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 synthesis parameters on morphology
- 3

4 Abstract

5 Fe₃O₄—Ag composite nanoparticles (NPs) were prepared by a new in situ reduction of Ag on the surface of Fe₃O₄ NPs using gallic acid as reducing agent in different NPs/gallic 6 7 acid ratio. The obtained NPs were characterized by Field-Emission Scanning/Scanning 8 Transmission Electron Microscopy and Fourier-transform infrared spectroscopy in order to evaluate their crystal structure, their morphology, the effective functionalization and 9 the presence of Ag. Different morphologies were obtained: polydispersed aggregates, 10 nanoflower-like and nanodumbbell nanocomposites, depending on the synthesis 11 12 conditions. Well-defined Fe₃O₄-Ag nanodumbbells were obtained in aqueous media, with 13 NPs/gallic acid ratio of 10:1. It was also assessed that silica shell is not a key issue to optimize the morphology of Ag NPs nucleated on the Fe₃O₄ core. 14

15

16 Structured Abstract

Aim: Synthesis of Fe₃O₄—Ag composite nanoparticles by a new *in situ* reduction of Ag
NPs on the surface of Fe₃O₄ NPs using gallic acid as reducing agent.

Materials & Methods: The influence of process parameters on NPs morphology and
 functionalization was evaluated by means Field-Emission Scanning/Scanning
 Transmission Electron Microscopy and Fourier-transform infrared spectroscopy.

Results and Conclusions: The synthesis conditions affect the morphology of the obtained nanoparticles, evidencing the formation of polydispersed aggregates, nanoflower-like or nanodumbbell nanocomposites. In particular, well-defined nanodumbbells were obtained in aqueous media, with NPs/gallic acid ratio of 10:1; while
the presence of a silica shell did not improve the morphology of Ag NPs nucleated on the
Fe₃O₄ core.

28

29 Lay abstract:

Nanoparticles (NPs) are extensively investigated in the biomedical field for the diagnosis
and the treatment of tumors. The aim of this work is to develop a simple and green method
to produce composite nanoparticles, formed by iron oxides (magnetic) and silver NPs.

Magnetic NPs can be moved using an external magnetic field and localized in the tumor; silver NPs can generate useful heat to kill tumor cells when exposed to light and possess antibacterial properties. The performed study showed that by varying the process parameters (e.g. amount of reagents, presence of coatings on the particles ...), it is possible to obtain multifunctional NPs with different shapes and sizes, potentially useful for the treatment of tumors.

39

40 Keywords

41 Magnetic nanoparticles, silver nanoparticles, *in situ* reduction, gallic acid.

42

43 Introduction

Magnetic nanoparticles (MNPs) are one of main objects of study in the current literature
concerning nanotechnology [1, 2]. MNPs are often suggested in biomedical applications
as drug carriers due to their ability, when exposed to an external magnetic field, to be
moved purposely to a target site in the human body. Most of the MNPs described in
literature are composed of iron oxide (mainly Fe₃O₄ or γ-Fe₂O₃) often used as magnetic

cores enclosed into an organic or inorganic shell (such as silica, gold, fatty acid, citric 49 50 acid and others) [3-5]. Iron oxide MNPs are among the most appropriate nanoplatforms for biomedical applications due to their chemical stability, superparamagnetism, 51 biocompatibility, easiness of synthesis and since they can be further functionalized with 52 53 molecules of particular interest, such as anticancer drug or specific bioactive molecules [3-5]. Iron oxide MNPs are used as contrast agents in magnetic resonance imagining 54 (MRI), drug delivery systems [6, 7] and deeply investigated for applications in the field 55 of cancer treatment by hyperthermia [2, 8, 9]. 56

57 The decoration of the magnetic core with ultra-small metal nanoparticles can further 58 enhance the variety of potential applications of this versatile category of nanoplatform in 59 biomedical field, merging the magnetic properties of the core with the peculiar properties 60 of metal nanoparticles [10, 11].

For example, silver nanoparticles are of great interest because they exhibit peculiar functionalities, such as, among others, antimicrobial, antiviral, optical and catalytic properties [12-15]. Their chemical stability, biocompatibility and long-term efficacy toward a broad bacterial spectrum make them suitable in many biomedical applications as targeted tool for *in situ* infection treatment [16].

Besides the well-known antimicrobial activity [14, 17, 18], silver nanoparticles are of
great interest in the field of surface-enhanced Raman spectroscopy [19] and photothermal
cancer therapy [20]. Silver nanoparticles exhibit strong absorption band at about 400 nm.
The position of the absorption band can vary depending on the size and shape of silver
nanoparticles.

Magnetic iron oxide nanoparticles and silver nanoparticles can be combined into
nanocomposite platforms implementing the above mentioned properties [6]. For example,

the decoration of Fe₃O₄ nanoparticles with Ag nanoparticles gives rise to enhanced optical
responses [21]. Moreover, the combination of magnetic (Fe₃O₄) and antibacterial (Ag)
properties, can be exploited for the magnetic targeting and removal of antimicrobial
agent.

77 Since magnetic nanoparticles are very sensitive to agglomeration, many methods have been investigated in literature to impart electrostatic or steric repulsion, such as the 78 79 coating with organic (e.g. surfactants, polymers) or inorganic materials (e.g. silica, metal oxides, metals) [22, 23]. Moreover, MNPs have been decorated with noble metals NPs, 80 especially Au NPs, by using different approaches [24]. One of the proposed methods to 81 82 decorate MNPs with metallic NPs is to cover the magnetic NPs with a silica layer, which can be easy functionalized and acts as intermediate layer between the magnetic core and 83 84 the metallic NPs [25]. Noble metal NPs can be also directly attached to the magnetic core 85 using silanes or coupling and reducing agents such as NaBH₄, hydroxylamine, plant extracts, or by the metal NPs adsorption on magnetic NPs surface [11, 24, 26-30]. 86

In the present work we report a new method to prepare Fe₃O₄—Ag composite 87 nanoparticles by in situ reduction of Ag on a magnetic core of Fe₃O₄, focusing the 88 attention on the effect of process parameters (synthesis media and functionalization steps) 89 on the nanoparticles nucleation and morphology. In particular, Gallic Acid (GA) has been 90 91 used for the first time, on the basis of authors knowledge, as *in situ* reducing agent for Ag 92 nanoparticles directly on Fe₃O₄ NPs' surface. GA has been chosen for its above mentioned double action, as well as for its intrinsic anti-inflammatory and anti-tumor 93 action, and for its potential sustainability, due to the possibility to be extracted from 94 vegetal wastes coming from the food production chains [31]. The obtained composite 95 96 NPs, due to both the magnetic properties of Fe₃O₄ and the optical/antibacterial properties

97 of Ag NPs, can be a useful tool for targeting bacterial infection as well as for treating98 tumor and associated complications.

