Ionic liquid-based microextraction method for the determination of silver nanoparticles in consumer products

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Abstract: A simple method to determine hazardous silver nanoparticles (AgNPs) based on ionic liquid dispersive liquid-liquid microextraction and back-extraction is herein described. This approach involves a first AgNPs stabilisation step using a cationic surfactant and a second extraction step from sample matrix by means of ionic liquid (IL) as an extraction phase. According to the high affinity of certain ILs towards metals, preliminary experiments have proven that those ILs consisting of imidazolium cation efficiently extracted AgNPs in the presence of a cationic surfactant and a chelating agent. Afterwards, histamine was used as a dispersing agent to promote phase transfer of differently-coated AgNPs from the IL in aqueous solution to be subsequently analysed by UV-visible spectrometry. The analytical procedure allows for AgNPs recovery from the sample matrix in aqueous medium, enrichment factor being up to 4, preserving both NP size and shape as demonstrated by transmission-electron microscopy images and the localised surface plasmon resonance band (SPR) characteristic for each AgNP. The present methodology displayed a linear response for AgNPs in the range 3 – 20 µg/mL, limit of detection being down to 0.15 µg/mL. Method efficiency was assessed in spiked orange juice and daily cream, yielding recoveries ranging from 75.7 to 96.6%. This method was evaluated in the presence of other nanointerferents namely gold nanoparticles (AuNPs). Based on diverse electrophoretic mobilities and SPR bands for metal nanoparticles, capillary electrophoresis was used to prove the lack of interaction of the target AgNPs with other AuNPs during the whole protocol, thus interferents do not affect AgNPs determination. As a consequence, the analytical approach herein described showed a great potential for the analysis of engineered nanosilver in consumer products.

Keywords: ionic liquid, imidazolium cation, extraction, histamine, silver nanoparticles, surface plasmon resonance

Abbreviatures: AgNPs, silver nanoparticles; AuNPs, gold nanoparticles; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; CTAC, hexadecyltrimethylammonium chloride; CTAB, hexadecyltrimethylammonium bromide; EDTA, ethylenediaminetetraacetic acid; CE, capillary electrophoresis; IL, ionic liquid; LOD, limit of detection; LOQ, quantification limit; NPs, nanoparticles; RSD, relative standard deviation; SPR, surface plasmon resonance; TEM, transmission electron microscopy;

1. Introduction

Nanotechnology has caused a huge economic impact in a growing market by the large number of benefits of engineering nanoparticles (NPs) in many fields of applications. Of special attention is their incorporation in many daily products [1]. Thus, a great variety of manufactured engineering NPs with diverse nature are being increasingly incorporated into various consumer's goods according to their unique properties to improve the product lifetime, flavor, texture, and odor, among others [2]. Despite these positive impacts, these nanoscale materials also cause unwanted effects at human health, especially AgNPs on daily basis [3]. Many are the reports about antibacterial, antiviral and antifungal AgNPs that showed great concerns about safety when exposed in cells and organs, and thus, the European Commission has been obligated to harmonize all the regulations to control the use of nanomaterials by enforcing companies to label the specifications and content of each NP added [4]. In this way, risk assessments and control of NP containing-consumer goods are a priority. These tasks are very challenging and standardized analytical strategies are required to achieve reliable information on the safest goods in the market.

In this regard, simple effective and affordable methods for testing AgNPs in complex matrices are required. On the one hand, a myriad of methods for determining engineering NPs uses more complex or expensive instrumental techniques that require personnel training, such as Raman spectroscopy [5], size exclusion chromatography [6], graphite furnace atomic absorption spectroscopy [7], high-resolution-continuum source atomic absorption spectrometry [8] and inductively coupled plasma mass spectrometry (ICP-MS) [9,10], which requires in some occasions the destruction of the sample (i.e., enzymatic digestion) causing loss of information related to the NP concentration, size distribution and stability. In other cases, additional extraction/enrichment steps are needed to avoid metal ion interferences, i.e. using a myriad of filtration/centrifugation protocols, or even other more effective ones like cloud point extraction [11]. Recent analytical trends for the determination of metal NPs in soils and food [12] with elevated metal ion content are focused on the coupling of equipment with costly detectors, i.e. the field flow fractionation techniques connected to ICP-MS among others [13,14,15]. On the other hand, few less expensive optical instrumentation were described for the determination of AgNPs [16,17,18]. However, we paid attention in the usefulness of the phase transfer processes of AgNPs [19] as an interesting approach for liquid-liquid extraction protocols for reducing the hazardous exposure to organic solvents during the analysis and for preserving the NP main characteristics from the aqueous matrix.

Ionic liquids (IL) [20] have attracted the attention from analytical scientists in the separation processes [21,22,23]. These organic liquid salts below 100ºC consist of a bulky unsymmetrical organic cation and a great variety of organic or inorganic anions. IL are recognized as green solvents with excellent solvation ability for molecules and materials, and are being considered as promising media already applied to a wide assortment of separation methods [24], as a result of their function as stabilization media, their low toxicity, high thermal stability and remarkable non-volatility and non-flammability. Imidazolium cations containing long alkyl chains resulted to be very suitable for metals extraction [25]. It is well-known that IL is considered an excellent stabilizer of nanoparticles [26]. In fact, the capability of phase transfer of AgNPs has been recently reported [27], in which AgNPs was extracted in dispersions of imidazolium-based IL (at 1%w/v) in methanol.

