

Tannic-acid-mediated synthesis and characterization of magnetite-gold nanoplatforms for photothermal therapy

*Original*

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1           **Tannic acid mediated synthesis and characterization of magnetite-gold**  
2                           **nanoplatfoms for photothermal therapy**

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18  
19           **Abstract**

20           Magnetite (MNPs) and gold (GNPs) nanoparticles attracted attention because of their  
21           potentialities in cancer treatment. In this paper, an original synthesis of MNPs and GNPs  
22           was designed through the innovative use of tannic acid (TA), which allows the synthesis  
23           and the stabilization of eco-friendly hybrid nanoplatfoms (HNPs) avoiding toxic  
24           chemicals. HNPs were characterized in terms of size, morphology, composition, magnetic  
25           and plasmonic properties, ability to generate heating under laser irradiation and their

26 hemotoxicity. The results revealed that the TA allowed the production of HNPs through a  
27 new, simple and green synthesis method. The HNPs preserved the peculiar properties of  
28 each nanomaterial, and did not show any hemotoxic effect, thus representing an  
29 innovative approach for magneto-photothermal therapy of cancer.

30

### 31 **Structured Abstract**

32 **Aim:** the design of new hybrid nanoplatfoms (HNPs) through the innovative and eco-  
33 friendly use of tannic acid (TA) for the synthesis and the stabilization of the NPs.

34 **Materials & Methods:** the size, morphology, composition as well as magnetic and plasmonic  
35 properties of HNPs were investigated together with their ability of HNPs to generate heating  
36 under laser irradiation and the hemotoxicity to explore their potential use for biomedical  
37 applications.

38 **Results and Conclusions:** the use of TA allowed the synthesis of the HNPs by adopting a  
39 simple and green method. The HNPs preserved the peculiar properties of both magnetic and  
40 plasmonic nanoparticles and did not show any hemotoxic effect.

41

### 42 **Keywords**

43 Magnetite nanoparticles; gold nanoparticles; magneto-plasmonic nanoparticles; photothermal  
44 therapy; tannic acid;

45

### 46 **1. Introduction**

47 In recent years, many studies have been focused on new applications of nanotechnology  
48 in biomedical field. Nowadays one of the main applications of nanotechnology in

49   biomedicine is the use of nanoparticles [1-7]. In particular, magnetite and gold  
50   nanoparticles (MNPs and GNPs respectively) have attracted a lot of interest in the  
51   scientific community thanks to the possibility to use them in different research fields [8-  
52   14]. MNPs have been considered as contrast agents for magnetic resonance imaging, heat  
53   sources for hyperthermia and vectors for drug delivery, especially in cancer therapy [15-  
54   21]. Moreover, they show low toxicity, high biocompatibility and great stability [22]. GNPs  
55   have attracted huge interest due to their easiness of synthesis and surface modification,  
56   high stability and excellent biocompatibility; in fact, they present very low toxicity even  
57   at high concentration [10-23]. Furthermore, GNPs possess a unique photo-physical  
58   phenomenon which is not present in massive metal: localized surface plasmon resonance  
59   (LSPR) [11,24-26]. The LSPR effect is the result of the nanoparticles interaction with light  
60   radiation in a specific wavelength, in fact, GNPs are able to transform the received light  
61   into thermal energy by producing heat. This could bring the cancer cells to apoptosis as a  
62   consequence of their high heat sensitivity [27]. Thus, GNPs are one of the most promising  
63   tools for photothermal therapy [11].

64   Combining MNPs and GNPs together, is possible to create a hybrid nanoplatform which  
65   preserves the specific properties of each nanomaterial, thus creating an innovative  
66   approach for magneto-photothermal therapy of cancer [28]. Indeed, magnetoplasmonic  
67   HNPs could be driven in a specific tumour site due to their ability to be activated through  
68   an external magnetic field while acting as photothermal system by exploiting the SPR  
69   effect when irradiated with a laser light. Moreover, it has been recently reported that the  
70   combined magnetic and optical properties of magneto-plasmonic HNPs could be  
71   successfully exploited in multimodal imaging techniques [29].

72   The aim of this work is to synthesize MNPs and GNPs creating a magneto-plasmonic  
73   nanoplatform (HNPs) through an innovative and eco-friendly synthesis of GNPs, which

74 uses tannic acid (TA) as the unique reagent able, at the same time, to reduce GNPs and  
75 stabilize HNPs without using any other toxic chemicals. This method faces up to the need  
76 to develop more environmentally friendly approaches in order to avoid the problems that  
77 chemical and physical NPs synthesis procedures usually present, such as the use of toxic  
78 chemicals that can lead to both health and environmental issues as well as the high  
79 exposure risk of the operator [30,31]. This new awareness can be achieved by using a  
80 wide range of biological resources, which could bring various advantages in the NPs  
81 synthesis, such as simplicity, low-cost, non-toxic procedures and compatibility for  
82 biomedical and pharmaceutical applications [32,33].

83 Tannic acid was selected since it is a polyphenolic compound extracted from plants [34]  
84 and can be used as stabilizing and reducing agent [12,35] avoiding the use of all other  
85 hazardous chemicals, creating a green and non-toxic synthesis of HNPs [36]. Moreover,  
86 TA is well known for its natural antioxidant, anti-inflammatory, antitumoral and  
87 antimicrobial properties [37,38]. This organic compound was used to allow GNPs  
88 nucleation directly on MNPs surface due to its high reducing power [36]. Furthermore,  
89 working under mild-acidic/basic condition, a partial hydrolyzation of TA take place  
90 generating glucose and gallic acid [39]: the glucose guarantee the property of being a good  
91 stabilizing agent while the gallic acid induces the formation of GNPs at room temperature  
92 thanks to its well-known reducing power [39,40].

93 A further aim of this work is to characterize the synthesized HNPs from different points  
94 of view, including size, morphology, composition, magnetic and plasmonic properties,  
95 ability to generate heating under laser irradiation. Moreover, it is well known that NPs  
96 can easily access blood cells, influencing their function and resulting in potentially toxic  
97 effects [41]. Therefore, a preliminary study on hemotoxicity of HNPs in contact with red

98 blood cells has been performed to attest the possibility to use the newly engineered  
99 nanoplatfoms for biomedical applications.

