

2 **Modified nanocellulose as promising material for the extraction of gold**  
3 **nanoparticles**

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10  
11 **Abstract:** Increasing number of engineering nanoparticles (NPs) in consumer products is unstoppable even  
12 after the toxic effect of metallic-based nanomaterials stated more than a decade ago; thus, selective methods  
13 for detecting metallic NPs are highly demanded and of great importance. A simple and rapid approach for  
14 detecting gold NPs (AuNPs) using ecofriendly sulfonated nanocellulose (s-NC) as sorbent material is  
15 described. The interaction between s-NC and AuNP is based on the affinity of sulfur atoms towards metals.  
16 We found that the use of cationic surfactant substantially benefitted the extraction and preconcentration of  
17 AuNPs by virtue of the NP stabilization. Good linear correlations were found in a concentration range spanned  
18 from 7-20 µg/mL with a detection limit of 0.26 µg/mL. Furthermore, the proposed method was successfully  
19 applied to detect AuNPs in presence of other nanowastes, opening new opportunities of smart materials as  
20 collectors and store nanoobjects for further uses. Further experiments are directed for reaching a generalized  
21 method towards the analysis of a bigger variety of NPs covering the trending topic third facet of the analytical  
22 nanoscience and nanotechnology.

23  
24 **Keywords:** Nanocellulose, gold nanoparticle, sorbent, dispersive solid phase extraction, electrophoresis.

25  
26 **Abbreviations:** AuNPs, gold nanoparticles; AgNPs, silver nanoparticles; CAPS,  
27 3-(cyclohexylamino)-1-propanesulfonic acid; CTAC, hexadecyltrimethylammonium chloride; CTAB,  
28 hexadecyltrimethylammonium bromide; EC, capillary electrophoresis; LVSS, large-volume sample stacking;  
29 TA, thiotic acid; NC, nanocellulose; NPs, nanoparticles; SDS, sodium dodecyl sulfate; SPR, surface plasmon  
30 resonance

## 1. Introduction

In recent years, the fascinating physicochemical properties of nanomaterials had entailed an exponential growth of applications, which includes many areas such as medicine, electronics, catalysts, cosmetics, food, and textile. With the nanotechnology promise to benefit the Society, this progress has forced the market with a vast of consumer goods containing large quantities of nanomaterials. As all revolutionary technologies, the impact of this growth on human health and environment is undeniable and a myriad of problems and consequences is coming to be faced. Nanotoxicology [1] is still in its infancy and there exist controversy around nanoparticle (NP) safety, which is stated to be dependent not only on their particle shape and size distribution but also on their chemical composition and stability, i.e., which is governed by their surface (both functionalities and charges) [2].

In this respect, pollution caused by nanotechnology has become one of the most urgent concerns for protecting the ecosystem [3]. Of great interest is the case of metallic NPs [4,5] by virtue of their antimicrobial properties, being able to interact with biomolecules, cells and even organs of living organisms for its nanosize and large surface area. On the other hand, it is expected that NPs accumulate in environment, for instance after their recent applicability as promising pesticides [6], or by their released from wasted consumer products. In reaction to this, the analytical community has paid attention in creating low-cost sophisticated analytical methods with ecofriendly sorbent materials for the detection, quantification and storage of hazardous NPs.

Most of methods described for separating and detecting AuNPs involved expensive instrumentation (i.e., field-flow fractionation [7], size exclusion chromatography [8], graphite furnace atomic absorption spectroscopy [9], electrothermal atomic absorption spectrometry [10], inductively coupled plasma mass spectrometry (ICPMS) [11]) with tedious enrichment steps (i.e. liquid-liquid and solid phase extractions, cloud point extraction (CPE) [10]) or disintegration of the sample (i.e., enzymatic digestion [12, 13]). No digestion of AuNPs were required in a reported method [14] in which an elaborated NPs ( $\text{Al}^{3+}$ -immobilized  $\text{Fe}_3\text{O}_4$ @silica@iminodiacetic acid) were used as sorbent materials for magnetic SPE to avoid gold ion interferences by ICPMS. Resins like Amberlite were also used for selective extracting plasmonic NPs via electrostatic interactions [9], being their elution easily performed with formic acid (8%) in methanol.

