, ¹³C, and ³¹P NMR and 2D spectra were recorded with Bruker Avance 300 and Ascend 400 spectrometers, respectively, working at 300–400 MHz for ¹H NMR, 75–100 MHz for ¹³C NMR, and 121–161 MHz for ³¹P NMR. Resonance frequencies are referred to tetramethylsilane and 85% H₃PO₄.

Functionalization of the Nanoparticles. MZN or mMZN (10 mg mL⁻¹) dispersed in 5 mL of ultrapure water was treated with the desired amount of BPODE (structure reported in the [Supporting Information](#)) with a final concentration of 10 mM. The suspension was stirred for 5 h, after which the nanoparticles were filtered and washed with ultrapure water.

Synthesis of the mMZN@AuNPs. A total of 0.7 mg of mMZN-8 nanoparticles, functionalized with BPODE, was dispersed in 1 mL of distilled water. The solution was centrifuged at 800 rcf for 3 min to eliminate aggregates. After magnetic separation, the zirconia-based nanoparticles were dispersed in 1 mL of a AuNPs aqueous colloidal solution (3×10^{-9} M) and synthesized by LASiS (see above). Functionalization of the mMZN-8 nanoparticles with the AuNPs (mMZN@AuNPs) was achieved after sonication for 2 h with the addition of a NaCl solution to a final concentration of 20 mM. Isolation from free AuNPs was achieved by magnetic sorting. Finally, the SERS reporter, malachite green functionalized with a thiol-containing side chain³² (20 μ L at 30 μ M), was added to the mMZN@AuNPs dispersed in 1 mL of distilled water. After sonication for 45 min, the mMZN@AuNPs were cleaned three times by magnetic sorting.

Synthesis of the MZN@AuNPs. A total of 0.7 mg of MZNs functionalized with BPODE was centrifuged at 650 rcf for 8 min to eliminate aggregates. Then the supernatant was centrifuged at 25000 rcf for 10 min, and after elimination of the supernatant, 1 mL of a AuNPs aqueous colloidal solution (3×10^{-9} M), synthesized by LASiS, was added. Sonication and a solution of NaCl were used in the case of mMZN@AuNPs. Cleaning from free AuNPs was obtained by centrifugation at 810 rcf for 6 min because denser AuNPs precipitated, whereas MZN@AuNP nanostructures were recovered from the supernatant. Texas Red functionalized with two thiol units^{3,32} (20 μ L at 30 μ M) was added, as a SERS reporter, to 1 mL of MZN@AuNPs in water. After sonication for 45 min, the nanostructures were cleaned three times from the reporter by centrifugation.

Characterization Methods. The obtained nanocomposites were studied by field-emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), powder X-ray diffraction (PXRD), and small-angle X-ray scattering (SAXS).

The size and shape of the nanoparticles were investigated by electron microscopy using a Zeiss Sigma VP field-emission scanning electron microscope, equipped with a Bruker Quantax energy-dispersive X-ray (EDX) spectrometry microanalysis detector. *ImageJ* software was used to evaluate the size of the nanoparticles: a minimum of 100 particles were used to determine the size distribution of all analyzed samples.^{50,51}

TEM images were taken with a JEOL 3010 microscope, operating at 300 kV, equipped with a GATAN (Warrendale, PA) multiscan CCD camera. Some drops of the samples ultrasonically dispersed in EtOH (ca. 1 mg mL⁻¹) were deposited on a holey carbon film grid for TEM observation.

The PXRD measurements were performed using nickel-filtered Cu K α radiation in Bragg–Brentano geometry with a X'Pert goniometer (Philips) equipped with a graphite monochromator. Line-broadening analysis was used to evaluate the crystallite sizes.⁵²

SAXS data were collected with a Kratky camera (PAAR) using nickel-filtered Cu K α radiation. The data were corrected for the absorption effect, and the sample holder scattering was subtracted.

A Micromeritics ASAP 2010 analyzer was used to collect the nitrogen adsorption–desorption isotherms at liquid-nitrogen temperature. The data were analyzed using the Brunauer–Emmett–Teller equation⁵³ to obtain the specific surface area and the Barrett–Joyner–Halenda model⁵⁴ to recover the pore-size distribution.

DRIFT-IR spectra were recorded with a Nexus FT-IR using a Nicolet AVATAR diffuse-reflectance accessory.