99

100 2. Experimental

Each experimental step is described in the following paragraphs. All reactants were
 purchased from Sigma Aldrich[®]/ Merck.

103

104 2.1 Synthesis of CA stabilized MNPs

The synthesis of Fe₃O₄ NPs (Magnetite-based - M NPs) was carried out by co-105 precipitation [32-34]. As reported also in [10, 11], M NPs were synthesized by mixing 106 aqueous solution of Fe²⁺ and Fe³⁺ salts in alkaline conditions for ammonium hydroxide 107 108 NH4OH (Merck, 25% aqueous solution) [5]. Two solutions 0.1 M of ferrous chloride $FeCl_2 \cdot 4 H_2O$ and ferric chloride $FeCl_3 \cdot 6H_2O$ (Sigma Aldrich, > 99%) were prepared 109 separately by dissolving the required quantities of $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ in 100 110 ml of bi-distilled water, magnetically stirred in two distinct beakers. The pH was around 111 112 3 for the ferrous chloride solution, and around 2 for the ferric chloride solution. After the complete dissolution of the salts, 50 ml of 0.1M FeCl₃ · 6H₂O and 37.5 ml of the 0.1M 113 $FeCl_2 \cdot 4H_2O$ were mixed in a beaker, reaching a pH value around 1.7. 114

115 The pH was then adjusted to about 10 by adding NH₄OH dropwise to the iron salts 116 solution, under continuous mechanical stirring: the mixture turned to black, due to the 117 formation of a suspension of iron oxide NPs (M NPs).

118 The as obtained suspension was then sonicated for 20 minutes and washed in bi-distilled

119 water, using a magnet to induce sedimentation. The as obtained M NPs were finally re-

suspended in 100 ml of bi-distilled water reaching a pH between 7-8.

121 Aiming to improve the MNPs suspension stability, the synthesis was followed by the M NPs stabilization with Citric Acid (CA) [32, 33]. 120 ml of a 0.05 M of CA (Sigma 122 Aldrich, > 99.5%) solution were added to the M NPs, previously separated by 123 sedimentation, adjusting the pH to a value of 5.2 by adding dropwise a concentrated 124 125 NH4OH solution. The new suspension was then heated to 80 °C and stirred (150 rpm) for 90 minutes. The duration of the heating step was optimized in order to promote the CA 126 127 adsorption on the M NPs while minimizing the magnetite decomposition to maghemite. Finally, the CA-capped MNPs were rinsed by means of an ultrafiltration device (Solvent 128 Resistant Stirred Cells - Merck Millipore)(4 steps in 50 ml of bi-distilled water at pH = 129 130 10.2) and suspended in 120 ml of bi-distilled water, adjusting the pH of the final water suspension to 10.2 with diluted ammonia. 131

132

133 2.2 Silica coating on MNPs

A part of CA-capped MNPs was coated with a silica shell following the Stöber method [34] by suspending them in a mixture of Tetraethyl orthosilicate (TEOS) as silica precursor, ethanol and water (ethanol:water volume ratio of 4:1), under stirring in orbital shaker at 37 °C and 150 rpm for 3 hours. Approximately 0.002 ml of TEOS for each mg of magnetic nanoparticles was used. Then, the obtained MS NPs were double-washed with bi-distilled water using an ultrafiltration device and re-dispersed in water.

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141 **2.3 Functionalization with aminopropyltriethoxysilane (APTES)**

To enhance the interaction between Ag NPs and magnetic NPs, terminal -NH₂ groups
were introduced on the surface of both M NPs (route A) and MS NPs (route B) by means
of functionalization with APTES (> 98%, Merck).

The functionalization with APTES on the M NPs was performed including a final step of washing and re-suspension in two different media (ethylic alcohol or water) in order to verify the role of the medium on GA grafting and, in turn, on the nucleation of Ag NPs. MS NPs were resuspended only in water.

For this step, 3 ml of each magnetic NPs suspension were diluted in 50 ml of absolute 149 150 ethanol (anhydrous, >99.9%, Carlo Erba Reagents) and sonicated for 3 minutes. 10 µl of APTES were then added to the resulting colloidal suspensions. The obtained suspensions 151 152 were then heated at 80 °C under mechanical stirring for three hours. The stirring time has 153 been prolonged respect to the conventional synthesis reported in literature, to enhance the 154 APTES grafting and assure a uniform presence of terminal amine group on the magnetic 155 nanoparticles surface, as reported in previous works [10, 11, 35]. Subsequently, the 156 mixtures were cooled at room temperature, the nanoparticles were magnetically separated and centrifuged (7500 rpm /20 minutes) for three times, washed in distilled water and re-157 158 suspended in water or ethanol (M NPs) or only in water (MS NPs) up to a volume of 50 159 ml. To induce positive charge at the surface of the APTES-coated nanoparticles, a diluted HNO₃ solution (0.05 ml of 6 M HNO₃ with 20 ml of water) was added drop by drop until 160 reaching a pH value \approx 5-6, and stirred for 10 minutes. 161

162

163 **2.4 Functionalization with GA**

Gallic acid (GA) was added to both ethanol and water-based M NPs suspensions using two different NPs/GA weight ratio (10:3 and 10:1) [37] and to water-based MS NPs suspension using a NPs/GA weight ratio of 10:1. The mixtures were stirred for 18 h at 80 °C, at the end of the treatment the NPs dispersed in ethanol were washed and centrifuged

- three times with ethanol and re-suspended in ethanol; the same procedure was adopted
- 169 for NPs dispersed in water, using bi-distilled water as washing and dispersion medium.
- 170 Figure 1 resumes the steps of the functionalization process.