On the contrary of these expensive methods, the optical properties (fluorescence, absorbance, scattering) of NPs have been also explored for AuNP monitoring using inexpensive optical instrumentation like fluorescence [15] and especially UV spectrometer [16-19] by using preliminary extraction approaches. This is the case of the ligand-assisted extraction of AuNPs from aqueous samples using dodecanethiol in a reversed phase C-18 column [16]; however, this methodology presented disadvantages such as the use of organic solvents, a prolonged sonication periods (3 h) at low temperatures for eluting AuNPs from sorbent and the need of additional evaporation and reconstitution steps of the eluting residue. Others reported the extraction of AuNPs by their encapsulation with resorcinarene with nonpolar organic solvents like chloroform and toluene [17]. Other interesting methodology demonstrated the extraction of AuNPs using the combination of a cationic surfactant (to stabilized AuNPs) and liquid-liquid extraction in imidazolium ionic liquids (IL) [18]. However, those described methods have the drawback of using toxic liquids such as organic solvents and IL. Recently, a

73 method for AuNP extraction and detection has been reported based on CPE using semiconductor quantum dots  
74 (SQDs) via optical incoherent light scattering of the resulting hybrid assembly [19]; however, the potential  
75 toxicity of SQDs composed of CdS is a negative aspect of the reported method.

76

77 Nanocellulose (NC), which is obtained from abundant renewable resources, is a good candidate in this matter  
78 because of their non-toxic features and impressive properties (e.g. compatibility, biodegradability, lightness,  
79 large surface area, high porosity and stiffness, mechanical and thermal stability, reactive surface, rheological  
80 properties, etc) [20, 21]. Although the NC developments are still in its infancy, there are great expectations as a  
81 competitor of graphene. In the last years, functionalization approaches of the NC surface for the development of  
82 innovative analytical tools had become crucial in the adsorption efficiency, the selectivity towards the target  
83 and also the separation capacity [22, 23]; Few are the analytical methods which demonstrated the advantages  
84 rendered by NC related to detection of other nanomaterials [24, 25] such as silver nanoparticles (AgNPs). Thus,  
85 in those examples, NC acted as sorbent material (stem from its multiple groups, porosity and large surface area)  
86 [24] and as sensing platform (resulted from the optical transparency and suitability to accommodate specific  
87 fluorophores) [25].

88

89 Herein, this paper proposes an ecofriendly method to extract, storage and quantify AuNPs using a low-cost  
90 material consisting on NC with superficial sulfonate groups (s-NC) based on simply monitorization of the  
91 surface plasmon resonance (SPR) absorption of AuNPs. The method displayed a good sorption towards  
92 plasmonic NPs by virtue of the affinity of sulfur towards metals.

93

94 This straightforward strategy fitted for the analysis of the nanoworld by means of using analytical nanotools,  
95 being an excellent example of the trend known as the third way of Analytical Nanoscience and Nanotechnology  
96 [26]. In this case, our method integrates low-cost instrumentation and biodegradable sorbent materials for  
97 targeting other nanosized contaminants.

98

99

## 2. Materials and Methods

100 Information of instrumentation and reagents are given in the electronic supplementary material (EMS)

### 101 2.1. Synthesis of nanoparticles

102 The synthesis of gold nanoparticles was performed as described elsewhere [27] following the Turkevich  
103 method [28]. An aqueous solution of  $\text{HAuCl}_4$  was mixed with trisodium citrate under reflux conditions. After  
104 cooling down, the red solution was stored in an amber bottle until further use. The average size of

105 The preparation of s-NC by acid hydrolysis of microcrystalline cellulose (MCC) was as reported elsewhere  
106 [24]. For more details, see EMS.

### 107 2.2 Protocols

#### 108 2.2.1. Determination of the superficial charge of nanocellulose

109 The net surface charge of s-NC was obtained by titrating a diluted suspension containing NC with NaOH (0.5  
110 and 0.1 M) and HCl (0.5 M) with Zetasizer instrument coupled with a titrator. The suspension was sonicated

111 with a sonicator tip to enhance the debundling. The charge density is the average of several measurements of  
112 each suspension.

### 113 **2.2.2. Extraction and elution of metallic nanoparticles with nanocellulose**

114 Previous to each experiment, the sorbent was activated by passing ultrapure water through an amount of s-NC  
115 (6 mg) placed in a 1.5 mL Eppendorf tube. Then, 50  $\mu$ L of CTAC (200 mM) was added to the solution  
116 containing metallic nanoparticles (ca. 1 mL) and then subjected to the dispersive solid phase extraction with the  
117 s-NC as sorbent. The extraction steps followed are: stirring on a vortex for 10 s, followed by sonication for 10  
118 min, and then, centrifuged for 3 min at 1800 rpm; thus, the separation of the sorbent containing the analyte from  
119 the aqueous sample is achieved. Afterwards, the supernatant was discarded and sorbent was washed with double  
120 distilled water. Then, sonication of the sorbent with a TA solution (75mM) for 10 min followed by  
121 centrifugation enabling the separation of the supernatant containing the eluted AuNPs. The sorbent was then  
122 washed once with methanol: water (ration 1:1) and reused for further uses. The eluted NPs were monitored by  
123 their SPR absorption band centred at 527 nm. The morphology of eluted AuNPs was proved to be intact after the  
124 extraction process by single particle measurement using nanosight (Figure S1).