Hence, this paper proposes an economic method for the determination of AgNPs with citrated and polyvinylpyrrolidone (PVP) covers (as typically referred in the label ingredients for stabilizing the NPs) using UV-visible spectrometry as inexpensive detector. The method consists of a liquid-liquid microextraction and preconcentration of AgNPs assisted by short alkyl chain imidazolium-based IL and their back-extraction onto

an aqueous solution using histamine as the dispersant agent. To corroborate the good extraction towards AgNPs and their desorption as well as their size and shape preservation to assess their original characteristic features, transmission electron microscopy and UV-visible spectrometry were used. Additionally, capillary electrophoresis (CE) was applied to study the influence of other interfering nanoobject such as AuNPs along the proposed analytical approach. This work contributes to the analytical challenge of finding solutions to problems of Society related to the widespread exposure of hazardous engineering nanoparticles.

2. Materials and Methods

2.1. Chemicals and Materials.

Sulfuric acid (95-98%), chloroform (≥98%), hexadecyltrimethylammonium bromide (CTAB, ≥99%),) and chloride (CTAC) (assay 25% in water), histamine dihydrochloride (≥99%), sodium phosphate dibasic heptahydrate (≥99.99%), sodium phosphate monobasic monohydrate (≥98%), mercaptosuccinic acid (97%), lipoic acid (TA, ≥98%), sodium dodecyl sulfate (SDS, 98%), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (98%), ethylenediaminetetraacetic acid (EDTA, 99.4-100.6%), citric acid trisodium salt (≥99%), silver nitrate (≥99%), silver chloride (99.999%) and AuNPs and AgNPs with citrate and PVP coatings (0.02 mg/mL in aqueous buffer, with averaged size of 10 and 20 nm) were supplied by Sigma-Aldrich (Madrid, Spain). The ionic liquids used were purchased by Merck. All reagents were used as received without further purification. Orange juice and cream samples were purchased from a local supermarket.

Ultrapure water purified through a Millipore system was used in all the experiments. All solutions were filtered through 0.45 μm pore-sized nylon membranes before the analysis by CE. During the experiments, all glassware was cleaned with aqua regia

2.2. Instrumentation.

Ultraviolet-Visible spectra were recorded on a Photon Technology International QuantaMasterTM Spectrofluorometer equipped with the model 814 PMT detection system and with the tungsten/halogen light source for the absorption measurements. Felix 32 software was used to obtain and process all absorption data. All optical measurements were performed at room temperature, using a micro quartz cuvette of 10 mm light path. AgNPs were monitored by their localized SPR band centered at 410 nm.

Transmission-electron microscopy (TEM) images were carried out with a Jeol JEM 2010 high-resolution electron microscope operating at average acceleration voltage of 200 kV and using a point-to-point resolution of 0.194 nm.

Capillary electrophoresis (CE) analyses were carried out on a Beckman Coulter P/ACE MDQ instrument (Palo Alto, CA) equipped with a UV-diode array detector and using a fused-silica capillary tube of an effective separation length of 57 cm (75 μM inner-diameter). 32 Karat software was used for the data acquisition and processing. The analysis was carried out taking into account the localized SPR bands of the metal NPs used.

A sonicator (J.P. Selecta; 50/60Hz; 110w), a vortex (Vórtex Heidolph Reax Top) and a centrifuge (Eppendorf Concentrator Plus) were used to carry out the phase transfer experiments.

2.3 Analytical protocols.

2.3.1. Extraction/preconcentration protocol of silver nanoparticles

Standard solutions containing the PVP or citrated coated AgNPs (at 3-20 µg/mL) were mixed with a solution containing the chelating agent EDTA (6 mM) and surfactant CTAC (20 mM) in phosphate buffer and then with 1-butyl-3-methylimidazolium hexafluorophosphate (HMIM•PF_6) (100 µL). After shaking the mixture for a few seconds, the NPs were immediately extracted from the aqueous solution into the IL. After the phase separation, the supernatant was discarded and the IL residue containing the stabilized AgNPs was washed twice with deionized water.

2.3.2. Back-extraction and detection of silver nanoparticles.

Firstly, histamine solutions (6-100 mM) were prepared in ultrapure water and adjusted to pH ranging from 6 to 12. The most favourable conditions for phase transferring the extracted AgNPs from the IL into aqueous medium were as follows: addition of 200 µL of $CHCl_3$ and 100 µL of histamine-aqueous solution (20 mM at pH 8) into the IL shaking it vigorously for only a few seconds. Thus, the stabilized AgNPs in histamine were efficiently spread out on the aqueous phase. Then, the tube was centrifuged to facilitate the phase separation and the upper-phase containing AgNPs was collected for characterization and quantitative detection by UV-visible spectrometry.

2.3.3. Analysis of samples

Orange juice and cream samples were enriched with standard solutions containing AgNPs at diverse concentrations and stored in brown flasks at 4ºC for a day before the analysis. For the enrichment step, sample aliquots were accurately weighted (1.5 g) and diluted with the standards at the appropriate concentration; after mixing for 20 min, the samples were stored for 24 h to reach the equilibrium; afterwards, samples were subjected to the extraction/preconcentration and back-extraction protocols before the analysis.