Figure 1: functionalization process (a) and scheme (b) of MAwG and MAeG synthesis.

173

174 2.5 Synthesis of Fe₃O₄—Ag composite nanoparticles

The reduction of Ag NPs was performed both for ethanol and water dispersed M NPs and MS NPs, using a AgNO₃/GA weight ratio of 1.7. AgNO₃ was dissolved in water and mixed with the NPs suspension at 37 °C for 15 minutes at 150 rpm; the pH of the mixture was adjusted to about 11 using NH₄OH. At the end of the synthesis the mixture was

179 washed with bi-distilled water, NPs were magnetically separated and re-dispersed in bi-

180 distilled water for further analysis.

	Magnetite	Silica	Synthesis in	APTES	Gallic acid	Silver
		shell	water (w) or		(GA)	
			ethanol (e)			
Μ	 ✓ 					
MAw	✓		W	~		
MAe	✓		e	~		
MAwG	 ✓ 		W	~	✓	
MAeG	✓		e	~	✓	
MAwGAg	✓		W	~	✓	~
MAeGAg	✓		e	~	✓	~
MS	✓	~				
MSAw	✓	~	W	✓		
MSAwG	✓	~	W	✓	 ✓ 	
MSAwGAg	✓	✓	W	✓	\checkmark	~

181 Table 1 reports the acronyms of the obtained NPs and resume their synthesis steps.

182



 Table 1: acronyms and resume of NPs synthesis steps.

184

185 **2.6 Characterization**

186 The structural characterization of M NPs was carried out by means of wide-angle (2θ 187 within 10-70°) X-ray diffraction analysis (XRD – X'Pert Philips diffractometer) using 188 the Bragg Brentano camera geometry and CuKa incident radiation. The identification of

189 crystalline phases was performed by using X'Pert HighScore program equipped with190 PCPDFWIN database.

To evaluate the effective functionalization with APTES and GA, all NPs before the *in situ* reduction of silver were analyzed by means of Fourier-transform infrared spectroscopy (FTIR Tensor 27, Bruker Optics S.p.A, Ettlingen, Germany) from 4500 to 300 cm⁻¹ and with 2 cm⁻¹ resolution. OPUS software (v. 6.5, Bruker S.p.A) was used for instrumental control and spectral acquisition.

196 In order to investigate the NPs functionalization after each synthesis step, the formation of Ag NPs and their dimension and distribution, all nanoparticles were characterized in 197 terms of morphology and composition by means of FESEM (Field-Emission Scanning 198 199 Electron Microscope - SUPRATM 40, Zeiss, Germany) and STEM (Scanning Transmission Electron Microscopy - Merlin Gemini Zeiss, Germany), equipped with 200 Energy Dispersive Spectroscopy (EDS). For STEM observation a drop of each NPs 201 202 suspension was placed on a copper grid with carbon film (SPI Supplies[®] Brand Lacey 203 Carbon Coated 200 Mesh Copper Grids - JEOL S.p.A.). STEM analyses were carried out 204 using different detectors and signals (secondary and backscattered electrons, the Bright 205 Field - BF and Dark Field – DF) in order to better distinguish the metallic NPs.

206

207 **3. Results**

208 **3.1 Route** A

Morphological (FESEM) and phase (XRD) analyses of M NPs (Figure 2) revealed the presence of NPs with spherical morphology and a diameter of about 5-20 nm. The XRD pattern reports the presence of only magnetite/maghemite phase, in accordance with the synthesis performed in air.



Figure 2: CA stabilized M NPs: a) morphological evaluation (FESEM), b) XRD
analysis

Table 2 reports the peaks and bands identified by FTIR analysis of M NPs (stabilized with 217 218 CA), MAw, MAwG, MAe and MAeG. Figure 3 shows as example the curves obtained 219 for M, MAw and MAwG NPs/GA 10:3. As it can be noticed, M NPs spectrum shows the presence of Fe-O bond between 500-600 cm⁻¹ typical of iron-oxide NPs [38], the 220 asymmetric stretching of C-O from the COOH group of CA at 1310 cm⁻¹, the large peak 221 between 1400 cm⁻¹ and 1660 cm⁻¹ can be assigned both to COO-Fe bonding (in literature 222 1431 cm⁻¹) and to the shift of peak characteristic of symmetric vibration of C=O due to 223 the CA interaction with magnetite; moreover a band at about 1700 cm⁻¹, relative to the 224 C=O stretching vibration from the free COOH group of CA, is still visible. In the curve a 225 band between 2600-2950 cm⁻¹ascribable to CH₂ stretching and a barely resolvable band 226 at about 3300 cm⁻¹ due to OH stretching are also observable [38-40]. The MAw NPs 227 spectrum shows a shoulder at 850-950 cm⁻¹ ascribable to the stretch of Si-OH symmetric 228 vibration or Si-O-Fe bond, and two bands around 960 and1000-1150 cm⁻¹ belonging to 229 230 the stretching of Si-O and Si-O-Si groups. The peaks at about 1330 and 1620 cm⁻¹ relative to C-N and NH stretching vibrations respectively, the band between 2920-2850 231 232 cm⁻¹ ascribable to the stretching and vibration of C-H and the bands at 3445 and 1640 cm⁻¹

¹, attributable to the N–H stretching vibration and NH₂ bending mode of free NH₂ groups
[41-43], are hardly attributable due to the overlapping with CA peaks.