### 125 **2.2.3. Evaluation of other metal nanoparticles as interference in the determination of AuNPs**

126 The selectivity of the method was evaluated in presence of other metal nanoparticles as TiO<sub>2</sub> and plasmonic  
127 AgNPs. Samples containing the target analyte and the interference were tested. On the one hand, TiO<sub>2</sub> resulted  
128 to be adsorbed onto the s-NC although no elution occurred under the protocol of the method, not altering the  
129 quantification of AuNPs. On the other hand, citrate AgNPs were retained by the sorbent and also eluted  
130 following the method conditions. Capillary electrophoresis was used to evaluate the effect of AgNPs eluted  
131 towards the determination of AuNPs; thus, electrophoretic mobilities of eluted nanoparticles were monitored by  
132 CE to inspect any AgNP-AuNP interaction. It is reported a “large-volume sample stacking” (LVSS) mode CE  
133 method that uses thiomalic acid (0.1%(w/v)) as buffer additive. In this electrophoretic modality, a large volume  
134 of sample is introduced in the capillary followed by a plug of buffer to increase the AuNP-additive interaction.  
135 Subsequently, a high negative voltage was applied for 4 minutes, and finally, the polarity is returned to positive  
136 to start the separation. All solutions were filtered through 0.45  $\mu$ m pore-sized nylon membranes before the  
137 analysis. The fused-silica capillary (57 cm length and 75  $\mu$ m inner-diameter) was conditioned using  
138 sequentially HCl (1M) for 5 min, NaOH (0.1 M) for 10 min and double distilled water for 5 minutes. The  
139 electrophoretic running buffer was composed of SDS (40 mM) and CAPS (10 mM) with 0.1 % methanol at pH  
140 9. Eluted metallic NPs were injected into the capillary (0.5 psi for 50 s) and subsequently separated (voltage of  
141 20 kV for less than 12 min). Between runs, the capillary was washed following the addition of HCl (1 M) for 1  
142 min, NaOH (0.1 M) for 2 min, double distilled water for 5 minutes and the running buffer for 5 min.

143

## 144 **3. Results and Discussion**

### 145 **3.1. Sorbent material suitability**

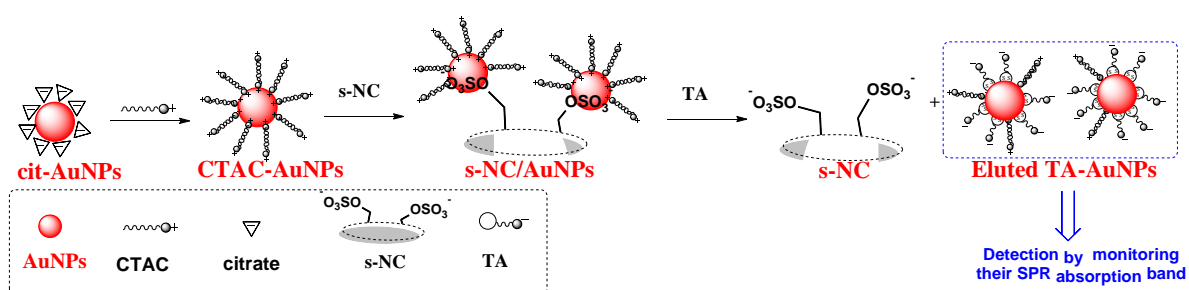
146 To succeed in the determination of AuNPs, the insertion of appropriate superficial groups in the sorbent  
147 material is required. Two negatively charged NC containing diverse ionizable groups (i.e. sulfonate esters  
148 (s-NC) and carboxylic groups (c-NC) on surface) were evaluated, resulting in the NC-AuNP interaction only  
149 when sulfonate groups were on NC surface. The selection of the appropriate sorbent surface for AuNPs was

150 based on our previous experience in the efficient determination of AgNPs with NC containing sulfonate  
 151 functional groups [24]. As previously reported [24], sulfonate groups contribute in less extent than thiols and  
 152 disulfide to noble metal binding; thus, the diverse capability of sulfur atoms binding metals is the basis of this  
 153 method (see Scheme 1). It is worthy to mention that this promising material (s-NC) may be used for extracting  
 154 and storage of nanowastes until their quantification and recycling for further uses.