2.3.4. Validation of the method

Slope, intercept and determination coefficient were estimated by linear regression analysis building a calibration curve obtained at five concentrations levels for 20 nm-citrated AgNPs ranging 3 – 20 µg/mL. The limit of detection (LOD) was established as three times the standard deviation of the blank signal divided by the slope of the calibration curve. The quantification limit (LOQ) was calculated as the ratio between ten times the standard deviation of the blank signal and the slope of the calibration curve. The repeatability and reproducibility were estimated in terms of relative standard deviation by analysing five replicates at the LOQ on a day and on three consecutive days, respectively. The enrichment factor was calculated as the ratio of analyte concentration in the enriched sample to the concentration in the histamine-aqueous phase. The absolute extraction recovery was estimated by the proportions of total analyte efficiently extracted into the IL and back-extracted into the histamine-aqueous solution. The matrix effect was evaluated by comparing the slopes of calibration curves in neat standard AgNP solution and in the enriched samples ranging 3.0 – 9.5 µg/mL.

2.3.5. Evaluation of the effect of AuNPs on the proposed method

In order to evaluate the selectivity of the method towards AgNPs as the target analyte, citrate-coated spherical AuNPs were selected for also displaying plasmonic properties and for their similarity in shape, shell and size.

Thus, samples containing both AgNPs and AuNPs at 10 µg/mL were tested using CE, firstly, to examine the presence of AuNPs in the back-extraction histamine solution by monitoring their localized SPR bands (at 520 nm for AuNPs whilst AgNPs appears at 410 nm) and, secondly, to evaluate any effect of AuNPs in the AgNP determination (possibly caused for any NP-NP interaction) by examining any modification in their electrophoretic mobility [28].

An electrophoretic method based on large-volume sample stacking (LVSS) mode was used to monitor the presence of AgNPs and AuNPs coated with citrate ions, as described elsewhere [29]. Mercaptosuccinic acid (0.1%(w/v)) was used as buffer additive in the running buffer (40 mM of SDS and 10 mM of CAPS with 0.1 %v/v methanol at pH 9). The back-extracted AgNPs in aqueous solution were injected into the capillary at 0.5 psi for 50 s and subsequently separated using a voltage of 20 kV. Conditioning of the fused-silica capillary tube was performed with sequentially 1 M of HCl (5 min), 0.1 M of NaOH (10 min) and deionized water (5 min) between runs.

3. Results and Discussion

3.1. Extraction protocol

The achievement of an efficient extraction and preconcentration process depends on the features of both target NPs and samples, and on the choice of a suitable extraction phase. Thus, these variables need to be carefully evaluated. Extracting AgNPs from a variety of complex matrices requires a strongly stable medium; thus, IL were selected as an extraction phase owing to their interaction with nanomaterials [26,27]. Most consumer goods are composed of an aqueous matrix containing different ingredients; thus, dispersive liquid-liquid microextraction was considered to be the best option for AgNPs determination in complex matrices. In fact, it is important to consider immiscible IL for a liquid-liquid extraction in a two-phase system.

Firstly, different AgNPs coatings were tested, shells being composed of citrated and PVP. Preliminary investigations for AgNPs extraction with a variety of ILs containing diverse cations (imidazolium, pyridinium) and anions (triflate, tetrafluoroborate, hexafluorophosphate, bis(trifluoromethylsulfonyl)imide) were evaluated. Those ones composed of hexafluorophosphate anion were suitable for extraction due to a higher immiscibility with aqueous medium. According to the hydrophobic nature of IL there is need for a liquid-liquid microextraction, higher extraction yields for AgNPs were accomplished for those ones containing imidazolium and hexafluorophosphate counterparts. These results are consistent with a study previously conducted on AuNPs determination by means of SERS [30]. It is noticeable that there are IL-NP bonds able to break nanomaterial interactions with matrix components.

As regards the effect of cation counterpart (1-hexyl-3-methylimidazolium, 1-butyl-3-dimethylimidazolium, 1-butyl-2,3-dimethylimidazolium, 1-methyl-3-octylimidazolium), alkyl chain substituents also alter the hydrophobicity, affinity and biodegradability of IL, and consequently their suitability for NPs extraction. It was observed that not only the cationic ring had an influence on extraction of citrate- and PVP-coated AgNPs, but also the alkyl chain length of the imidazole ring.

After evaluation, we observed that butyl and hexyl chains happened to be more suitable for the extraction. The more hydrophobic IL containing long alkyl chains (e.g. octyl side chain) provoked NPs turbidity in the reported conditions, unless dilution with alcohol were incubated for 30 min as described elsewhere [27]. Because of the rapid biodegradability of the longer alkyl chain length of imidazolium derivatives [31], to address the issue of green analytical methods, 1-hexyl-3-methylimidazolium hexafluorophosphate (HMIM•PF_6) was selected instead of the butyl derivative for NPs extraction from the aqueous solution. These results suggested that extraction of metal NPs was governed by both anion and cation counterparts of IL.