The functionalization with GA introduces three peaks between 1600-1200 cm⁻¹ ascribable

to the C=C bond, a small peak at about 1700 cm⁻¹ relative to the C=O stretch of carboxylic 236 group and a band centered at about 3400 cm⁻¹ relative to OH groups of GA [44, 45]. 237 FTIR spectra of M, MAe and MAeG (obtained peaks reported in table 2) evidence a band 238 between 400 and 500 cm⁻¹ ascribable to Fe-O bond and the peak at 1310 cm⁻¹ due to the 239 240 asymmetric stretching of -CO from the COOH group, the peak probably due to both the COO-Fe bond and to the C=O of COOH group that interact with magnetite NPs, the C=O 241 vibration from the free COOH group of CA at about 1700 cm⁻¹ [38, 40]. The 242 243 functionalization with APTES is confirmed by the presence of a shoulder at about 970 cm⁻¹ ascribable to Si-O stretching, a peak at about 1100 cm⁻¹ due to the Si-O-Si stretching 244 and the shoulder at about 2870 cm⁻¹ is related to the stretching vibration C-H. Even if 245 after the synthesis the NPs were washed, some characteristic peaks of ethanol (not 246 reported in table 2) can be observed in the spectrum of MAe at about 1260-1000 cm⁻¹ 247 ascribable to the stretching of C-O and between 3400-3300 cm⁻¹ related to the OH 248 stretching. Both the influence of the residual ethanol and the CA disturb the identification 249 of the peaks relative to the C-N stretching vibration and, the free NH stretching vibration 250 [41-43]. The spectrum of MAeG shows three peaks between 1600-1200 cm⁻¹ ascribable 251 to the C=C bond and a very small peak at about 1700 cm⁻¹ relative to the C=O stretch of 252 carboxylic group, the band at about 3400 cm⁻¹ relative to OH groups of GA in this case 253 254 is poor visible [44, 45].

255

Assignment	Position (cm ⁻¹)	Remarks

Fe-O	500-600	Typical of iron-oxide NPs (M, MAw, MAwG,
		MAe, MAeG)
Si-OH, Si-O-Fe	850-950	A shoulder related to the stretch of Si-OH
		symmetric vibration or Si-O-Fe bond (MS,
		MSAw, MSAwG)
Si-O	960-970	A band due to the Si-O stretching (MAw,
		MAwG, MAe, MAeG)
Si–O–Si	1000-1150	A band belonging to the stretching of Si–O–Si
		groups (MAw, MAwG, MAe, MAeG)
C=C	1200-1600	A peak ascribable to the C=C bond (MAeG,
		MAeG)
C-O	1310	The asymmetric stretching of C-O from the
		COOH group of CA (M, MAw, MAwG, MAe,
		MAeG)
C-N	1330	The peak related to C-N stretching vibration
		(MAw, MAwG, MAe, MAeG)
COO-Fe, C=O	1400-1600	The large peak can be assigned both to COO-
		Fe bonding (in literature 1431 cm ⁻¹) and to the
		shift of peak characteristic of symmetric
		vibration of C=O due to the CA interaction
		with magnetite (M, MAw, MAwG, MAe,
		MAeG)
NH	1620	A broad band at 1628 cm-1 can be ascribed to
		the N–H stretching vibration (MAw, MAe)

NH ₂	1640	The band attributable to NH ₂ bending mode of
		free NH ₂ groups (MAw, MAe)
C=O	1700	Stretching vibration from the free COOH group
		of CA (M, MAw, MAwG, MAe, MAeG)
C=O	1700	A small peak related to the C=O stretch of
		carboxylic group (MAwG, MAeG)
CH ₂	2600-2950	A band ascribable to CH ₂ stretching (M, MAw,
		MAwG, MAe, MAeG)
С-Н	2850-2920	The band ascribable to the stretching and
		vibration of C-H (MAw, MAeG, MAe, MAeG)
ОН	3300-3400	A band related to OH groups (M, MAw,
		MAwG, MAe, MAeG)
N-H	3445	The bands attributable to the N–H stretching
		vibration (MAw, MAwG, MAe, MAeG)

Table 2: List of the main peaks detected in the FTIR spectra of M, MAw, MAwG,

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MAe, MAeG, MS, MSAw and MSAwG NPs.





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The morphological and compositional analysis of MAeG (Figure 4a-c) shows the presence of spherical particles with a diameter between 5-20 nm. The EDS analysis (Figure 4c, MAeG with NPs/GA 10:1 as example) shows the Si peak ascribable to APTES functionalization. Analogously, the morphological analysis of MAwG (Figure 4d-e) reveals the presence of NPs with pseudo-spherical morphology and a diameter of about 5-20 nm. Also in this case the compositional analysis performed on MAwG with NPs/GA 10:1 (Figure 4f) evidences the peak of Si, due to the APTES functionalization.



271	Figure 4: FESEM-EDS analysis of a) MAeG 10:1, b) MAeG 10:3, c) MAeG 10:1, d)
272	MAwG 10:1, e) MAwG 10:3, f) MAwG 10:1.

The STEM-EDS analysis of MAeGAg is reported in figure 5; as it can be observed 274 MAeGAg performed using NPs/GA ratio 10:1 show the presence of Ag nanometric 275 particles (< 50 nm) agglomerated with magnetite nanoparticles, evidenced with arrows in 276 277 figure 5a together with big silver particles (100-150 nm) with different shape (Figure 5b). 278 The EDS of area (Figure 5c) shows the peak of Ag, together with elements characteristic of MAeG (Fe, Si and O) and elements acribable to sample holder (Cu, Al, C). 279 MAeGAg performed using NPs/GA ratio 10:3 show the presence of very small Ag NPs 280 281 (< 10 nm), which forms a nanoflower structure with magnetic NPs (Figure 5d, evidenced by arrows and schematized in the inset). However, also in this case, in some area of the 282 sample big silver crystals were observed (Figure 5f and e) as confirmed by the EDS 283 analysis (Figure 5f). 284



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Figure 5: FESEM-EDS analysis of MAeGAg 10:1 (a-c) and MAeGAg 10:3 (d-f).

Figure 6 shows the STEM-EDS analysis of MAwGAg; in this case only Ag nanoparticles 288 with dimension comparable with magnetite NPs were observed both for NPs/GA ratio 289 290 10:1 (Figure 6a and b) and 10:3 (Figure 6d and e). Thus, in both NPs/GA ratio conditions, the synthesis in water allowed to obtain nanodumbbell structures with magnetite NPs 291 connected with Ag NPs (Figure 6b and 6e). EDS analysis (Figure 6c and f) shows the Ag 292 peak in addition to magnetic NPs peaks. As far as concerned the stability of composite 293 NPs, their visual inspection suggests that they form a fairly stable suspension. Future test 294 295 will be carried out to experimentally evaluate the stability of the nanostructures over time.

296



Figure 6: STEM-EDS analysis of MAwGAg 10:1 (a-c) and MAwGAg 10:3 (d-f).