100

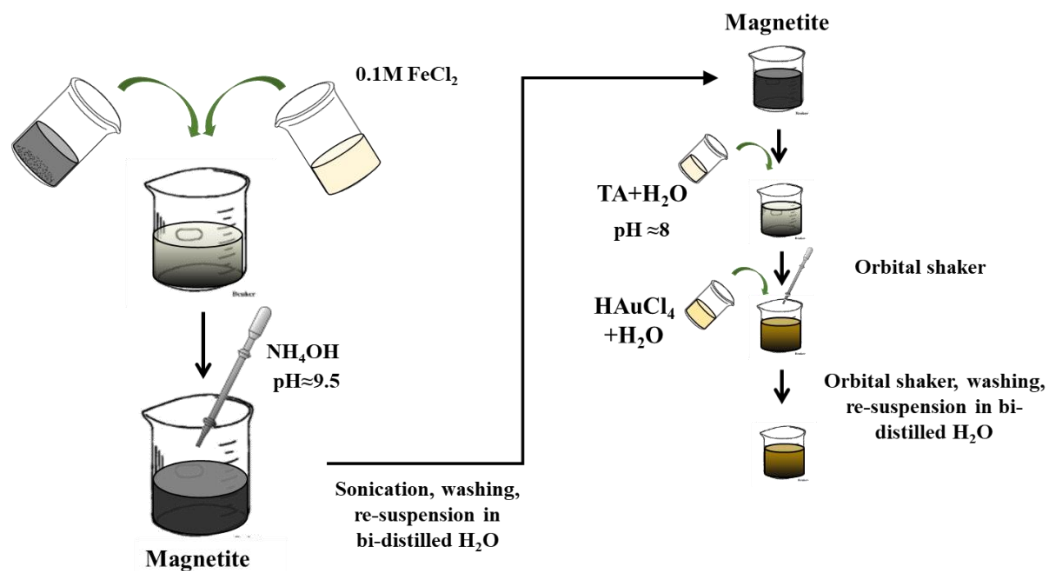
## 101 **2. Materials and methods**

### 102 **2.1 HNPs synthesis**

103 HNPs were prepared by improving a synthesis route reported in our previous papers  
104 [12,35]. The syntheses differ from each other because in the previous ones the MNPs were  
105 stabilized with citric acid and functionalized with APTES (3-Aminopropyl-  
106 triethoxysilane) to promote the GNPs attachment, while in the synthesis here described,  
107 the TA was used as unique benign reagent that works both as stabilizing and reducing  
108 agent. Briefly, Fe<sub>3</sub>O<sub>4</sub> NPs were firstly synthesized by the co-precipitation method in which  
109 37.5 ml of 0.1 M FeCl<sub>2</sub> and 50 ml of 0.1 M FeCl<sub>3</sub> were mixed together until the salts were  
110 completely dissolved. To induce the magnetite formation, NH<sub>4</sub>OH was added drop by drop  
111 until the pH reached a value of 9.5 and the suspension turned black, indicating the  
112 precipitation of MNPs. Then the suspension was sonicated in an ultrasonic bath (SONICA  
113 Ultrasonic Cleaner) for 20 minutes and washed two times before re-suspended in 100 ml  
114 of bi-distilled water [42,43].

115 TA solution was prepared by dissolving 2.55 mg of TA in 1.2 ml of bi-distilled water and  
116 buffered at pH= 8 in order to improve its reducing power. Then the TA solution was added  
117 to the MNPs dispersion with a ratio TA(ml) : MNPs(ml) of 0.3 and left at 70 °C for 5  
118 minutes under agitation to allow the TA binding on NPs. All the steps were carried out  
119 rapidly in order to avoid the Fe<sub>3</sub>O<sub>4</sub> NPs aggregation. Finally, 60 mg of HAuCl<sub>4</sub> were  
120 dissolved in 12 ml of bi-distilled water and added to the TA-MNPs suspension and left  
121 under continuous stirring at 70 °C for 5 minutes. This step would allow the GNPs  
122 nucleation directly on MNPs surface. All the reactants were purchased by Sigma Aldrich®.

123 The obtained HNPs were then characterized in terms of size, morphology, composition,  
124 magnetic and plasmonic properties. Figure 1 reports schematically the above-mentioned  
125 synthesis steps.



126

127

**Figure 1.** Experimental procedure for the HNPs synthesis.

## 128 2.2 HNPs characterization

### 129 2.2.1 Morphological and compositional characterization

130 In order to assess the dimension, the shape and the morphology of the as-synthesized  
131 HNPs, electron microscopes FESEM (Zeiss supra 40 GEMINI Field Emission Scanning  
132 Electron Microscopy) and - TEM (FEI Tecnai F20 TWIN transmission electron microscope  
133 with a Schottky emitter operated at 200 KV) were used; the chemical composition and the  
134 correct reduction of GNPs on MNPs were detected by Zeiss supra 40 GEMINI X-ray  
135 spectroscopy (EDS). For these analyses 5 $\mu$ l of sample solution were placed on a Lacey  
136 carbon coated 200 mesh copper grid and then located on the appropriate support for the  
137 analysis.

138 To verify the effective functionalization and perform elemental analysis a JASCO 4000

139 Fourier transform infrared spectroscopy (FT-IR) was used, spectra were acquired from

140 4000 to 500  $\text{cm}^{-1}$ , to perform FT-IR analysis, the solution was left at room temperature  
141 until the powder were completely dried.

142

### 143 *2.2.2 Optical characterization*

144 The UV-Visible spectrophotometry, UV-2600 Shimadzu (UV-VIS) was employed to  
145 identify at which wavelength the HNPs are able to absorb and to provide information on  
146 their size and aggregation, for this analysis the HNPs were maintained in their original  
147 water suspension.