155

156 Preliminary dispersion experiments of NC corroborate other previous report [24], in which negatively charges  
 157 of s-NC afford greater dispersion rate in an aqueous media, avoiding agglomerating and bundling of the  
 158 cellulosic sorbent, aiding in the practical application of this nanofibers. This is in accordance with the Zeta  
 159 potential calculated for s-NC (-34.9 mV), being a convenience for its use as sorbent in dispersive extraction  
 160 methodologies.

161



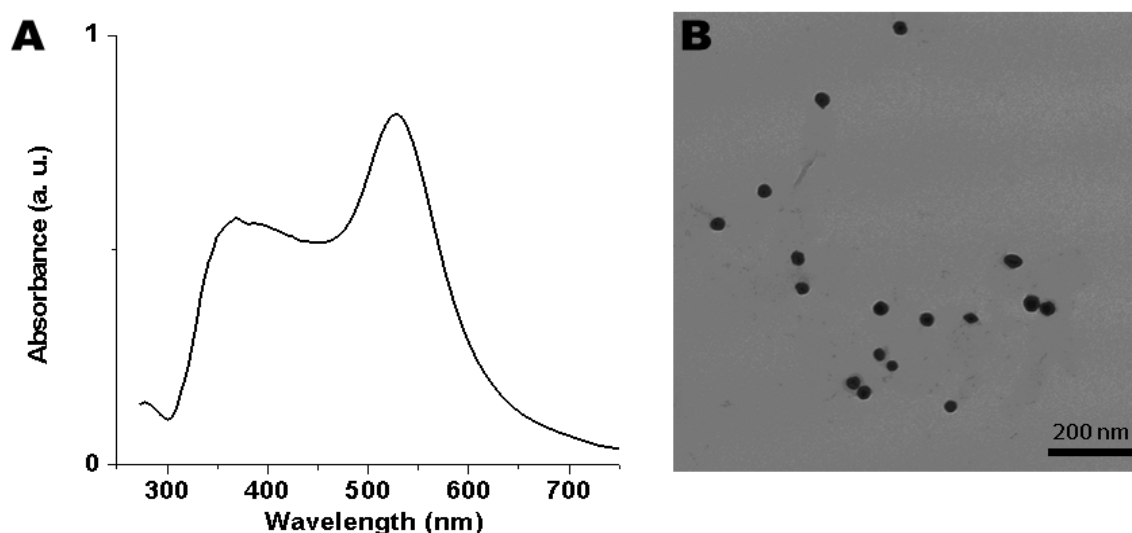
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164 Scheme 1. Illustration of the ligand exchange of citrated gold nanoparticles during the analytical method based  
 165 on dispersive solid phase extraction with sulfonated nanocellulose as sorbent material.

166

### 167 3.2. Evaluation of the optimal conditions for the extraction of gold nanoparticles with sulfonated 168 nanocellulose

169 It is important to mention that the location of SPR band is an indicative of the AuNP stability. Isolated AuNPs of  
 170 average size of 35 nm display its maximum absorption centred at 527 nm whereas they weakly absorb at 700 nm  
 171 (Figure 1). Thus, the ratio of both intensities was collected to evaluate the SPR intensity during the experiments.  
 172 The agglomeration of AuNPs is characterized by the red shifting of the SPR which resulted in a colour change  
 173 to violet.



174  
175 Figure 1. UV-visible spectrum (A) and TEM micrograph (B) of the as-prepared AuNPs coated with citrate.  
176

177 In order to make the NC surface selective for metallic NPs, the use of cationic surfactants (e.g.  
178 cetyltrimethylammonium salts) resulted to be essential for the adsorption of AuNPs into the well-dispersed  
179 s-NC and produced no apparent aggregation of AuNPs derived from the UV-spectra. In contrast, evaluation of  
180 anionic surfactants like SDS resulted in no extraction of AuNPs by the selected sorbent material. In fact, these  
181 results are attributed to two effects of the cationic surfactant, on the one hand, a stabilization of the AuNPs in  
182 presence of s-NC and, on the other hand, a strengthening of the interaction between the sorbent and the metallic  
183 NPs [18]. No differences between the extraction of AuNPs using the chloride (CTAC) and bromide (CTAB)  
184 counterions were observed; thus, we decided to use the chloride derivative.