An Agilent Cary 5000 spectrophotometer and a quartz cuvette (2 mm optical path) were used to collect the UV–vis–near-IR spectra. SERS spectra in solution were recorded in a 2 mm quartz cuvette with a Renishaw inVia micro-Raman instrument using a 5 \times objective and a helium–neon laser with a power of 1.5 mW on the cuvette.

ζ -potential measurements were recorded with a Malvern Nano ZS instrument equipped with a 633 nm helium–neon laser.

Direct-current (dc) magnetization measurements were performed with a Quantum Design SQUID magnetometer, equipped with a superconducting coil, which produces magnetic fields in the range of ± 5 T. The nanoparticles were dispersed in an epoxy resin to avoid any movement during the measurements.

Magnetophoretic characterization was performed by recording the decreasing UV–vis extinction of a solution of magnetic nanosystems under a magnetic gradient obtained with a small magnet.²¹ The solution was inserted into the UV–vis spectrometer in a $3 \times 10 \times 10$ mm³ (optical path 10 mm) cuvette, whereas the magnet (Supermagnete S-10-20-N; $D = 10$ mm diameter and $h = 20$ mm height) was inserted perpendicularly to the optical path and in contact with the solution.

RESULTS AND DISCUSSION

MZNs were successfully obtained by sol gel using a neutral surfactant combined with inorganic salts.⁴⁸

It is worth noticing that, in order to obtain zirconia nanoparticles with large surface area preserving the porosity, the surfactant was removed at 120 $^{\circ}$ C in a vacuum extraction process. The nanoparticles obtained with this method were amorphous, spherical, and monodispersed, with a mean diameter of 200 nm ([Figures 1](#), left, and [S14](#)).

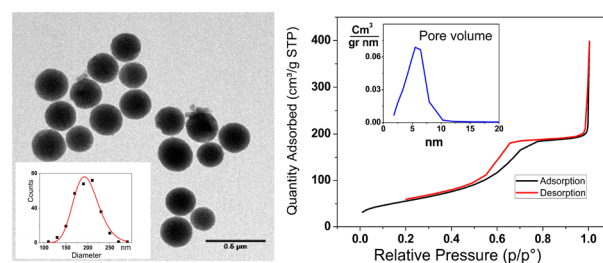


Figure 1. Left: TEM images of MZNs and particle size distribution (inset). Right: Nitrogen physisorption analysis and pore-size distribution before thermal treatment (inset).

The nanoparticles have a surface area of about 200 m² g⁻¹ with a pore volume of 0.3 cm³ g⁻¹ and a pore diameter distribution centered at 5 nm, sufficiently large to host several types of molecular guests. The isotherms display the type IV profile with a H1 hysteresis loop (according to the IUPAC classification; [Figure 1](#), right),⁵⁵ typical for mesoporous materials. If treated at temperatures higher than 300 $^{\circ}$ C, ZrO₂ crystallizes, with collapse of the structure of the MZNs with a loss of porosity and the formation of large aggregates.^{48,56,57}

Upon high-temperature treatment (700 °C), the nanoparticles impregnated with cobalt and iron salts do not aggregate and the roundness of the nanoparticles is maintained (Figure 2).

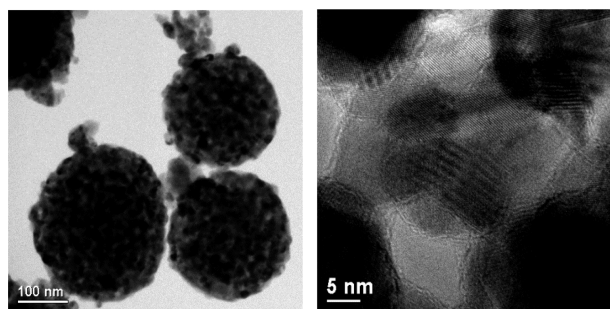


Figure 2. TEM images of mMZN-8.

All samples, after thermal treatment, have a surface area of around $20 \text{ m}^2 \text{ g}^{-1}$ (nitrogen physisorption analysis; Figure S15), 10 times lower than the initial surface area and close to the surface area of full density ZrO_2 nanoparticles of similar size: pores are now filled with metal oxides and/or shrunken because of the crystallization of zirconium oxide.