148

### 149 *2.2.3 Magnetic characterization*

150 The magnetic properties of HNPs were investigated by means of a DC magnetometer  
151 (LakeShore 7225) equipped with a Cryogen-Free Magnet, useful to study the  
152 superparamagnetic behavior of the MNPs and the ability of the sample to be activated  
153 with an external magnetic field by means of an induction heating system (FELMI-EGMA  
154 6-10.15 REV.01). Magnetic hysteresis cycle measurements were performed at room  
155 temperature in quasi static condition using an applied magnetic field up to 800 kA/m  
156 using the samples in form of powder.

157

### 158 *2.2.4. Plasmonic behavior under laser irradiation*

159 The HNPs were subjected to a 10 minutes NIR laser irradiation of 808 nm (model FC-808,  
160 CNI Optoelectronics Tech, Changchun, China) in order to detect their ability to be  
161 activated with an external light stimulus and exploit their SPR effect of increasing  
162 temperature. The HNPs concentration used for the analysis was 0.1 mg/ml (determined  
163 using ICP-MS analysis) in a total volume of 1 ml. The laser power used was set at 1  $\text{W}/\text{cm}^2$   
164 and the spot size of the laser beam was 1 cm in order to irradiate the entire volume of the



165 vial. Temperature of the samples was monitored in real time using a J-type Teflon  
166 thermocouple.

167 This analysis is useful to study their ability to be used as photothermal agent in cancer  
168 treatment, for this reason, the HNPs were maintained in their original water suspension.

169

### 170 *2.2.5 In-vitro hemotoxicological analysis*

171 Once attested all the physical, chemical, magnetic and optical properties, a preliminary in-  
172 vitro cytotoxicity evaluation of the as-produced HNPs was performed, in which a  
173 nanoplateforms concentration of 35 and 100 µg/ml (determined via ICP-MS analysis) were  
174 put in contact with red blood cells for 5 hours.

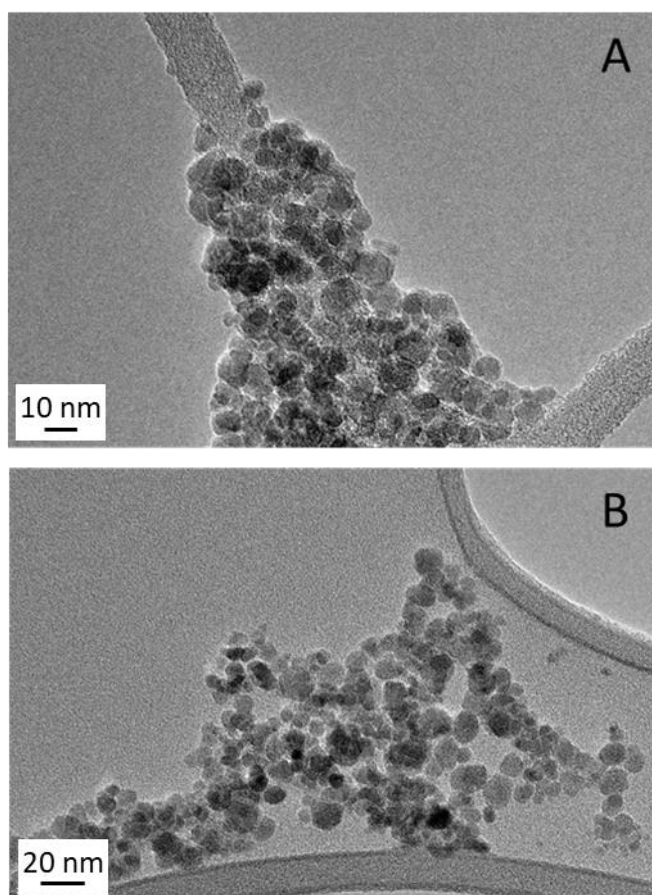
175 For the hemolysis study, red blood cells were isolated from the whole sheep blood,  
176 supplied by the Veterinary Faculty (University of Ljubljana, Slovenia) in Alsever's medium  
177 (TCS Biosciences Ltd, UK) and used within one week. Red blood cells were isolated via  
178 centrifugation (2500 rpm/10 min) and washed 3-times with phosphate-buffered saline  
179 (PBS) buffer (tablets, Sigma Aldrich). Nanoparticles suspended in PBS were incubated  
180 with 5 vol.% of red blood cells (pH 7.4) for 5 h at 37 °C with constant orbital shaking in  
181 1.5 ml tubesEppendorfof, Germany, volume of samples 1 mL, all samples in triplicates).  
182 After incubation, tubes were centrifuged (1500 rpm/4 min) to sediment cells and  
183 supernatant was analyzed in triplicates. Hemolysis was evaluated by measuring released  
184 hemoglobin absorbance (A) at 541 nm using a plate reader (Synergy H4, BioTek,  
185 Winooski, VT, USA). Samples representing positive control (100% dead) were prepared  
186 by lysing control samples with deionized water via hypotonic osmotic shock. Percent  
187 hemolysis was then calculated as follows: Hemolysis (%)=100·(Asample - Acontrol)/(  
188 A100% dead - Acontrol). One-way ANOVA and Student's t-test was used for our statistical  
189 analysis. The data were presented as mean ± SD for all experiments.

190

### 191 3. Results and discussion

192 In this paragraph, the morphological, compositional and chemical characterizations concerning  
193 the HNPs are described. In particular, a preliminary characterization on bare iron oxide  
194 nanoparticles was firstly performed in order to verify the correct formation of MNPs followed  
195 by the analyses achieved to attest the correct binding of TA on MNPs.

196 To verify the size and morphology of MNPs, they were firstly characterized by means of  
197 TEM analysis. In figure 2 TEM images of bare iron oxide nanoparticles are shown, in which  
198 is visible their pseudo-spherical shape and a dimensional range between 5-15 nm.