185 Optimization of the CTAC concentration, amount of s-NC used as sorbent material and the pH are given in the  
186 ESM. The experimental results indicated that best results for the extraction of AuNPs were achieved with 6 mg  
187 of sorbent material and 200 mM of CTAC. Experiments at pH values from 6 – 8 resulted in not significant  
188 different in the extraction capability of the sorbent towards AuNPs.

189

### 190 3.3. Elution and detection of gold nanoparticles.

191 As expected, the retained AuNPs into the s-NC surface were eluted only in presence of substances containing  
192 sulfur atoms with higher affinity to gold atoms than s-NC, as organosulfur compounds are. In this case, the  
193 foul-smelling organosulfur were avoided; thus, water-soluble disulphides were under investigation to avoid the  
194 use of organic solvents. Interestingly, the structural characteristics of the sodium salt of TA was a good  
195 candidate for such matter; thus, as shown in Scheme 1, TA gave rise to a complete elution of AuNPs from NC  
196 surface in a short period of time. Experimentally, by shaking the colourless TA solution in presence of the  
197 sorbent retaining AuNPs for few seconds, all AuNPs were released from the sorbent observing a pinkish  
198 solution, i.e., the selected organosulfide were successfully adsorbed on AuNPs making possible the NP  
199 redispersion in aqueous media with no apparent aggregation neither change of the NP morphology, as evidence  
200 by the SPR band. This seems to be a favourable step for two reasons: *i*) no evaporation/reconstitution of the  
201 eluting is required for avoiding organic solvents; *ii*) the elution is directly performed without the need of drying  
202 the sorbent.

203

204 Concentrations of TA ranging 20-100 mM were also tested, being 75 mM suitable to guarantee the complete  
205 elution of AuNPs. As reported elsewhere [29], TA is self-assembled on AuNPs to guarantee their stabilization  
206 thanks to the disulfides, which bind strongly gold as thiols do. It is important to note that in this method the TA  
207 concentration is higher than in other reported method for eluting AgNPs [24]; it is clear from these results that  
208 AuNPs needs higher TA for stabilizing and redispersing again in an aqueous solution.

209

210 After centrifugation of the mixture at 3000 rpm for only 4 min, the white sorbent was then washed for its reuse  
211 with simply water:methanol whereas the reddish supernatant is subjected to analysis.

212

### 213 **3.4. Feasibility of the sorbent for the determination of gold nanoparticles.**

214 The analytical performance of the method was evaluated in terms of linearity, precision and limits of detection  
215 and quantification. The linearity of the method was obtained using distilled water standards enriched at different  
216 AuNP concentrations (0-20  $\mu\text{g/mL}$ ). The equation resulted from the reflected linear trend ( $y = 0.1697x -$   
217  $0.0402$ ) exhibits a correlation coefficient of 0.998. Each concentration level was performed in triplicate.

218

219 The detection and quantification limits, calculated as  $3S_a/b$  and  $10S_a/b$  (where  $S_a$  is the standard error of the  
220 intercept and  $b$  is the slope of the calibration curve), resulted to be of 0.26 and 0.87  $\mu\text{g/mL}$ , respectively. The  
221 precision of the method, expressed as relative standard deviation (RSD), resulted to be of 12.26%.

222

223 Interestingly, when using tap water enriched with AuNPs, the data indicates lack of matrix effect. Assuming  
224 quantitative extraction, the preconcentration factor, defined by the volume of the initial and final aqueous  
225 phases, was calculated by the method proposed by Fang [30], resulted to be of 8 for a suitable detection.

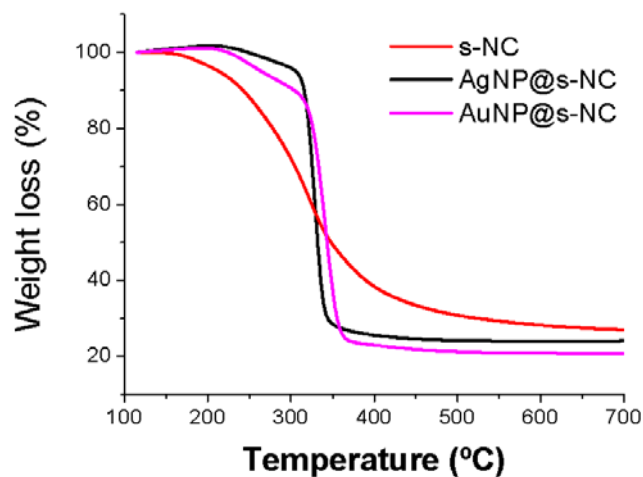
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227 Nanoparticle tracking analysis was used to understand the behaviour of the as-prepared AuNPs in diverse  
228 aqueous media. Figure S2 reports on how the stability of citrate AuNPs in tap water is lower if compared to the  
229 same AuNPs in the elution solvent once subjected to the extraction protocol. This result showed that TA acted  
230 as redispersing agent as well as a stabilizer. In contrast, the as-prepared AuNPs showed aggregation after  
231 spiking river water samples as a result of the color change from red to blue due to bigger aggregates.