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300 **3.2 Route B**

The peaks obtained by means of FTIR analysis of MS, MSAw and MSAwG nanoparticles are reported in table 2. MS NPs evidence both the peak ascribable to Fe-O bond at about 500-600 cm⁻¹ and the peak characteristic of silica shell: at 780 cm⁻¹ the stretch of Si-OH, the broad band at about 850-990 cm⁻¹ can be attributed to the Si-O symmetric vibration or to the Si-O-Fe bond and the peak at 1060 cm⁻¹ the stretch of Si-O-Si [46, 47]. The spectrum of MSAw shows an increase of the intensity of the peak related to Si-O-Si bond attributable to the APTES functionalization, together with the peak of Fe-O bond. The spectrum of MSAwG shows the peeks between 1600-1200 cm⁻¹ ascribable to the C=C bond, the peak at about 1700 cm⁻¹ relative to the C=O stretch of carboxylic group, the band at about 3400 cm⁻¹ relative to OH groups of GA [44, 45].

- The morphological analysis of MSAwG (Figure 7a) evidences the presence of pseudo-
- spherical nanoparticles in the range 10-20 nm. The EDS spectrum (Figure 7b) shows a
- very intense peak of Si ascribable to the silica shell, together with Fe and O peak; C peak
- is due to the grid.



315

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Figure 7: STEM image and EDS analysis of MSAwG.

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The STEM analysis of MSAwGAg (Figure 8a) shows the formation of Ag NPs of ≤ 20 nm not well dispersed among magnetic NPs, but aggregated in some area (schematic representation in Figure 8a); compositional analysis of an area (Figure 8b) shows the effective presence of the Ag peak.



- **Figure 8**: STEM-EDS analysis of MSAwGAg obtained using a NPs/GA ratio of 10:1.
- 324

325 **4. Discussion**

Aiming to improve the MNPs suspension stability, useful also for further functionalization steps, the prevention of their aggregation is a crucial aspect. In fact, the stability of MNPs in water is not optimal, due to anisotropic dipolar attraction [48] that could limit the nucleation of the metal nanoparticles on their surface [49]. For this purpose, CA was added to the MNPs suspension as stabilizing agent, promoting electrostatic repulsion, as reported also by several authors [48, 50, 51].

Concerning the functionalization with APTES and GA, no significant differences were noticed by FTIR analysis, although in the synthesis performed in ethanol it was difficult to identify some characteristic groups due to probable traces of residual ethanol.

The morphological and compositional analysis of samples synthesized in water evidenced the nucleation of smaller Ag nanoparticles by using a NPs/GA ratio of 10:3. We supposed that the higher amount of GA used in this synthesis introduced more nucleation sites allowing the formation of a lot of very small Ag NPs. In any case, even in this synthesis some larger Ag crystals, with a multibranched morphology, were noticed. These crystaks could have been originated due to the seeding role of the nanoflwoers-like structures, as reported for ternary heterostructures by H. Zeng and S. Sun [52], further enhanched bythe excess of reducing agent in the synthesis environment.

The sintheses performed in water revealed the formation of small Ag nanoparticles both using a NPs/GA ratio of 110:1 and 10:3. This result could be explained considering that, as reported by [52], the partial exposure of Fe(II) on the nanoparticles to aqueous phase could act as self-catalytic center for the reduction of Ag⁺ ions to Ag^o nanoparticles, leading to the heterodimer morphology.

In summary, the synsthesis in water allows to obtain more homogenoeus heterodimer nanostructures, while the ethanol-based synthesis seems to have some critycal aspects in the control of the dimension and structure of silver NPs. Moreover, in the syntesis in water, the different ratio between NPs and GA amount seems to not significantly influence the amount and dimension of Ag NPs. For this reason, the route B was perfomed only in water using a NPs/GA ratio of 10:1.

The NPs coating with a shell of silica seems to not improve the NPs functionalization and 354 355 most of all the *in situ* reduction of Ag NPs; in this case some small aggregates of Ag NPs, 356 not uniformely distributed were observed. As reported in other studies [47, 53], the 357 structure of nanocomposites is significantly influenced by charges, reactive groups or functional moieties on the nanoparticles surface. The role of silica layer in the 358 precipitation of the above mentioned aggregates can be explained considering that bi-359 360 component hybrid nanoparticles are generally obtained by sequential nucleation and growth of the second component on a preformed seed of the first one [52]. The successful 361 362 synthesis of heterodimers strongly depends on the ability of suppressing the formation of dispersed NPs of the second component (homogeneous nucleation) and promote their 363 seed-mediated heterogeneous nucleation on the first component NPs. This could be 364

365 achieved if the lattice spacing between the two components is well-matched to ensure epitaxial growth of the second component on the first one, which often participate to the 366 reaction as catalyst, lowering the activating energy for heterogeneous nucleation. The 367 obtained results demonstrated that the silica shell seems to not positively influence the 368 369 decoration of MS NPs, probably due to its shield effect on the heterogeneous nucleation of Ag NPs, while promoting their homogeneous nucleation in the synthesis medium and 370 371 their aggregated with the MS NPs, due to electrostatic interactions. Thus, the silver in situ 372 reduction by GA and the heterogeneous nucleation are promoted when magnetic NPs are 373 not coated by the silica shell; these two mechanisms are synergistic and lead to a better 374 decoration of magnetite NPs. In particular, if magnetic NPs are not coated by silica, they 375 are able to expose Fe(II) supporting the heterogeneous nucleation of Ag NPs, in addition 376 to the *in situ* reduction due to GA.