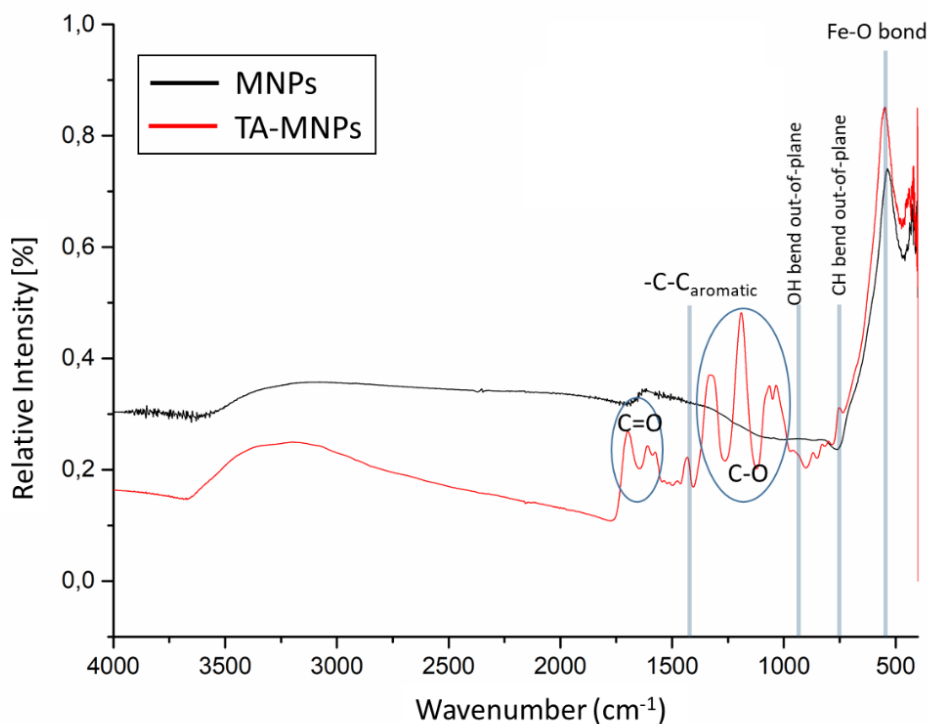


199

200 **Figure 2:** TEM image of MNPs. Scale bar: figure A 10 nm; figure B 20 nm

201 In order to confirm the correct binding of TA on MNPs surface, the FT-IR and UV-Vis  
202 analyses were performed as shown in figure 3 and figure 4 respectively. In FT-IR graph

203 (figure 3), the patterns of MNPs and TA functionalized MNPs are shown. Both spectra  
204 display the strong vibrational modes of Fe-O bonds of magnetite located at  $585\text{ cm}^{-1}$   
205 [44,45], while from the TA-MNPs pattern (red line) the main characteristic peaks of TA  
206 [46] are visible, in particular, the  $758\text{ cm}^{-1}$  peak indicates out of plane CH bending of  
207 phenyl groups, the peak at  $923\text{ cm}^{-1}$  is referred to OH out of plane bending of acid groups,  
208 the C=O stretching vibration at  $1730\text{-}1705\text{ cm}^{-1}$  and C-O at  $1100\text{-}1300\text{ cm}^{-1}$ , while around  
209  $1452\text{ cm}^{-1}$  the stretching vibrations of -C-C aromatic groups appear. Moreover, as  
210 confirmation of correct functionalization of MNPs with TA, the broad peak at  $3400\text{ cm}^{-1}$ ,  
211 which represent the hydroxyl groups and surface-adsorbed water molecules, is visible  
212 together with the vibrational modes of Fe-O bonds of magnetite  
213



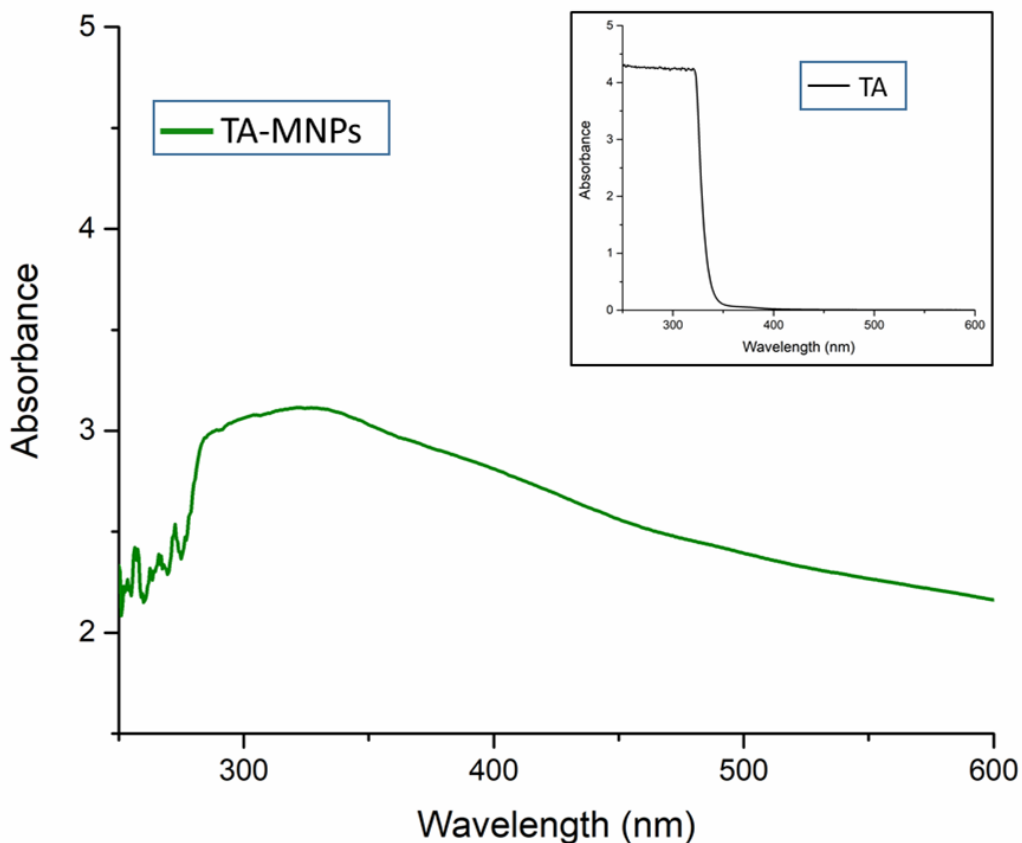
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215