The use of diverse surfactants as phase-transfer enhancers was studied. Non-ionic surfactants namely Triton (X100 and X114), anionic surfactants such as sodium dodecyl sulfate (SDS) and cationic surfactants specifically cetyltrimethylammonium chloride or bromide (CTAC or CTAB) were tested. Cationic surfactants were the only ones promoting the extraction by means of AgNPs stabilisation within the IL phase. It was experimentally observed that AgNPs aggregates were present on the interphase between aqueous medium and IL, then minimising phase transfer (Electronic Supplementary Material Fig.S1). However, addition of cationic ammonium surfactants into the aqueous phase caused the formation of micelles stabilising AgNPs and allowing them to be transferred into the IL phase without NP aggregation. It is expected that the presence of citrated anions onto the surface of AgNPs was responsible for the appearance of small aggregates onto the IL surface, then decreasing phase exchange. Extraction with CTAC was proven to be 10 times higher in respect of CTAB. Interestingly, citrate-coated AgNPs extraction was achieved with 20 mM CTAC, whilst for PVP-coated AgNPs only 15 mM CTAC is needed. Surfactant concentrations beyond 30 mM led to destabilisation of citrate-coated AgNPs. Therefore, so as to obtain a general extraction and preconcentration protocol for most types of AgNPs, a 20-mM concentration was selected as adequate.

Due to the high affinity shown by IL towards metal ions, as reported elsewhere [29], there was a need to add a chelating agent, namely EDTA, during the extraction for binding such metal interferences.

Extraction time was also assessed and results indicated that AgNPs were transferred from the aqueous medium to the IL-bearing phase in less than 2 minutes after shaking both by hand and in an ultrasonic bath.

The effect of pH value of the aqueous solutions containing NPs on the extraction process was evaluated. Phosphate buffer was selected to adjust the pH value. Results showed that pH values ranging from 4 to 9 were appropriate for an efficient AgNPs extraction. Acid pH values, in fact, destabilised nanoparticles, in particular those ones coated by citrate ions.

3.2. Back-extraction and detection of silver nanoparticles.

Due to interferences and likely overlay caused by IL when the signal for the target analyte is acquired with certain detectors, it is convenient to transfer the extracted AgNPs back into an aqueous phase indeed suitable for analysis. However, it is difficult to find strong chemisorptive molecules able to undergo a rapid ligand exchange on the retained NPs allowing their dispersion in aqueous medium, without any NP degradation or flocculation.

Back-extraction of AgNPs from HMIM·PF_6 into an aqueous medium was examined using diverse organic molecules as phase-transfer exchangers. The molecules tested contain sulphur and nitrogen atoms because of

239 their affinity towards metals. Results for the back-extraction of AgNPs with diverse families of organic
240 molecules are described below:
241 *i)* The use of carboxylic acids containing a thiol or a disulfide groups (e.g. mercaptosuccinic acid and lipoic
242 acid) allowed AgNPs to be transferred from IL to an aqueous medium via ligand exchange. Results
243 demonstrated that a thiol-containing aqueous solution promote the rapidly phase-transfer of the extracted
244 AgNPs with recoveries of ca. 50%. No shifts in SPR band were found for AgNPs in the UV-visible absorption
245 spectrum providing evidence of the ligand exchange onto the NPs surface without altering their original size
246 and shape upon basic pH values (8-12) studied.
247 *ii)* The use of an aqueous solution of 0.1 M 4-dimethylaminopyridine (DMAP) also led to phase transition of
248 AgNPs resulting in recoveries lower than 50%; it was observed that back-extraction occurs maintaining the
249 original AgNPs size, as reported by TEM analysis and UV-visible spectroscopy. However, the main
250 inconvenient found using DMAP was the long-time period (ca. 35 minutes) needed for AgNPs to be spread out
251 in the aqueous solution assisted by an ultrasonic bath.
252 *iii)* Another possible dispersant for promoting phase transition of AgNPs from the IL into the aqueous phase
253 was sulfonic acid containing a free amine group namely taurine. However, the back-extraction process was
254 significantly reduced due to the formation of partial NP aggregates strongly attached onto IL surface.
255 *iv)* Biogenic amines specifically the monoamine histamine and polyamine spermidine were also evaluated. Only
256 histamine bearing two basic centers led to AgNPs phase transfer in a fast way, resulting in recoveries above
257 70%.
258 Figure 1 displays a comparison of averaged recoveries found for three amine-containing organic compounds,
259 demonstrating that hydrophilic histamine is an appropriate phase-transfer enhancer.
260 Influence of pH value for the histamine-containing aqueous solution on the back-extraction process of AgNPs
261 were also evaluated for its importance. The extraction efficiency was determined at diverse pH values in the
262 range from 6-12. The suitable pH value of the aqueous solution for citrate- and PVP-coated AgNPs back-
263 extraction resulted to be 8, in which the histamine is monoprotonated in the aliphatic amino group (see
264 Electronic Supplementary Material Fig. S2A). The concentration of histamine (6-100 mM) for the back-
265 extraction of AgNPs was also tested. Interestingly, PVP-AgNPs requires only 6 mM of histamine for the phase
266 transfer whereas citrate-coated ones (Electronic Supplementary Material Fig. S2B) were back-extracted into 20
267 mM of histamine aqueous solution. Thus, a general back-extraction protocol for differently-coated AgNPs was
268 achieved at 20-mM histamine-containing solution as an appropriate concentration in a basic medium.
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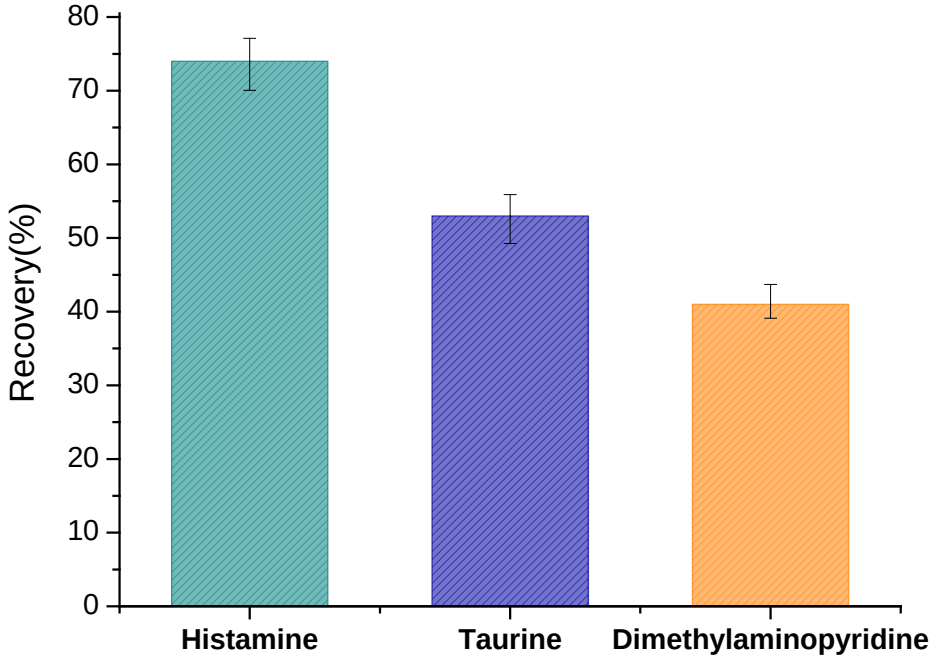