The sample mMZN-12.5, i.e., with a higher loading of CoFe_2O_4 , shows a big change in the morphology and microstructure and a large amount of iron and cobalt oxide particles outside the ZrO_2 nanospheres, while the sample mMZN-8 is the best compromise for the microstructure, loading, and presence of cobalt–iron oxide nanoparticles outside the ZrO_2 nanospheres (see the SEM images in Figures S16 and S17).

TEM images of mMZN-8 confirm that the shapes and sizes of the nanoparticles are maintained and show that they have a crystalline microstructure (Figure 2, right) with sizes in the range of 15–30 nm (further confirmed by SAXS and wide-angle X-ray scattering measurements; Table S1).

The PXRD plots in Figure 3 show both the MZN patterns before (amorphous) and after the thermal treatment of mMZN.

Crystallization of MZN (Figure 3d) provides mainly the thermodynamically stable monoclinic ZrO_2 with a very low

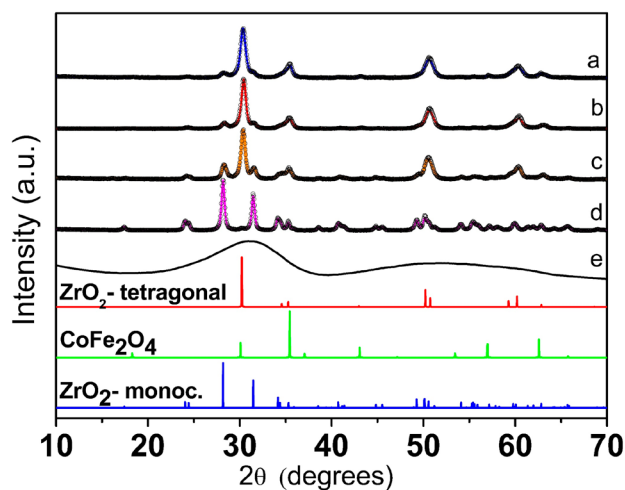


Figure 3. PXRD patterns of (a) mMZN-12.5, (b) mMZN-8, (c) mMZN-4, (d) MZN@700 °C, and (e) amorphous MZN.

content of tetragonal ZrO_2 probably stabilized by the small size of the crystallites (Table S1). On the other hand, crystallization of all of the mMZN-X samples produces both tetragonal and monoclinic ZrO_2 . Stabilization of the most symmetric tetragonal phase is probably due to the formation of a solid solution between the zirconia and iron or cobalt oxides.^{35,58–60} The weight fraction of the tetragonal phase of ZrO_2 increases with the amount of Co and Fe ions until about 70% for the 8% and 12.5% samples (Table 2).

Table 2. PXRD Quantities of Different Phases of the Different Samples (in Parenthesis, the Expected Nominal Values)

sample	t- ZrO_2 (wt %)	m- ZrO_2 (wt %)	CoFe_2O_4 (wt %)	CoFe_2O_4 (mol %)
MZN@700 °C	2	98		
mMZN-4	56	38	6	3.2 (3.8)
mMZN-8	73	19	8	4.4 (7.4)
mMZN-12.5	68	17	15	8.5 (11.1)

Furthermore, the crystalline CoFe_2O_4 fraction, determined by Rietveld analysis with some difficulty because of superposition of the peaks (Figure S18), is always lower than the loaded one, in accordance with the hypothesis that part of the iron–cobalt oxides form solid solutions, with ZrO_2 stabilizing the tetragonal zirconia fraction. We can also observe that the missing fraction of cobalt–iron oxide saturated at about 3.0 mol %.

SAXS analysis allows measurement of the sizes of the CoFe_2O_4 particles that have an electron density lower than the electronic density of the ZrO_2 matrix.

The size of the large sphere ($\sim 200 \text{ nm}$) is higher than the resolution of our Kratky camera, so only the asymptotic h^{-3} behavior (Porod asymptotic law for a slit-smear camera) is visible as the background.

SAXS data are fitted assuming a distribution of spherical CoFe_2O_4 inclusion, and the obtained average sizes agree with the PXRD pattern analyses reported in Table S1: the CoFe_2O_4 crystallite sizes are smaller or equal to the sizes of the particles. Figure S19 shows the fit and size distribution obtained for the 8% sample.