232

233 The method was evaluated in presence of other metallic nanoparticles as potential interferences. On the one  
234 hand,  $\text{TiO}_2$  were selected as interfering system. Preliminary results indicated that no extraction of these NPs by  
235 the sorbent occurred at the conditions described in the method. On the other hand, citrated AgNPs were also  
236 tested and the experiments confirmed that the extraction of both AuNPs and AgNPs occurred [24]. It is an  
237 indication that both plasmonic nanoparticles displayed similar sorption abilities towards the selected sorbent. In  
238 order to to understand the types of NP-NC interaction, evaluation of the thermal stability of the sorbent in  
239 absence and presence of AuNPs and AgNPs was performed. Figure 2 represents thermal degradation of NC as  
240 an indicative on how both plasmonic NPs interact with the sorbent. On the one hand, in absence of metal NPs,  
241 there is a gradual degradation of the fibers reaching up to 400  $^{\circ}\text{C}$ , as expected for its sugar skeleton (rupture of  
242 glycoside bonds, ring opening, dehydration and decomposition). The retention of both plasmonic NPs on  
243 surface of s-NC significantly affected the functionality of the sorbent; thermogravimetric curves of both  
244 NP-retained nanofibers showed two main regions of mass lost; one onset of combustion at 230-310 $^{\circ}\text{C}$ , possibly

245 due to organic coatings of nanoparticles, and a maximum mass lost at 340°C, associated with the nanofiber  
246 decomposition. These data support that plasmonic nanoparticles coating the nanofibers altered the degradation  
247 of the s-NC in similar extend.  
248



249  
250 **Figure 2.** Thermogravimetric analysis of sorbent material (s-NC) in absence and presence of metallic  
251 nanoparticles in nitrogen atmosphere.  
252

253 The elution of both the target analyte and the interfering AgNPs occurred upon the protocol conditions. In this  
254 respect, it is important to know that the eluted plasmonic NPs were not interacting each other in the elution step  
255 for the further quantification of AuNPs at 527 nm. This experiment is feasible since citrate-coated AgNPs and  
256 AuNPs exhibit different strong SPR absorption bands at ca. 400 and 527 nm, respectively. CE was selected to  
257 prove hybrid formation in the elution solution based on the variation in the electrophoretic mobilities of both  
258 types of NPs. It is described a LVSS mode in which a large amount of sample is injected and retained owing to  
259 a high negative voltage; at that moment, the electroosmotic flow moves towards the inlet while anionic  
260 nanoparticles move towards the detection end stacking at one side of the boundary between the sample zone and  
261 the running buffer. Then, the separation starts in a normal mode. Lack of NP-NP interaction resulted from this  
262 electrophoretic modality, as depicted in Figure S3. Interestingly, AuNPs did not interact with the interference as  
263 a result of the shorter migration time. This is an indicative that the quantification of AuNPs is not affected by the  
264 presence of citrate AuNPs.  
265

266 In summary, although the high detection limit of this method in contrast to other previously published (Table  
267 S1), this analytical approach poses many advantages such as the use of ecofriendly materials for the extraction  
268 and storage of AuNPs, the lack of handling organic solvents, the use of non-toxic odourless disulphide, rapid  
269 analysis without the need of reconstitution of the eluent neither drying the sorbent before the elution, and the use  
270 of cheap instrumentation as a mere UV-spectrometer.  
271

272 This work is in line with the current analytical nanoscience and nanotechnology challenge involved in  
273 developing fast analytical methods for monitoring and collecting newly emerging nanocontaminants from  
274 aqueous environments. The presented method enables detecting different plasmonic NPs by the use of EC for  
275 their separation, detection and identification, as demonstrated in this work.