Figure 1: Averaged recoveries of AgNPs using different amine-containing organic molecules as phase-transfer agent.

Interestingly, we found that addition of an apolar solvent caused substantial influence on the AgNPs back-extraction process. After evaluation of diverse organic solvents as possible phase-transfer promoters, only chloroform resulted to be able to produce a fast spread out of AgNPs in aqueous phase preserving the NP main properties. Figure 2 shows that the appearance and main features of AgNPs (i.e. size and SPR band) kept unchanged after being subjected to the described analytical protocol using histamine.

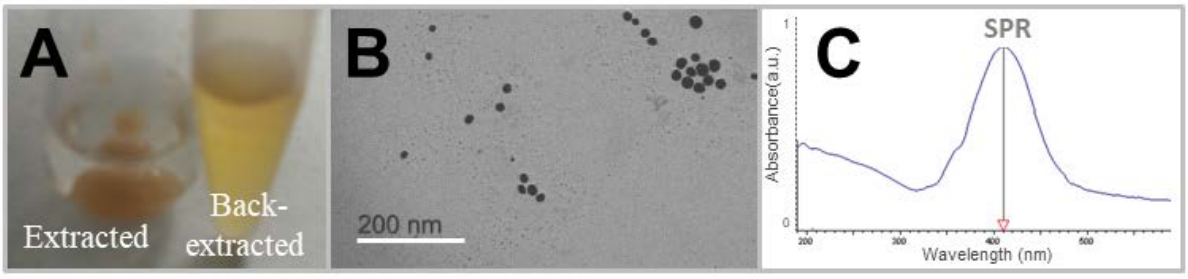
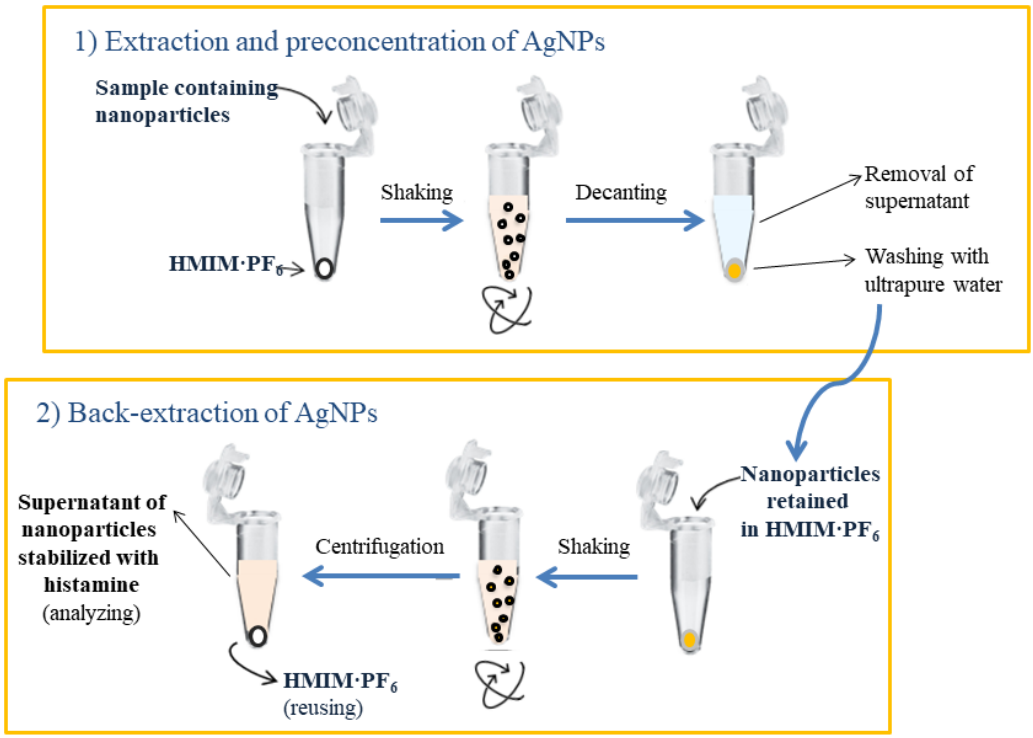