DC magnetization measurements with a SQUID magnetometer were also performed (Figure S20). As expected, at low temperature, all of the samples show hysteretic behavior. mMZN-4 shows an evident kink around zero field, together with strong but not saturated character at high field.

This feature can be ascribed to the presence of a soft magnetic phase, probably due to separation of Co_3O_4 , as was already observed in the literature,⁶¹ inducing an off-stoichiometry in the CoFe_2O_4 nanoparticles. The presence of soft-magnetic-phase mMZN-4 affects the value of the coercivity field (H_c) and, especially, the value of saturation magnetization (M_s) at 300 K, which is extremely low (Table 3) with respect to the other samples, thus confirming the presence of some impurities (e.g., Co_3O_4). Because of the behavior of this sample, it seems to be not physically possible to determine a value of the reduced remanence magnetization (M_r/M_s). On the other hand, samples with 8% and 12.5% of cobalt ferrite show quite high coercivity, compatible with the presence of stoichiometric nanostructured CoFe_2O_4 .

Table 3. DC Magnetization Measurements with a SQUID Magnetometer: Coercive Field ($\mu_0 H_c$), Magnetization Recorded at 5 T (M_{5T} , 5 K), Reduced Remanent Magnetization (M_r/M_s), and Saturation Magnetization Recorded at 300 K (M_s , 300 K)^a

sample	$\mu_0 H_c$ at 5 K (T)	M_{5T} at 5 K ($A\ m^2\ kg^{-1}$)	M_r/M_s	M_s at 300 K ($A\ m^2\ kg^{-1}$)
mMZN-4	0.92(1)			29(1)
mMZN-8	1.4(2)	88(4)	0.8	63(4)
mMZN-12.5	1.4(2)	68(5)	0.7	51(2)
mMZN-8-BPODE	1.4(2)	91(4)	0.5	45(3)

^aUncertainties in the last digit are given in parentheses.

At low temperature, a strong nonsaturating tendency is observed probably because of the high surface anisotropy.⁶² However, the values of magnetization measured at 5 T are also compatible with the presence of $CoFe_2O_4$ nanoparticles, with a M_{5T} value for mMZN-8 that is very close to the bulk value ($93\ A\ m^2\ kg^{-1}$),⁶³ indicating the good quality of the particles in this sample (Table 3). The lowering of M_{5T} with respect of the bulk value observed in mMZN-12.5 can be ascribed to the complex interplay between the surface spin disorder (i.e., spin canting) and cationic distribution.⁶⁴ The good magnetic properties of the mMZN-8 sample are confirmed by the value of the reduced remanent magnetization at 5 K around 0.7, which is close to the theoretical value for the cubic anisotropy ($M_r/M_s = 0.8$) and definitively in agreement with that of the crystalline particles of $CoFe_2O_4$.^{65,66} All of the samples show nonzero coercivity and remanence magnetization at 300 K (Figure S20), clearly indicating that at this temperature nanoparticles in the “blocked state” are still present. The value of saturation magnetization confirms again the better performance of the mMZN-8 sample, showing the highest saturation magnetization.

The 8% sample has been successfully functionalized with BPODE, as proven by EDX, IR, ζ -potential, and SQUID analysis. This simple alternative method for functionalization of the metal oxide nanoparticles does not use the organosilane precursors,^{67,68} which often also require a silica interface layer

to obtain an effective functionalization. DRIFT-IR spectra (Figure 4, left) show changes of the MZNs after functionalization: as one can see in the red line, peaks under $3000\ cm^{-1}$ and around $1000\ cm^{-1}$ were found, corresponding to aliphatic C–H and bounded P–O vibrations, respectively. Moreover, as one can see from the spectra of the bis(phosphonic acid) before coupling (blue line) and of the coupled system (red line), peaks concerning P–O vibrations are shifted at higher wavenumbers, proving the bonding of the molecules on the nanoparticles.^{69,70} ζ -potential titrations were also performed to confirm surface modification of the nanoparticles (Figure 4, right). As one can see, charge values change substantially with the samples: MZNs show positive ζ -potential values at acidic pH and negative ones in basic conditions, having an isoelectric point around pH 7.