377

5. Conclusions

A novel method to prepare composite nanoparticles Fe₃O₄—Ag, by in situ reduction of 379 Ag on a magnetic core of Fe₃O₄ was developed. The synthesis allowed at first to prepare 380 Fe₃O₄ NPs stabilized by CA with spherical shape and size in the range 5 - 20 nm. The 381 382 obtained CA capped Fe₃O₄ NPs were successfully functionalized with APTES to graft GA, as reducing agent for Ag NPs, in different media and with different NP/GA ratios. 383 384 Depending on the investigated synthesis conditions, different morphologies were 385 obtained, from polydispersed aggregates to nanoflower-like and nanodumbbell 386 nanocomposites. The best results were obtained in aqueous media, with NPs/GA ratio of 10:1, producing Fe₃O₄—Ag well defined nanodumbbells of about 20 nm size. 387

A further sol-gel coating of the CA-capped Fe₃O₄ NPs with a silica shell, functionalized 388 with APTES and GA in aqueous medium, was performed to assess its influence on the 389 390 nucleation of Ag NPs. In the presence of the silica coating, Ag NPs with size < 20 nm nucleated as polydispersed aggregated among Fe₃O₄ NPs, evidencing that the silica shell 391 392 is not a key issue to optimize the morphology of Ag NPs nucleated on the magnetic core. Further optimizations of synthesis parameters (such as time and reagent ratio) are still 393 394 necessary to improve the size and distribution of Ag NPs on Fe₃O₄ NPs. The 395 electromagnetic and optical characterizations are still in progress, as well as the 396 evaluation of suspensions stability.

397

398 Summary Points

- 399 1. Development of a novel method to prepare composite nanoparticles Fe_3O_4 —Ag,
- 400 by in situ reduction of Ag on a magnetic core
- 401 2. Use of gallic acid, a green anti-inflammatory and anti-tumor phenolic compound,
- 402 as *in situ* reducing agent for Ag NPs
- 403 3. Evaluation of different synthesis media and functionalization steps
- 404 4. Estimation of different gallic acid/NPs rations
- 5. Successfully functionalization of magnetic NPs with APTES and grafting withGA, both in water and ethanol
- 407 6. Polydispersed aggregates, nanoflower-like and nanodumbbell nanocomposites408 were obtained according to the synthesis conditions
- 409 7. Well-defined Fe₃O₄—Ag nanodumbbells (about 20 nm) were obtained using
 410 aqueous media, a NPs/GA ratio of 10:1

411	8. The presence of a silica coating was not found to be useful to optimize the
412	morphology of Ag NPs nucleated on the magnetic core
413	
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418	References
419	[1] Rahman MM, Nanomaterials, 356. ISBN: 978-953-307-913-4, (2011). DOI:
420	10.5772/1371
421	[2] Wu W, Wu Z, Yu T, Jiang C, Kim WS. Recent progress on magnetic iron oxide
422	nanoparticles: synthesis, surface functional strategies and biomedical applications. Sci.
423	Technol. Adv. Mater. 16, 023501(2015). DOI: 10.1088/1468-6996/16/2/023501.
424	[3] Tartaj P, Morales MP, Veintemillas-Verdaguer S, Gonzalez-Carreno T, Serna CJ.
425	The preparation of magnetic nanoparticles for applications in biomedicine J. Phys. D.
426	Appl. Phys. 36, 182-197 (2003). DOI: 10.1088/0022-3727/36/13/202
427	[4] Veiseh O, Gunn J, Zhang M, Design and fabrication of magnetic nanoparticles for
428	targeted drug delivery and imaging. Adv. Drug. Deliv. Rev. 62(3), 284–304 (2010). doi:
429	10.1016/j.addr.2009.11.002
430	[5] Li Z, Kawashita M, Araki N, Mitsumori M, Hiraoka M, Do M. Magnetite
431	nanoparticles with high heating efficiencies for application in the hyperthermia of
432	cancer. Mater. Sci. Eng. C. 30, 990–996 (2010). Doi:10.1016/j.msec.2010.04.016

- 433 [6] Prucek R, Kilianová TJ, Panáček M et al. The targeted antibacterial and antifungal
- 434 properties of magnetic nanocomposite of iron oxide and silver nanoparticles.
- 435 *Biomaterials* 32, 4704-4713 (2011). doi: 10.1016/j.biomaterials.2011.03.039
- 436 [7] Kluchova K, Zboril R, Tucek J, et al.
- 437 Superparamagnetic maghemite nanoparticles from solid-state synthesis their
- 438 functionalization towards per oral MRI contrast agent and magnetic carrier for trypsin
- 439 immobilization. *Biomaterials* 30, 2855 (2009). doi: 10.1016/j.biomaterials.2009.02.023
- 440 [8] Corr SA, Rakovich YP, Gun'ko YK. Multifunctional magnetic-
- 441 fluorescentnanocomposites for biomedical applications. Nanoscale Res. Lett. 3, 87
- 442 (2008). doi: 10.1007/s11671-008-9122-8
- 443 [9] Srivastava P, Sharma PK, Muheem A, Warsi MH. Magnetic Nanoparticles: A
- 444 Review on Stratagems of Fabrication and its Biomedical Applications. *Recent Patents*
- 445 *on Drug Delivery & Formulation*, 11, 101-113 (2017).
- 446 doi.:10.2174/1872211311666170328150747
- [10] Miola M, Ferraris S, Pirani F. et al. Reductant-free synthesis of magnetoplasmonic
- 448 iron oxide-gold nanoparticles *Ceramics International* 43, 15258–15265 (2017). DOI:
- 449 10.1016/j.ceramint.2017.08.063
- 450 [11] Multari C, Miola M, Laviano F, et al. Magnetoplasmonic nanoparticles for
- 451 photothermal therapy. *Nanotechnology* 30, 255705 (9pp) (2019). doi:10.1088/1361-
- 452 6528/ab08f7
- 453 [12] Mohan YM, Lee K, Premkumar T, Geckeler KE. Hydrogel networks as nano-
- 454 reactors: a novel approach to silver nanoparticles for antibacterial applications. *Polymer*
- 455 48, 158 (2007). Doi:10.1016/j.polymer.2006.10.045