Figure 2: (A) Photograph of the extracted 20-nm sized AgNPs into HMIM·PF_6 (left) and their back-extraction process in histamine-aqueous solution (right). (B) TEM image of the back-extracted AgNPs in histamine-containing solution. (C) Localized surface plasmon resonance (SPR) of the back-extracted AgNPs in aqueous solution.

3.3. Feasibility of the method for silver nanoparticles determination

According to the imminent risk coming from the extensive use of AgNPs in dairy products may cause to the human health, a general low-cost, effective and fast method for detecting such hazardous NPs is needed. Consequently, the proposed method based on the use of IL dispersive liquid-liquid microextraction and back-extraction protocol (summarized in Scheme 1) meets these requirements in screening for AgNPs by means of quickness at low cost. Interestingly, after the analytical procedure, IL can be reused (after chloroform evaporation) at least in two successive cycles without significant loss of extraction capability, as proven by analyses of three consecutive back-extracted AgNPs by UV-visible spectrometry monitoring their SPR band at 410 nm (see Electronic Supplementary Material Fig. S3).



Scheme 1: Illustration of the analytical separation protocol of silver nanoparticles using ionic liquid dispersive liquid-liquid microextraction and their back extraction into histamine-aqueous solution.

The validation of the proposed method was carried out in terms of linearity, precision and limits of detection and recovery. To this end, the calibration curve was constructed from standard solutions containing 20 nm-citrated AgNPs at concentrations ranging 3 – 20 µg/mL with a determination coefficient R^2 of 0.99. The sensitivity of the method was evaluated in terms of detection and quantification limits. The limits of detection (LOD) and quantification (LOQ) resulted to be of 0.15 µg/mL and 0.50 µg/mL. Regarding the precision of the method, the repeatability and reproducibility (quintuplicate) were of 3.3 and 4.3%, respectively. The enrichment factor of the method was 4 and the absolute extraction recovery was 73.2%.

Because the extensive use of antimicrobial AgNPs in goods consumed on a daily basis, the applicability of the method was thus evaluated in face-care creams and orange juices. Preliminary analyses suggested no AgNPs were found in the chosen samples. Therefore, samples were enriched at two concentration levels as described in the experimental section. All enriched samples were then subjected to the analytical methods for AgNP determination. Results showed no matrix effect for orange juice samples whereas a slight matrix effect was found for cream (from a small decrease in the slope) which is solved by 10%-sample dilution. The recoveries obtained in both types of samples varied in the range of 73.2- 96.6 %, being satisfactory results taking into account the complexity of the sample matrices. Additionally, the precision was determined by means of RSD from four independently samples (n=4). The percentage recoveries and RSD for citrated-AgNPs using the proposed method are summarised in Table 1.

Table 1. Recoveries of AgNPs in consuming products previously enriched with citrated AgNPs of 20 nm at diverse spiked levels. Each concentration was analysed in quadruplicate.

Samples	Concentration found (µg/mL)	Recovery (%)	RSD (%)
Orange juice	3.28	82.0	3.33
	4.91	92.2	6.51
	7.02	90.3	5.92
	9.50	89.3	7.10
Face cream	3.03	75.7	5.53
	4.83	96.6	6.99
	7.22	73.2	10.2

3.3.1 Interfering studies

Metal species, in particular those ions coming from AgNPs dissolution, were evaluated to assess the proposed method. In presence of silver nitrate and silver chloride no effect in the AgNP quantification was observed. Moreover, influence of other metal NPs, namely AuNPs, in the AgNP determination as a potential interference was also studied using CE for monitoring the SPR characteristic bands for AgNPs at around 410 and for AuNPs at ca. 520 nm. Preliminary experiments demonstrated that both citrate-coated AuNPs and AgNPs were extracted into the IL, as shown in Electronic Supplementary Material Fig. S4A. However, the extracted AuNPs within $HMIM{\cdot}PF_6$ did not undergo back-extraction process into the histamine-aqueous solution at 20 mM. Thus, a mixture of AgNPs and AuNPs was subjected to the proposed method and the resulting histamine-containing solution after back-extraction were analysed by CE. As expected from the preliminary experiments, no significant effect on the determination of AgNPs in presence of AuNPs was observed, as can be evidenced by the absence of the characteristic SPR band of AuNPs in the histamine-containing aqueous solution (Figure 3)

and any shifting of electrophoretic mobility of AgNPs [32,33]. These results suggested that AgNPs have higher affinity to histamine functional groups thus promoting the ligand exchange and phase transition.