On the other hand, for modified MZNs, different behavior are shown: they present more negative values of the surface charge at almost all tested pH values. The mMZN-8 samples have slightly positive ζ -potential values at the lowest pH tested, showing an isoelectric point around pH 4.5, and decrease with increasing pH.

This behavior could be related to the introduction of a magnetic phase, which differently interacts with aqueous buffered solutions. mMZN-8-BPODE shows very negative values of the surface charges; the presence of several polyanionic BPODE molecules that modify the surface of the mMZNs, thanks to the high charge, prevent aggregation and ensure high colloidal stability for several days. It is worth noticing that, in the pH range 4–9, modification with bis(phosphonic acid) provides the nanoparticles with a superficial negative charge with an overall potential below $-20\ mV$, which is highly responsible for the good colloidal stability. Moreover, repeated measurements on the same samples showed good reproducibility of the colloidal solutions, as was clearly demonstrated by the rather small error scale bar for each ζ -potential data point.

Functionalization of the particles is also confirmed by variation of the magnetic hysteresis loops (Figure S21) recorded at 5 K due to the interaction of BPODE with cobalt ferrite on the surface of the nanoparticles, as was already reported in the literature,^{71–73} as well as EDX measurements

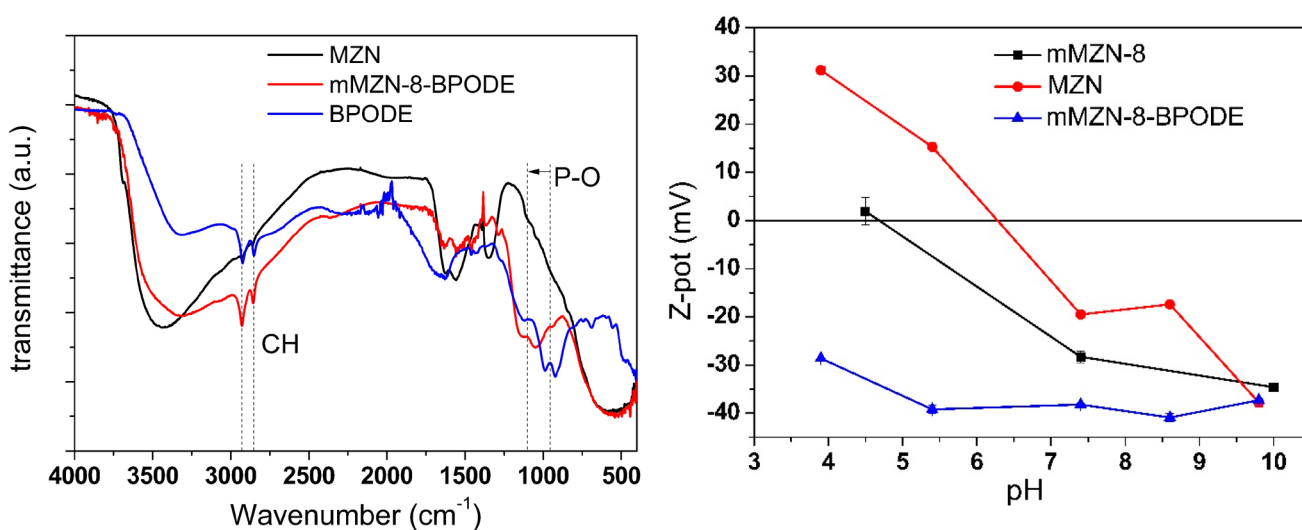


Figure 4. Left: DRIFT-IR spectra (a) of MZN (black line), BPODE (blue line), and MZN-BPODE (red line). Right: ζ -potential (b) titrations of MZN (red line), mMZN-8 (black line), and mMZN-BPODE (blue line).

(Figure S22) that show the sulfur peak at 2.30 keV in the mMZN-BPODE spectrum, a peak that is evident when compared with the pattern of the unmodified MZN.

For plasmonic applications, mMZN-8 functionalized with BPODE was coupled with AuNPs obtained by laser ablation (see the Experimental Section). The TEM and FE-SEM images, reported in Figure 5, clearly show the presence of the AuNPs on the surface of the mMZN-8 nanoparticles.