Figure 3: FTIR spectra of MNPs and TA-MNPs

216

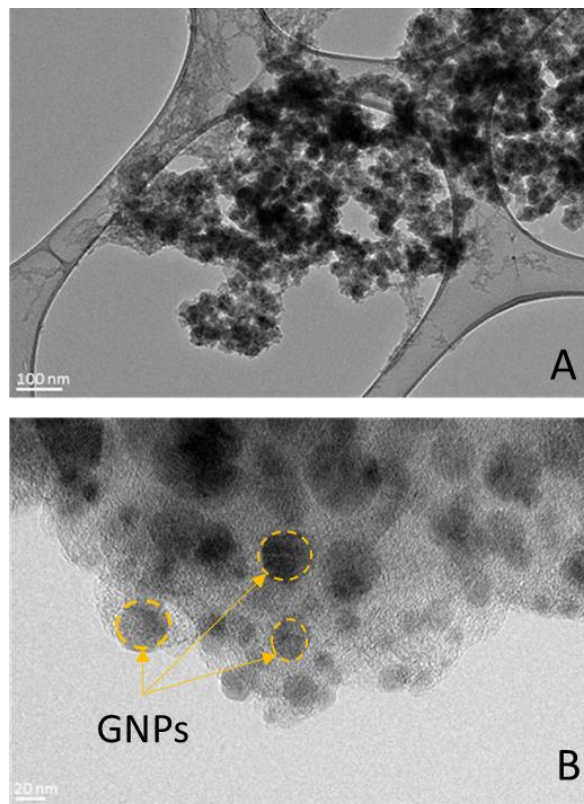
217 Figure 4 reports the UV-Vis analysis in which the peak associated to the tannic acid at  
218 around 300 nm is visible. This result corroborates the outcomes obtained from FT-IR and  
219 confirms the correct binding of tannic acid on MNPs surface, thus confirming that TA can  
220 be grafted onto the surface of magnetic particles, without interposing other spacer  
221 molecules (e.g. APTES).



222  
223 **Figure 4:** UV-Vis spectra of TA-MNPs. In the inset: UV-Vis spectra of TA alone.

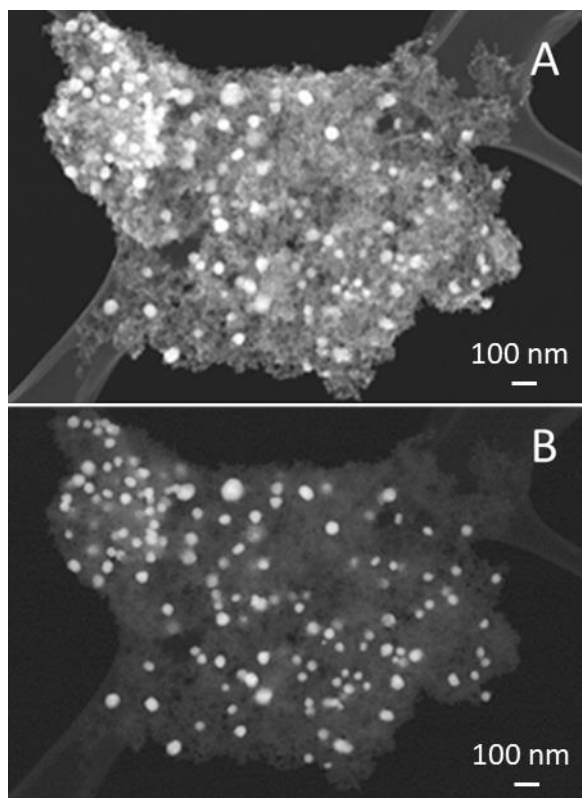
224  
225 Once attested the correct functionalization with TA, the TEM analysis was used to verify  
226 the size and morphology of HNPs, as well as to attest the correct attachment of GNPs on  
227 iron oxide core. Figure 5 shows the TEM images of HNPs, in which the GNPs are visible;  
228 they appear darker than MNPs due to their higher atomic number and electron density.  
229 GNPs show an approximately spherical shape and a dimension around 10-20 nm,

230 confirming the presence of GNPs on MNPs core creating a sort of nano-dumbbell  
231 structures.



232  
233 **Figure 5:** TEM image of HNPs. In figure 4 (B) GNPs are evidenced. Scale bar: figure A  
234 100 nm; figure B 20 nm.

235  
236 In figure 6 the STEM images acquired in dark field mode are shown, in which high mass  
237 materials (such as gold) appear bright. The figure shows the morphological aspect of  
238 HNPs, in which the GNPs result to be homogeneous and well-dispersed on magnetite  
239 surface. This analysis corroborates the TEM results previously obtained, in which it is  
240 possible to observe a high concentration of GNPs attached to the MNPs with a dimensional  
241 range between 10 nm and 20 nm. The correct reduction of GNPs on MNPs is also  
242 supported by the EDS analysis (figure 7), that evidence the presence of all the elements  
243 characteristic of MNPs and GNPs.