- 456 [13] Wang AQ, Liu JH, Lin SD, Lin TS, Mou CY. A novel efficient Au-Ag alloy
- 457 catalyst system: preparation, activity, and characterization. J Catal 233, 186. (2005)
- 458 doi.org/10.1016/j.jcat.2005.04.028
- 459 [14] Lee D, Cohen RE, Rubner MF. Antibacterial properties of Ag nanoparticle loaded
- 460 multilayers and formation of magnetically directed antibacterial microparticles.
- 461 *Langmuir* 21, 9651 (2005). Doi:10.1021/la0513306
- 462 [15] Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis,
- 463 Characterization, Properties, Applications, and Therapeutic Approaches. Int. J. Mol. Sci.
- 464 17(9) 1534 (2016). doi: 10.3390/ijms17091534
- 465 [16]Markova Z, Šiskova KM, J. Filip et al. Air Stable Magnetic Bimetallic Fe–Ag
- 466 Nanoparticles for Advanced Antimicrobial Treatment and Phosphorus Removal.
- 467 Environ. Sci. Technol. 47, 5285–5293 (2013). Doi:10.1021/es304693g
- 468 [17] Lee HJ, Yeo SY, Jeong SH. Antibacterial effect of nanosized silver colloidal
- solution on textile fabrics. J. Mater. Sci. 38, 2199 (2003).
- 470 Doi:10.1023/A:1023736416361
- 471 [18] Pal S, Tak YK, Song JM. Does the antimicrobial activity of silver nanoparticles
- depend on the shape of the nanoparticle? a study of the gram-negative
- 473 bacterium Escherichia coli. Appl. Environ. Microbiol. 73, 1712. (2007) DOI:
- 474 10.1128/AEM.02218-06
- ⁴⁷⁵ [19] Wu YX, Liang P, Dong Q, et al.Design of a silver nanoparticle for sensitive
- ⁴⁷⁶ surface enhanced Raman spectroscopy detection of carmine dye. *Food Chemistry* 237,
- 477 974-980 (2017). Doi: 10.1016/j.foodchem.2017.06.057

- 478 [20] Zhang H, Chen G, Yu B, Cong H. Emerging advanced nanomaterials for Cancer
- 479 photothermal therapy. *Rev.Adv. Mater. Sci.* 53, 131-146 (2018). DOI: 10.1515/rams-
- 480 2018-0010
- 481 [21] Ching G, Jiang J, Ying JY, Ji W. Fe₃O₄-Ag nanocomposites for optical limiting:
- 482 broad temporal response and low threshold. *Opt Express*18, 6183 (2010). DOI:
- 483 10.1364/OE.18.006183
- 484 [22] Laurent S, Forge D, Port M, et al. Magnetic Iron Oxide Nanoparticles: Synthesis,
- 485 Stabilization, Vectorization, Physicochemical Characterizations, and Biological
- 486 Applications. Chem. Rev., 108(6), 2064–2110 (2008). DOI: 10.1021/cr068445e
- 487 [23] Kharisov BI, Rasika Dias HV, Kharissova OV, Vázquez A, Peña Y Gómez I.
- 488 Solubilization, dispersion and stabilization of magnetic nanoparticles in water and non-
- 489 aqueous solvents: recent trends. *RSC Adv* ,4, 45354-45381 (2014). DOI:
- 490 10.1039/C4RA06902A
- 491 [24] Stafford S, Serrano Garcia R, Gun'ko YK. Multimodal Magnetic-Plasmonic
- 492 Nanoparticles for Biomedical Applications. *Appl. Sci.* 8, 97 (2018).
- 493 doi:10.3390/app8010097
- 494 [25] Abbas M, Ramulu Torati S, Kim C. A novel approach for the synthesis of ultrathin
- silica-coated iron oxide nanocubes decorated with silver nanodots (Fe₃O₄/SiO₂/Ag) and
- their superior catalytic reduction of 4-nitroaniline. *Nanoscale*, 7, 12192-12204 (2015).
- 497 DOI: 10.1039/C5NR02680F
- 498 [26] Lee B, Koo S. Silver reduction on the surface of magnetite nanoparticles using a
- 499 coupling agent. J.Ind. and Eng. Chem. 18(3), 1191–1195(2012). Doi:
- 500 10.1016/j.jiec.2012.01.009

- 501 [27] Ballesteros CAS, Cancino J, Marangoni VS, Zucolotto V. Nanostructured Fe₃O₄
- satellite gold nanoparticles to improve biomolecular detection. Sensors and Actuators B
- 503 198, 377–383 (2014). doi.: 10.1016/j.snb.2014.03.079
- 504 [28] Fantechi E, Castillo PM, Conca E, Cugia F, Sangregorio C, Casula MF, Assessing
- the hyperthermic properties of magnetic heterostructures: The case of gold–iron oxide
- 506 composites. Interface Focus 6, 20160058 (2016). doi: 10.1098/rsfs.2016.0058
- 507 [29] Ivashchenko O, Gapinski J, Pepli´nska B. et al.Self-organizing silver and ultrasmall
- iron oxide nanoparticles prepared with ginger rhizome extract: Characterization,
- 509 biomedical potential and microstructure analysis of hydrocolloids. *Mater. Design* 133,
- 510 307–324 (2017). doi.: 10.1016/j.matdes.2017.08.001
- 511 [30] Amala Jayanthi S, Manovah David T, Jayashainy J, Muthu Gnana D, Nathan T,
- 512 Sagayaraj P. A convenient two-step bottom-up approach for developing Au/Fe₃O₄
- 513 nanocomposites with useful optical and magnetic properties. J. Alloys and Compounds
- 514 606, 254–226 (2014). DOI: 10.1016/j.jallcom.2014.03.186
- 515 [31] Ferraris S, Miola M, Cochis A. et al.In situ reduction of antibacterial silver ions to
- 516 metallic silver nanoparticles on bioactive glasses functionalized with Polyphenols. Appl.
- 517 Surf.Sci. 396, 461–470 (2017). doi.: 10.1016/j.apsusc.2016.10.177
- 518 [32] Borroni E, Miola M, Ferraris S. et al. Tumor targeting by lentiviral vectors
- combined with magnetic nanoparticles in mice. *Acta Biomaterialia* 59, 303–316 (2017).
- 520 doi: 10.1016/j.actbio.2017.07.007.
- 521 [33] Multari C, Miola M, Ferraris S. et al.Synthesis and characterization of silica-coated
- 522 superparamagnetic iron oxide nanoparticles and interaction with pancreatic cancer cells.
- 523 Int J Appl Ceram Technol. 15, 947–960 (2018). Doi: 10.1111/ijac.12897