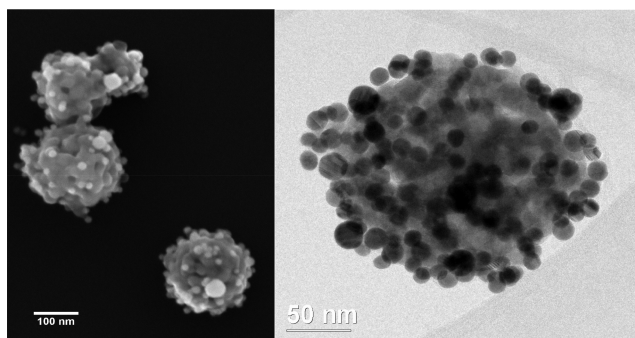


Figure 5. FE-SEM (left) and TEM (right) images of mMZN-8@AuNPs.

An important property of magnetoplasmonic nanoparticles is their mobility in a magnetic gradient, which can be exploited for applications in different research fields like increasing signals of the analytes or for cell separation.⁷

Particle migration, under a magnetic gradient, can be followed with UV–vis extinction variation of the corresponding colloidal solution.²¹ Then, changes of the recorded extinction spectrum can be fitted considering the attraction force generated by the magnetic gradient and the viscosity of the solvent, which counterbalance the accelerated motion of the particles toward the magnet.⁷⁴ The fitting of the extinction decrease, when the nanoparticles are attracted by the magnet, can be obtained considering the distribution of the nanoparticle diameters recorded with SEM images.

The magnetophoretic experiment was performed by recording the extinction at $\lambda = 370$ nm, where the magnetic nanoparticles show a good extinction spectrum (Figure S23).

First, we evaluated the magnetophoretic properties of mMZN-8 (Figure 6 (red points)) as a function of time.

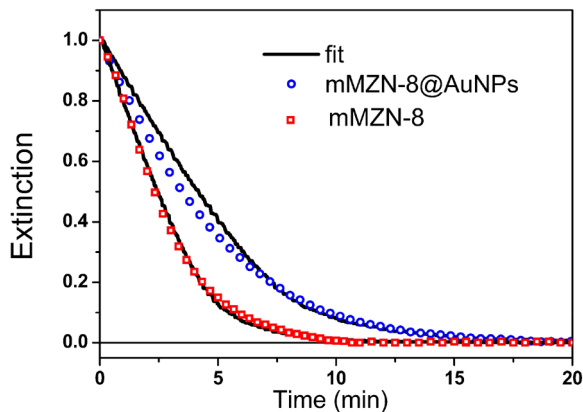


Figure 6. Magnetophoretic curves (normalized at 0 min) and their fittings for mMZN-8 (red) and mMZN@AuNPs (blue) with the parameter values reported in Table S2.

The fitting was obtained with the parameters reported in Table S2. A parameter of the fitting is the percentage of the magnetic phase, observing that the fitting suggests a value very similar to that obtained with the XRD measurements (Table 2). The nanoparticles show a good response to the magnetic gradient because, in only 6 min, more than 90% was recovered within 3 mm from the magnet and almost 100% after 10 min.

The same experiment was performed with the mMZN@AuNP magnetoplasmonic nanostructures. The extinction spectra of MZN@AuNPs and of mMZN-8@AuNPs show the characteristic bands of aggregated AuNPs, namely, a plasmonic band around 520–550 nm, also observed for isolated AuNP, and other plasmonic bands at longer wavelengths (600–800 nm), characteristic of aggregated AuNPs³¹ (Figure S24). Aggregation of the nanoparticles is very important because hot spots, where SERS is particularly enhanced, are created between the nanoparticles (see below).

The extinction data at 370 nm for the magnetophoretic experiment with mMZN@AuNPs and their fittings (see the parameter values in Table S2) are reported in Figure 6 (blue points). It is possible to observe that the hydrodynamic diameter obtained is larger than that in the previous case, which is justified by the different hydrodynamic diameters of the particles with AuNPs on their surface. The magnetophoretic response of mMZN@AuNPs is always good because almost 100% of the particles was recovered within 18 min.

mMZN@AuNPs were then functionalized with thiolated malachite green (MG-SH): Figure 7a shows the SERS spectrum of the obtained nanostructures, with the characteristic band of MG-SH at 1617 cm^{-1} .³²