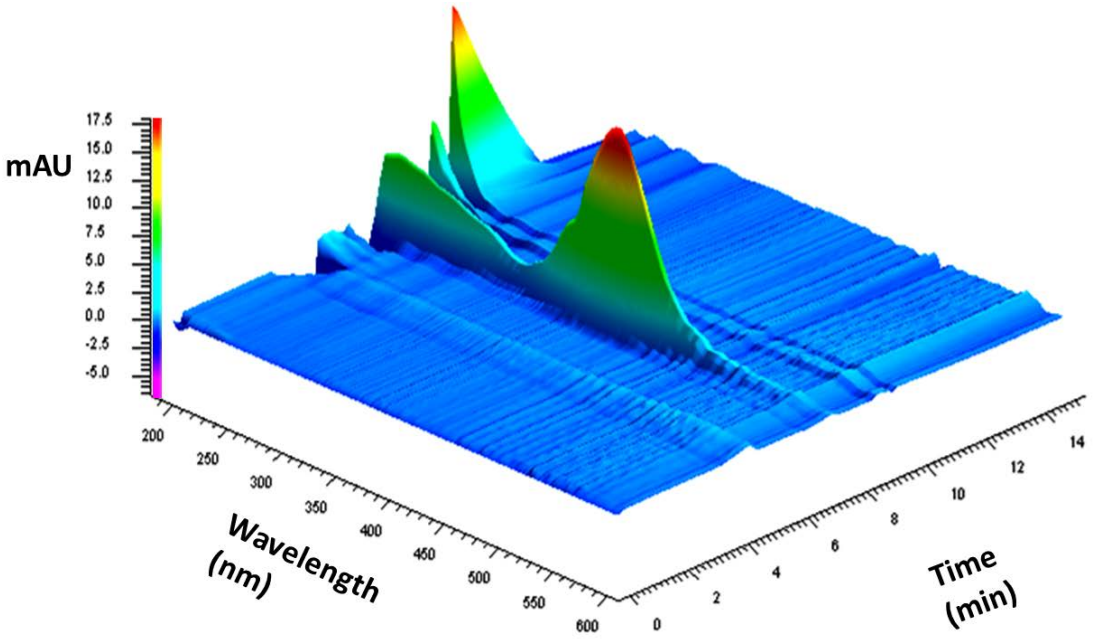


Figure 3: Three-dimensional (absorbance vs. time vs. wavelength) graph showing the presence of SPR band of AgNPs when analysing the histamine-containing solution for a mixture of citrate-coated AuNPs and AgNPs which were subjected under the proposed analytical method using capillary electrophoresis equipped with UV-diode array detector to monitor any characteristic localized surface plasmon resonance band.

This method has several positive aspects for the determination of AgNPs, such as *i)* the use of small amount of IL (considered as green solvent) for NPs extraction, *ii)* the resuspension of the extracted AgNPs into an aqueous medium that helps manipulation and avoids IL interference in certain instrumental techniques, and *iii)* the possibility of using low-cost instrumentation as a mere UV-spectrometer for AgNPs quantification in very complex matrices, iv) fast AgNPs monitoring in consumer goods when regulations policies will be well defined.

4. Conclusion

To sum up, this work proposes an AgNPs determination protocol based on both ionic-liquid dispersive liquid-liquid microextraction and back-extraction. This approach is distinguished by ease, quickness and low cost. It was demonstrated that an imidazolium-based IL containing hexyl chains and PF_6 anions was suitable for differently-coated AgNPs extraction and stabilisation onto the IL. To avoid IL interfering effects in the AgNPs determination, phase transfer of AgNPs into an aqueous phase (back-extraction) was performed to monitor the target analyte in an easy-handling and less toxic aqueous phase. Back-extraction into aqueous phase for the

extracted NPs was achieved preserving physical features by simply using histamine as a dispersant. Interestingly, histamine concentration varies according to the type of NP coating, stablishing an appropriate concentration of 20 mM for determining AgNPs. A limitation of this method is the use of chloroform to promote NPs back-extraction into the histamine-containing aqueous solution. This unprecedented analytical approach renders the unique feature of AgNPs separation from complex matrices (e.g. goods consumed on a daily basis) and quantification by UV-visible spectrometry.

The proposed analytical method allows to recover AgNPs in a rapid way at a low cost. By means of this strategy it may be possible to open new ways aimed at evaluating the presence of similar nanomaterials in a wide application range, for instance biological specimens.

Conflict of interest

All authors declare that they have no competing interests.

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FIGURE AND TABLE CAPTIONS

Figure 1: Averaged recoveries of AgNPs using different amine-containing organic molecules as phase-transfer agent.

Figure 2: (A) Photograph of the extracted 20-nm sized AgNPs into HMIM·PF_6 (left) and their back-extraction process in histamine-aqueous solution (right). (B) TEM image of the back-extracted AgNPs in histamine-containing solution. (C) Localized surface plasmon resonance (SPR) of the back-extracted AgNPs in aqueous solution.