- 524 [34] Muzio G, Miola M, Ferraris S, et al.A. Follenzi. Innovative superparamagnetic
- 525 iron-oxide nanoparticles coated with silica and conjugated with linoleic acid: Effect on
- tumor cell growth and viability. *Mat. Sci.Eng. C* 76, 439–447 (2017). doi:
- 527 10.1016/j.msec.2017.03.063.
- 528 [35] Stöber W, Fink A, Controlled growth of monodisperse silica spheres in the micron
- 529 size range, J. Colloid Interface Sci. 26, 62–69 (1968).
- 530 [36] Miola M, Multari C, Debellis D, Laviano F, Gerbaldo R, Vernè E,
- 531 Magneto-plasmonic heterodimers: Evaluation of different synthesis approaches. J Am
- 532 *Ceram Soc*. 1–10 (2021).
- 533 [37]Panagiota S, Louloudi M, Deligiannakis Y, EPR study of phenolic radical
- stabilization by grafting on SiO₂. *Chemical Physics Letters* 472, 85-89 (2009). Doi:
- 535 10.1016/j.cplett.2009.02.080
- 536 [38] Cheraghipour E, Javadpour S, Mehdizadeh, AR, Citrate capped superparamagnetic
- 537 iron oxide nanoparticles used for hyperthermia therapy J. Biomedical Science and
- 538 *Engineering*, 5, 715-719 (2012). doi: 10.4236/jbise.2012.512089.
- 539 [39] Nigam S, Barick KC, Bahadur D. Development of citrate-stabilized Fe3O4
- 540 nanoparticles: Conjugation and release of doxorubicin for therapeutic applications a, J.
- 541 Magn. Magn. Mat. 323, 237–243 (2011). Doi: 10.1016/j.jmmm.2010.09.009
- 542 [40] Kulshrestha P, Gogoi M, Bahadur D, Banerjee R. In vitro application of paclitaxel
- 543 loaded magnetoliposomes for combined chemotherapy and hyperthermia. Colloids Surf
- 544 *B Biointerfaces* 96, 1-7(2012). doi: 10.1016/j.colsurfb.2012.02.029.
- 545 [41] Bini RA, Marques RFC, Santos FJ, Chaker JA, Jafelicci Jr M, Synthesis and
- 546 functionalization of magnetite nanoparticles with different amino-functional

- 547 alkoxysilanes. J. Magn. Magn. Mat. 324, 534–539 (2012). Doi:
- 548 10.1016/j.jmmm.2011.08.035
- 549 [42] Khatiri R, Reyhani A, Mortazavi SZ, Hossainalipour M. Preparation and
- 550 characterization of Fe3O4 /SiO2 / APTES core-shell nanoparticles. Proceedings of the
- 4th International Conference on Nanostructures. (2012).
- 552 [43] Kanimozhi S, Perinbam K. Synthesis of amino-silane modified superparamagnetic
- 553 Fe3O4 nanoparticles and its application in immobilization of lipase from Pseudomonas
- fluorescens Lp1. *Materials Research Bulletin*, 48, 1830-1836 (2013). Doi:
- 555 10.1016/j.materresbull.2013.01.024Get rights and content
- 556 [44] Wang W, Chen Q, Jiang C, Yang D, Liu X, Xu S, One-step synthesis of
- 557 biocompatible gold nanoparticles using gallic acid in the presence of poly-(N-vinyl-2-
- 558 pyrrolidone), Colloids and Surfaces A: Physicochem. Eng. Aspects 301, 73–79 (2007).
- 559 doi:10.1016/j.colsurfa.2006.12.037
- 560 [45] Deligiannakis Y, Sotiriou GA, Pratsinis SE. Antioxidant and Antiradical SiO2
- 561 Nanoparticles Covalently Functionalized with Gallic Acid. ACS Appl. Mater. Interfaces
- 562 4, 6609–6617 (2012). doi: 10.1021/am301751s.
- 563 [46] Nadeema K, Ali L, Gul I, Rizwand S, Mumtaz M, Effect of silica coating on the
- structural, dielectric, and magnetic properties of maghemite nanoparticles. J. Non-
- 565 *Crystal. Sol.* 404, 72–77 (2014). Doi: 10.1016/j.jnoncrysol.2014.07.036
- 566 [47] Emadi M, Shams E, Kazem Amini M, Removal of Zinc from Aqueous Solutions
- 567 by Magnetite Silica Core-Shell Nanoparticles. Journal of Chemistry 2012, ID 787682
- 568 (2013). Doi: 10.1155/2013/787682

- 569 [48] Cheraghipour E, Javadpour S, Mehdizadeh AR, Citrate capped superparamagnetic
- iron oxide nanoparticles used for hyperthermia therapy, J. Biomed. Sci. Eng. 5, 715–719
- 571 (2012) . DOI: 10.4236/jbise.2012.512089.
- 572 [49] Cozzoli PD, Pellegrino T, Manna L, Synthesis, properties and perspectives of
- 573 hybrid nanocrystal structures, *Chem. Soc. Rev.* 35, 1195–1208 (2006).
- 574 Doi:10.1039/B517790C
- 575 [50] Goetze TC, GansauBuske N, Roeder M, Gornert P, Bahr M, Biocompatible
- 576 magnetic core/shell nanoparticles, J. Magn. Magn. Mater. 252, 399–402 (2002).
- 577 Doi:10.1016/S0304-8853(02)00624-8
- 578 [51] Campelj S, Makovec D, Drofenik M, Preparation and properties of water-based
- 579 magnetic fluids, J. Phys. Condens. Matter 20, 204101 (2008). doi: 10.1088/0953-
- 580 8984/20/20/204101 * One of the most significant examples of the use of citric acid as
- 581 stabilizing and dispersant agent.
- 582 [52] Zeng H, Sun S. Syntheses, Properties, and Potential Applications of
- 583 Multicomponent Magnetic Nanoparticles. Adv. Func. Mat. 18, 391-400 (2008). Doi:
- 584 10.1002/adfm.200701211 * Complete and detailed review concerning the development
- 585 of multicomponent nanoparticles.
- 586 [53] Cham-Fai Leung K, Xuan S, Zhu X, et al.Gold and iron oxide hybrid
- nanocomposite materials. *Chem. Soc. Rev.* 41, 1911–1928(2013). Doi:
- 588 10.1039/C1CS15213K
- 589
- 590 Table of Contents graphic