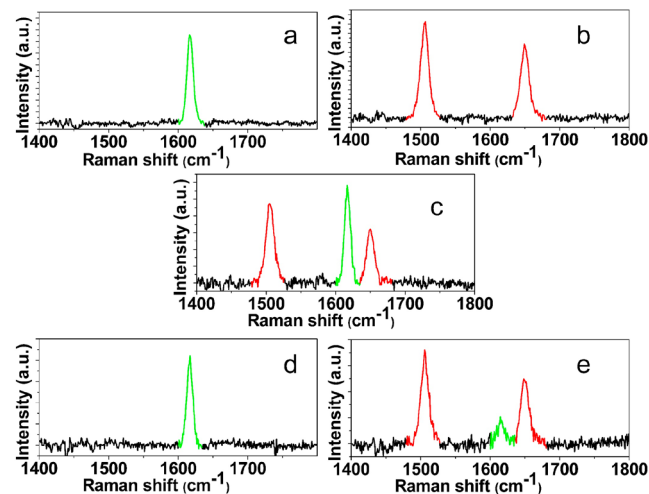


Figure 7. Raman spectra of (a) mMZN@AuNP-MG, (b) MZN@AuNP-TR, and (c) MZN@AuNP-TR and mMZN@AuNP-MG mixed together. (d and e) Raman spectra after magnetic sorting of the two systems mMZN@AuNP-MG and MZN@AuNP-TR, respectively.

The nonmagnetic MZNs were also functionalized with AuNPs, providing MZN@AuNPs (see the Experimental Section) after functionalization with BPODE. The extinction spectrum of these nanoparticles, as in the case of mMZN@AuNPs, shows that aggregated AuNPs were present (Figure S24). MZN@AuNPs were functionalized with a different SERS reporter, thiolated Texas Red (TR-SH), and in Figure 7b is reported the SERS spectrum of these nanoparticles, with the characteristic bands of TR-SH^{3,7} at 1504 and 1650 cm^{-1} .

The strong SERS spectra of mMZN@AuNPs and of MZN@AuNPs show that these nanocomposites can be used as efficient SERS tags for applications in which their presence has to be monitored like in tumor cell targeting.^{7,32}

The extinction spectrum of the ZrO₂ nanoparticles with AuNPs aggregated onto their surface and SERS enhancement in the hot spots of these aggregates can be calculated with a boundary element method (BEM) calculation.^{31,75,76} A model structure for the calculation considers a ZrO₂ sphere with a diameter of 200 nm and seven AuNPs of 25 nm each, organized like a “flower” on the surface of the zirconia sphere, at a distance of 0.5 nm from each other (Figure S25). This distance is appropriate for AuNPs, which are nearly in contact to avoid quantistic coupling effects.³¹ The calculated spectra (absorption, scattering, and their sum, extinction) for this structure are reported in Figure S26a. These spectra show that the scattering component due to the ZrO₂ particles is the most important contribution to the extinction spectrum and that the small absorbance component is mostly due to the AuNP “flower”. This is evident from the calculated spectra reported in Figure S26b for an isolated “flower”.

The same BEM calculations allow evaluation of the enhancement of the SERS spectrum. The largest enhancement is found, as expected, within the gaps between the AuNPs (the so-called hot spots). With an excitation line at 633 nm, one calculates, for the AuNP “flower” on the ZrO₂ sphere, a SERS enhancement value of 4.0×10^{10} . The calculated enhancement is characteristic of the cluster of AuNPs and shows that the presence of the ZrO₂ sphere has a small influence on the SERS enhancement because for the isolated flower one calculates an enhancement of 5.8×10^{10} .

A proof-of-concept experiment for exploiting the magnetic properties of mMZN@AuNPs was conducted by mixing these nanostructures with MZN@AuNPs and then operating a magnetic sorting. The efficiency of the separation can thus be monitored with the signals of the SERS reporters of the two nanostructures.

Magnetic separation was performed with four magnets (Supermagnete S-5-25-N; $D = 5$ mm diameter and $h = 25$ mm height) on the side of a small vial containing the colloidal solution for 1 h.

Then, the solution was separated from the nanostructures attracted by the magnets. The SERS spectrum of the solution is reported in Figure 7c, whereas the spectrum of the nanostructures attracted by the magnets and dispersed again in water is reported in Figure 7d.