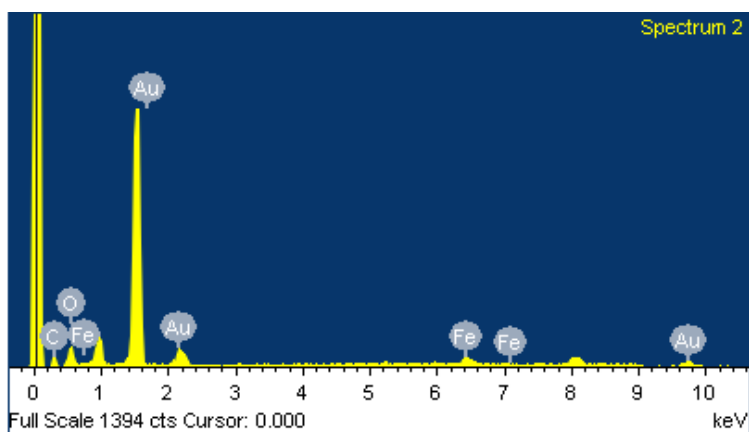


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245

**Figure 6:** STEM images of HNPs. Scale bar: 100 nm.

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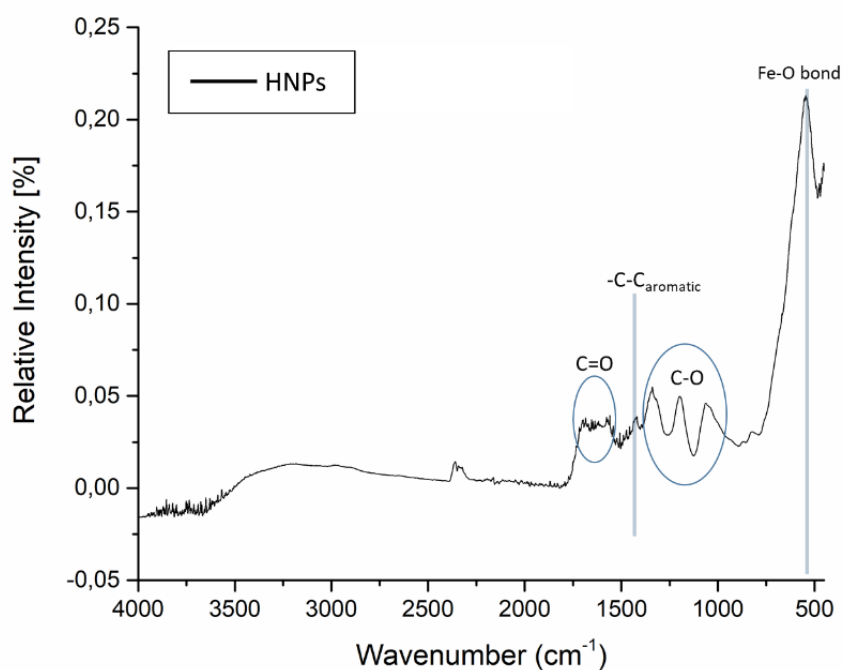
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**Figure 7:** EDS analysis of HNPs.

249

250 Moreover, in order to identify the functional groups present in the TA, which is  
251 responsible for the reduction of GNPs and stabilization of HNPs, FT-IR measurement of  
252 the final HNPs solution was carried out as shown in figure 8. In the image it is possible to

253 observe an altered intensity and position of the TA peaks with respect to the FT-IR of TA-  
254 MNPs (figure 3) as indication of the correct reduction of GNPs. In particular, the shift of  
255 the broad peak from  $3400\text{ cm}^{-1}$  to lower wavenumber suggests the involvement of OH  
256 functional groups in the reduction process as well as the altered intensity of CO groups  
257 and C-C aromatic rings, which indicate the involvement of TA-MNPs in immobilization of  
258 GNPs (47). On the basis of data, it could be inferred that the TA remains bound to the HNPs  
259 surface and that the TA phenolic hydroxyls may be responsible for the reduction of metal  
260 ions. During the metal reduction process, the COO- group present in the TA, together with  
261 the rest of the molecule, can work as surfactant on the HNPs surface stabilizing them  
262 through electrosteric stabilization (48).



263

264

**Figure 8:** FT-IR spectra of HNPs

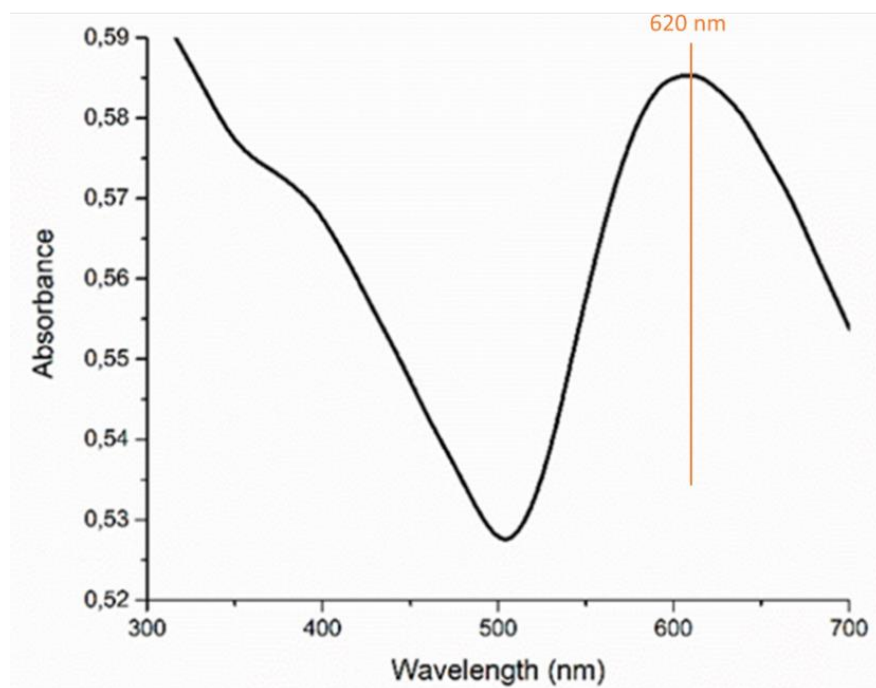
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266 Once attested the correct HNPs formation and the correct role of TA to work as reducing  
267 and stabilizing agent, the UV-Visible spectrophotometry was employed to identify at

268 which wavelength the HNPs are able to absorb. The graph in figure 9 shows a high signal  
269 in the GNPs absorbing window with sharp absorbing peak at 620 nm. This analysis is  
270 useful to attest the great ability of GNPs to absorb light as well as to confirm the high  
271 concentration, homogeneous dimension and very good dispersion of GNPs in the solution  
272 [11]: this is confirmed by the broad gold extinction peak which in case of aggregation it  
273 would show a decrease in intensity (due to the depletion of stable nanoparticles) and a  
274 wider peak towards longer wavelengths (due to the formation of aggregates) [49].