276

**277 4. Conclusions**

278 A fast and ecofriendly method based on biocompatible and ecofriendly s-NC for the extraction and  
279 preconcentration of AuNPs and its determination by a mere spectrophotometer is an attractive alternative for  
280 routine analysis of consumer products and environmental water containing AuNPs. The method is characterized  
281 by the use of relatively low-cost instrumentation and renewable sorbent material and by the lack of organic  
282 solvents, of reconstitution step and of drying the sorbent before the elution. Other interfering metal  
283 nanoparticles were evaluated and CE was used to inspect the mobilities of plasmonic nanoparticles resulting in  
284 no interaction AuNP-AgNP. Although this extraction approach holds promise for the fast identification of  
285 AuNPs in waters, which is one of the trending topics nowadays in analytical science [31]. Further experimental  
286 investigations will include strategies towards lowering the detection levels from a variety of environmental  
287 water samples.

288 It is described a LVSS mode suitable to monitor any possible interaction between both the analyte and the  
289 interfering nanoobject,

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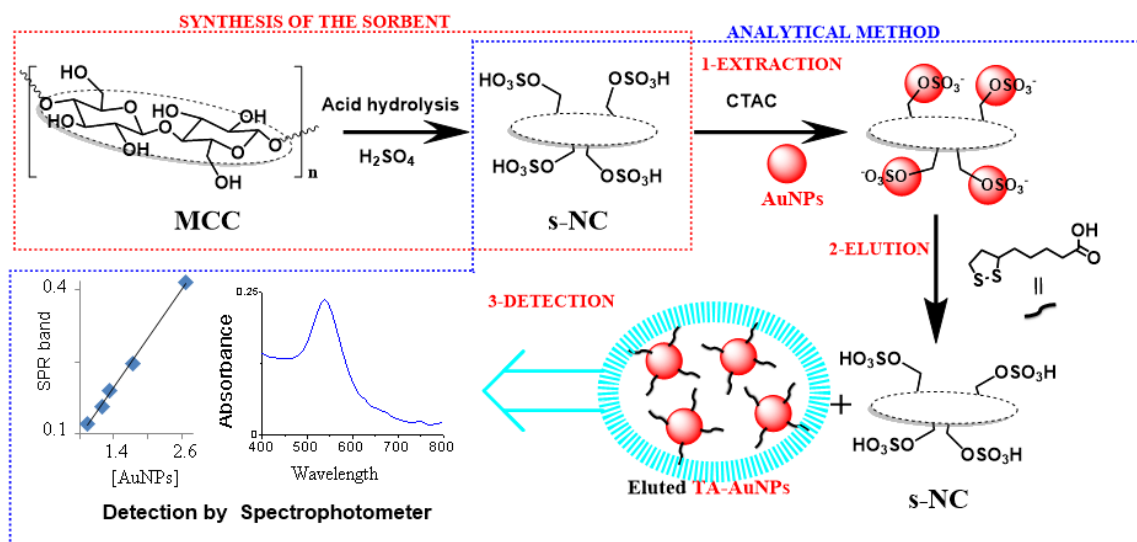
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## FOR GRAPHICAL ABSTRACT ONLY

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300 Schematic illustration of the synthesis of sulfonated nanocellulose (s-NC) from microcrystalline  
 301 cellulose (MCC) and its use as sorbent material in the proposed analytical method devoted to the rapid  
 302 determination of gold nanoparticles.

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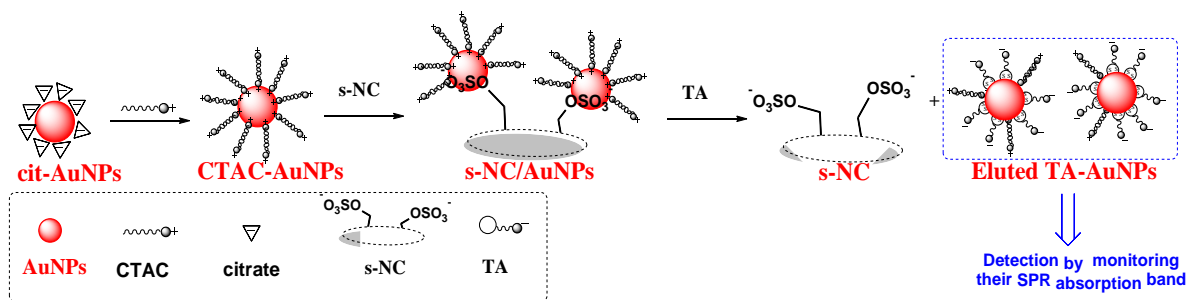
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## 305 FIGURES AND FIGURE CAPTIONS