It is clear that the spectrum of the nanostructures present in solution (Figure 7e) shows the SERS signals of TR-SH at 1504 and 1650 cm⁻¹ and only a small signal of MG-SH at 1617 cm⁻¹, whereas the nanostructures attracted by the magnets (Figure 7d) show only the spectrum of MG-SH at 1617 cm⁻¹. The separation is very satisfactory considering that the residual signal of MG-SH in the solution of nonattracted nanoparticles derives from the difficulty of pipetting the solution without perturbing the nanoparticles attracted by the magnet.

Separation of the nanostructures can be considered to be quite satisfactory also referring to the high efficiency of the SERS spectra, which allows the detection of a very small quantity of SERS reporters.

CONCLUSION

This work reports a new strategy based on MZNs for the construction of a new nanotool exploiting both the magnetic

and plasmonic properties. This was obtained through the synthesis and characterization of cobalt ferrites/zirconia nanocomposites, showing magnetic properties comparable to those of bulk cobalt–iron oxides. A deep investigation was performed for a better understanding of their physical–chemical properties, such as crystal structures and magnetic properties. The spherical nanoparticles were modified with a thiol-functionalized bis(phosphonic acid), which was used to link AuNPs, obtaining nanostructures with magnetic and plasmonic properties. It is worth noticing that the use of thiol-containing bis(phosphonic acid) as a grafting unit to join together the zirconia and AuNPs is a new straightforward approach simply based on the addition of an organic linker in solution endowed with specific organic functional groups, while more traditional methods are based on chemical functionalization via organosilane derivatives, requiring longer multistep functionalization procedures. The nanostructures were further functionalized with SERS reporters. Magnetophoresis characterizations were performed, highlighting the possibility of a fast recovery, using a commercial magnet, of all of the nanostructures. BEM calculations showed, and experimental spectra confirmed, that the magnetoplasmonic nanostructures provide strong SERS signals like those for the isolated plasmonic nanostructures. Experiments for the separation of magnetic and nonmagnetic systems were very satisfactory, opening the possibility of using these nanostructures for the controlled separation of specific biological moieties in complex matrixes like CTCs in whole blood. Such applications, as well as the use of the nanostructures for therapeutic applications like drug delivery or photo/magneto-thermal therapies, which will make these nanostructures theranostic agents, will be further investigated.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnm.9b01982>.

Detailed synthesis of bis(phosphonic acid) and NMR characterizations, TEM and SEM images of the synthesized nanoparticles, PXRD pattern of mMZN-8 compared to those related to t-ZrO₂ (ICSD 164862), m-ZrO₂ (ICSD 417639), and CoFe₂O₄ (ICSD 41257), hysteresis loops at 5 K of mMZN-8 and the functionalized mMZN-8-BPODE, EDX spectra of the functionalized MZN-BPODE, N₂ physisorption of MZN and mMZN-8, extinction spectra of MZN and mMZN-8, and models for the calculation of extinction spectra (PDF)

AUTHOR INFORMATION

Corresponding Author

Pietro Riello – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy; orcid.org/0000-0002-6087-3802; Email: riello@unive.it

Authors

Anna Del Tedesco – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy; Department of Translational Research, National Cancer Institute–CRO-IRCSS, 33081 Aviano, Italy; orcid.org/0000-0002-3433-6472

Valentina Pioletto – Nanostructures and Optics Laboratory, Department of Chemical Sciences, University of Padova, 35131 Padova, Italy

Gabriele Sponchia – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy

Khohinur Hossain – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy

Lucio Litti – Nanostructures and Optics Laboratory, Department of Chemical Sciences, University of Padova, 35131 Padova, Italy; orcid.org/0000-0001-6247-5456

Davide Peddis – Institute of Structure of Matter, National Research Council, 00015 Monterotondo Scalo, Rome, Italy; Department of Chemistry and Industrial Chemistry, Università of Genova, 16146 Genova, Italy; orcid.org/0000-0003-0810-8860

Alessandro Scarso – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy; orcid.org/0000-0001-6114-9181

Moreno Meneghetti – Nanostructures and Optics Laboratory, Department of Chemical Sciences, University of Padova, 35131 Padova, Italy; orcid.org/0000-0003-3355-4811

Alvise Benedetti – Department of Molecular Sciences and Nanosystems, Università Ca' Foscari Venezia, 30170 Mestre, Venezia, Italy

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsnm.9b01982>

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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