275

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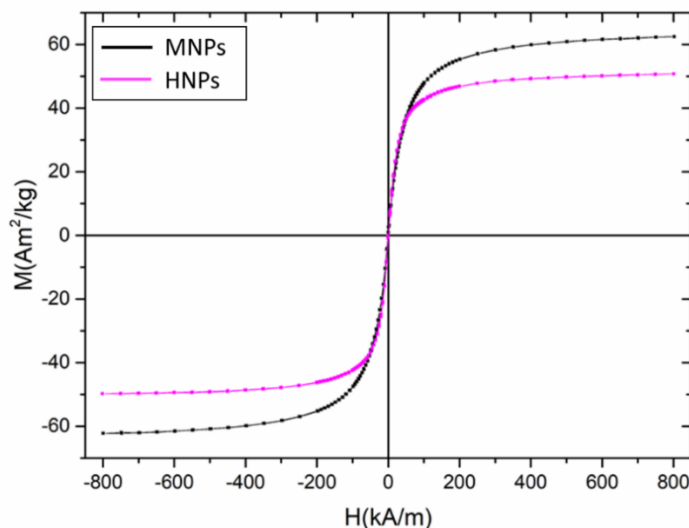
**Figure 9:** UV-Vis of HNPs.

279

280 With the aim to determine the effect that GNPs have on the magnetic properties of pure  
281 MNPs, the magnetic properties of the suspension were evaluated by means of  
282 magnetization measurements system and induction heating system. In fact, it is important



283 to consider that the ability to manage the HNPs using a magnetic field is one of the main  
284 reasons for utilizing Fe<sub>3</sub>O<sub>4</sub> NPs as support for GNPs.  
285 In particular, in figure 10 magnetic hysteresis cycle curves at room temperature of bare  
286 MNPs (black curve) and the as synthesized HNPs (pink curve) are reported. Here it is  
287 possible to observe that both the samples exhibit a superparamagnetic behaviour as  
288 confirmed by the negligible coercive field and remanence magnetization [50]. The higher  
289 magnetization values of MNPs with respect to HNPs, could be linkable to its lack of any  
290 additional element that lower these properties (such as the TA and GNPs); in fact, the  
291 decrease in the saturation magnetizations of HNPs, could be due to the diamagnetic  
292 nature of GNPs anchored on MNPs surface, as well as the minor amount of magnetic NPs  
293 in the sample in which gold is also present. Despite this, the HNPs show no hysteresis,  
294 indicating that GNPs are not influencing excessively the magnetic properties of the  
295 precursor [51].



296

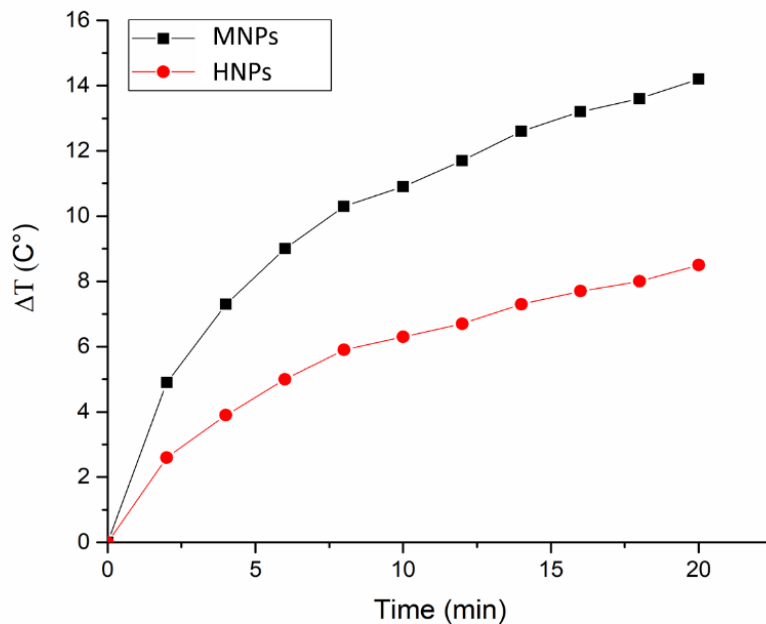
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**Figure 10:** Magnetic hysteresis cycles of MNPs and HNPs.

298 In figure 11, the temperature-time curves obtained by submitting the samples to an  
299 external magnetic field are reported. This analysis is useful to attest the ability of the

300 sample to be activated with an external mediator. In particular, it is possible to notice that  
301 MNPs (black curve) are able to produce higher heating than HNPs (red curve), due to the  
302 same reasons for which the magnetization appeared lower in figure 10. Despite this, the  
303 synthesized HNPs are able to be externally activated with the applied magnetic field: this  
304 means that the GNPs decoration is not influencing the magnetic properties of HNPs,  
305 corroborating the magnetization measurement results previously obtained.

306



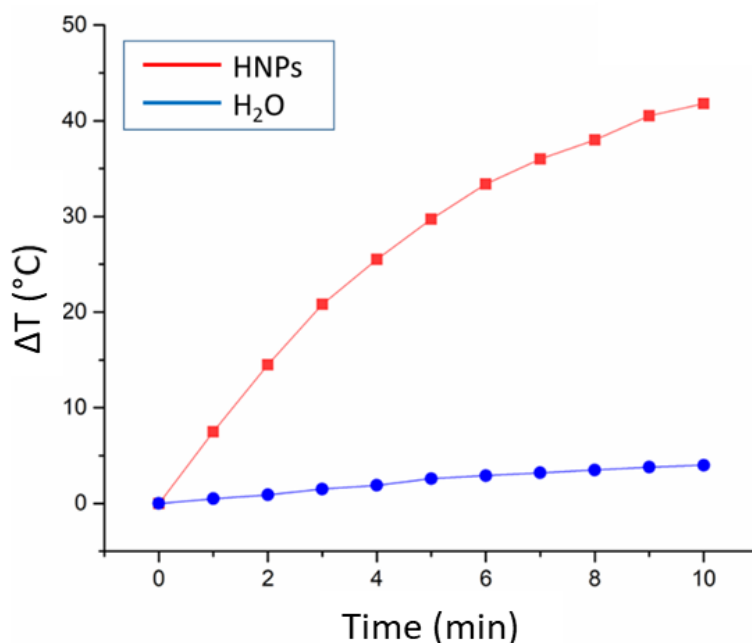
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308 **Figure 11:** Temperature-Time curve of MNPs and HNPs after 20 min of an external  
309 magnetic field application. Applied magnetic field: 800 kA/m.