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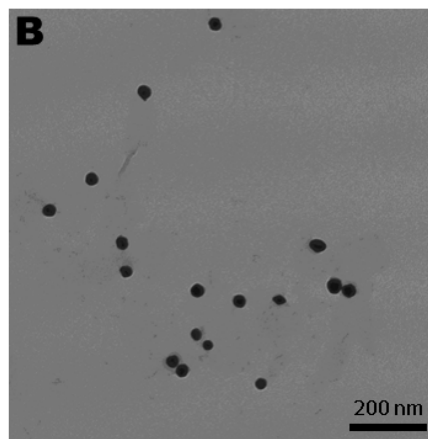
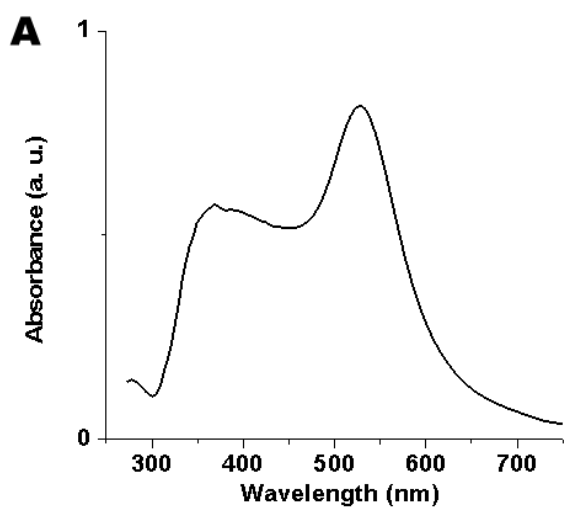
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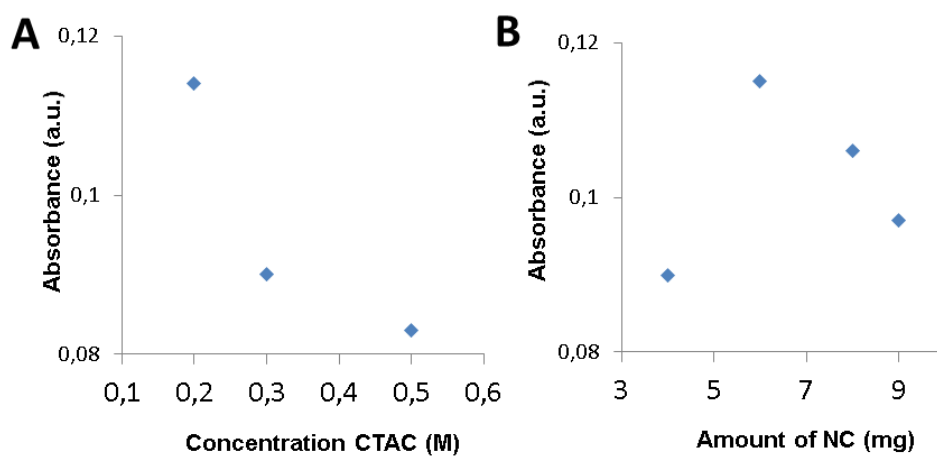
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316 **Figure 1**

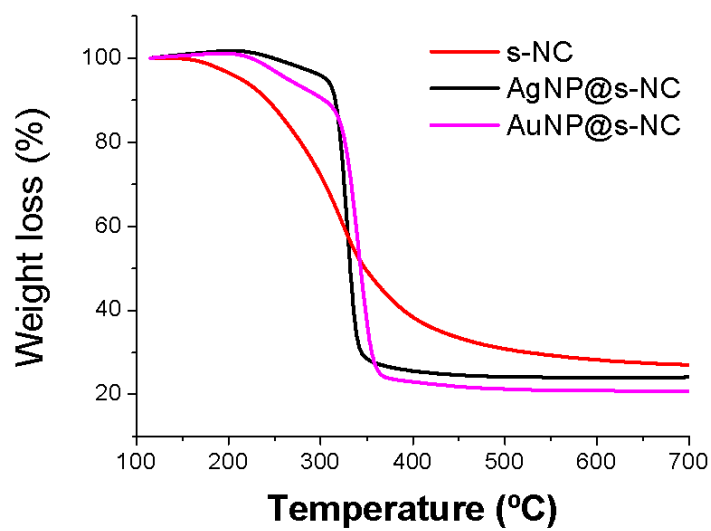
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Figure 2.

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325 **Figure 3.**

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