310

311 Finally, the HNPs were subjected to a laser irradiation in order to detect their ability to be  
312 activated with an external light stimulus and to exploit their SPR effect increasing their  
313 temperature. This analysis is useful to study their ability to be used as photothermal agent  
314 in cancer treatment. The same test was performed both with HNPs dispersed in water at

315 concentration of 0.1 mg/ml than with only water, to observe the difference between the  
316 two solutions. In figure 12, it is visible that after 10 minutes of laser irradiation, the HNPs  
317 are able to raise a temperature of 40/45°C ascribable to the high absorption spectra of  
318 GNPs at the characteristic wavelength of the irradiation showed in UV-Vis graph (figure  
319 9), while, as expected, the water is not showing any effect when irradiated. This result  
320 confirms the excellent ability of GNPs to exploit SPR effect demonstrating the ability of  
321 the synthesized HNPs to be used in photothermal therapy.  
322

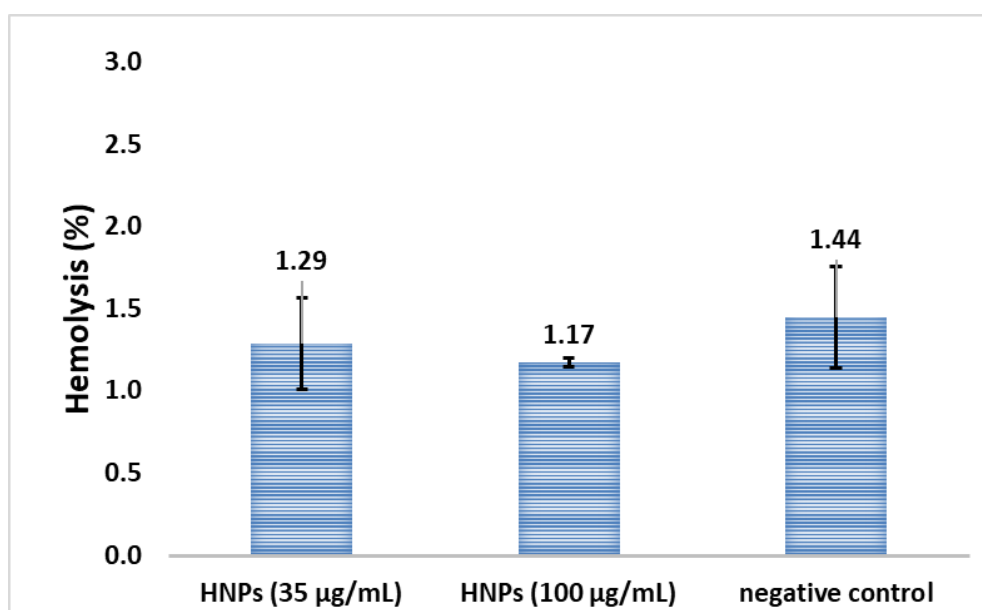


323  
324 **Figure 12:** Photothermal results of nanocomposites after 10 minutes of laser  
325 irradiation. HNPs concentration: 0.1 mg/ml; Laser power: 1 W/cm<sup>2</sup>.  
326

327 Once characterized the sample in terms of size, morphology, chemical, magnetic and  
328 optical properties, a preliminary hemotoxicological analysis was performed in order to  
329 evaluate the toxicological effect of the nanocomposites in contact with red blood cells  
330 (RBCs). With this analysis it is possible to evaluate the hemoglobin absorbance using a

331 spectrophotometer, which is useful to detect the RBCs hemolysis before and after  
332 incubation with nanoparticles. The results concerning the RBCs hemolysis after  
333 incubation with 35 and 100  $\mu\text{g}/\text{ml}$  concentration of HNPs, are shown in figure 13 in which  
334 it is possible to notice that after 5 h incubation, the HNPs did not show any hemotoxicity,  
335 which is comparable to that of the negative control sample. In fact, for each sample of RBC  
336 it is observed that the hemolysis is very low (lower than 1.4%), which means that the NPs  
337 hemotoxicity at these concentrations is negligible and therefore they are potentially  
338 usable for biomedical applications [52,53].

339



340

341 **Figure 13:** Hemotoxicity results. Cell viability of RBCs was evaluated after 5 h  
342 incubation at 37  $^{\circ}\text{C}$  (n=3). Data analysis revealed no statistically significant difference in  
343 cell viability values between tested groups ( $p < 0.05$ ).

344

#### 345 4. Conclusions

346 In this this work a facile and reproducible synthesis method was optimized and used to  
347 develop new hybrid nanoplatforms composed by magnetic core and GNPs decoration. The

348 main goal was to prepare the HNPs through a green and simple synthesis by means of the  
349 innovative use of tannic acid, a polyphenolic compound as both reducing and stabilising  
350 agent. This approach allowed to prepare the HNPs without using any toxic chemical in the  
351 process, thus improving the synthesis procedure in terms of number of reagents used,  
352 properties, scalability, cost-efficiency and eco-sustainability. Further, another aim of the  
353 research was the complete characterization of the obtained HNPs including a preliminary  
354 hemotoxicological evaluation. The obtained structures are able to preserve the peculiar  
355 properties of each nanomaterial and display negligible hemotoxicity, creating a novel  
356 approach for magneto-photothermal therapy of cancer.

357

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361

### 362 **Summary Points**

- 363 • A facile and reproducible synthesis method was developed to prepare new hybrid  
364 nanoplateforms (HNPs) composed by magnetic core and gold nanoparticle  
365 decoration.
- 366 • The innovative use of tannic acid as both reducing and stabilising agent was used  
367 to prepare the HNPs
- 368 • The HNPs were prepared without any toxic chemical in the process.
- 369 • A complete characterization revealed that the obtained HNPs are able to preserve  
370 the peculiar properties of each nanomaterial.
- 371 • The obtained HNPs display negligible hemotoxicity.

- 372 • The obtained HNPs represent a novel approach for magneto-photothermal  
373 therapy of cancer.

374

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