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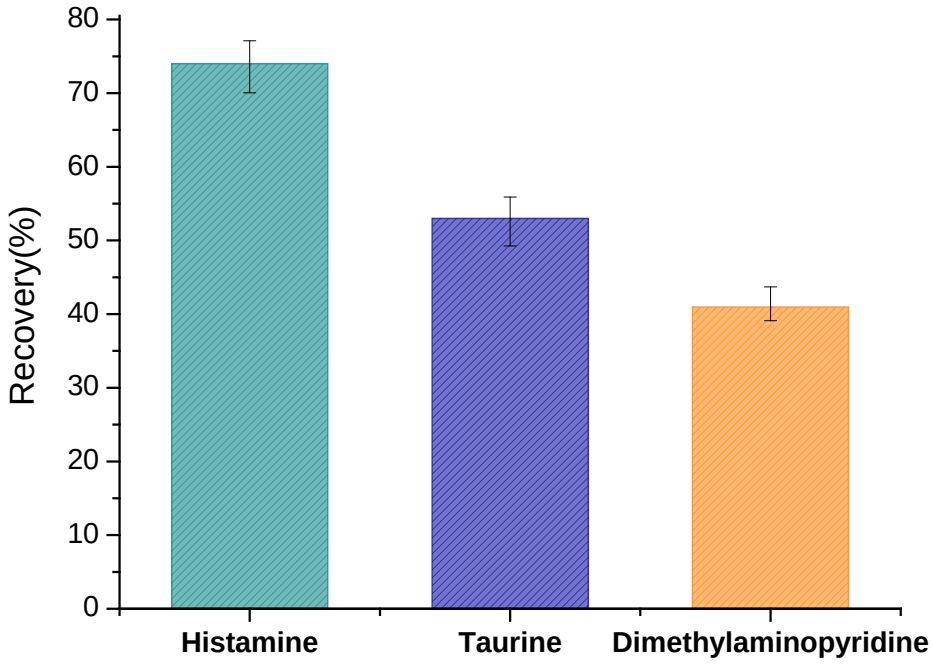
402 **FIGURES AND TABLE**



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404 **Figure 1.**

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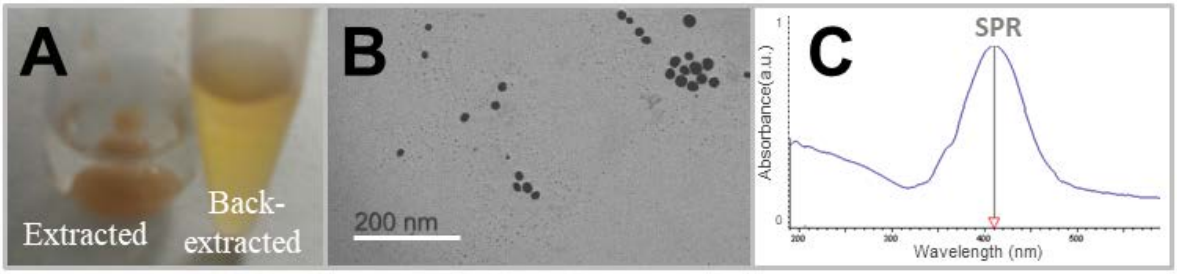

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407 **Figure 2.**

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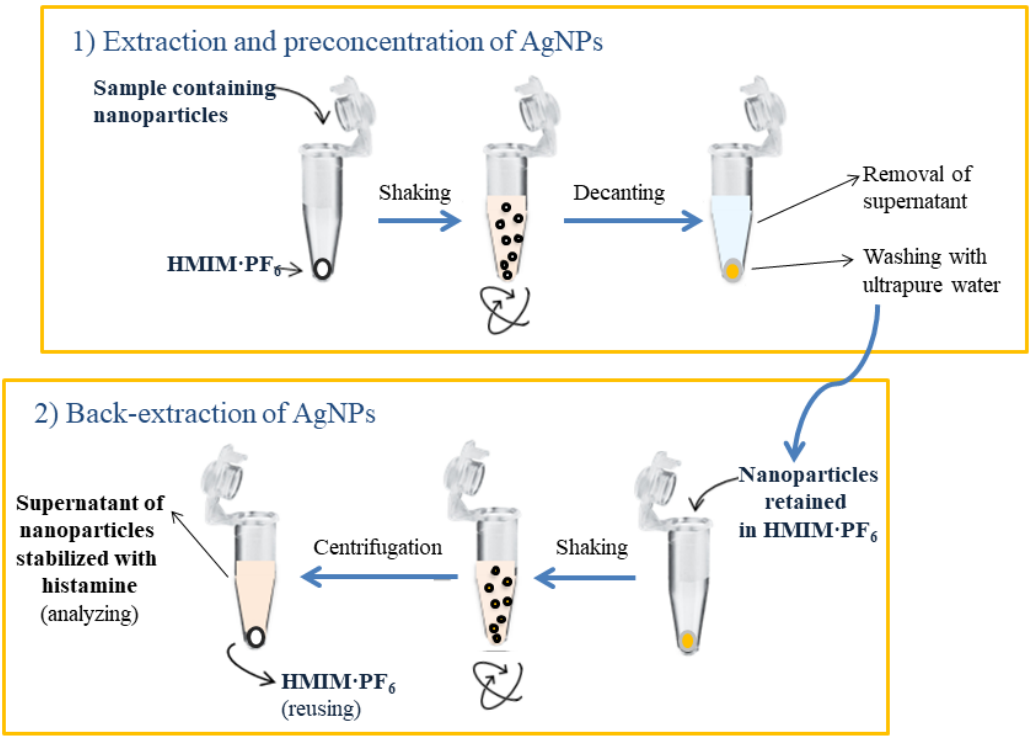

411

412 **Scheme 1.**

413

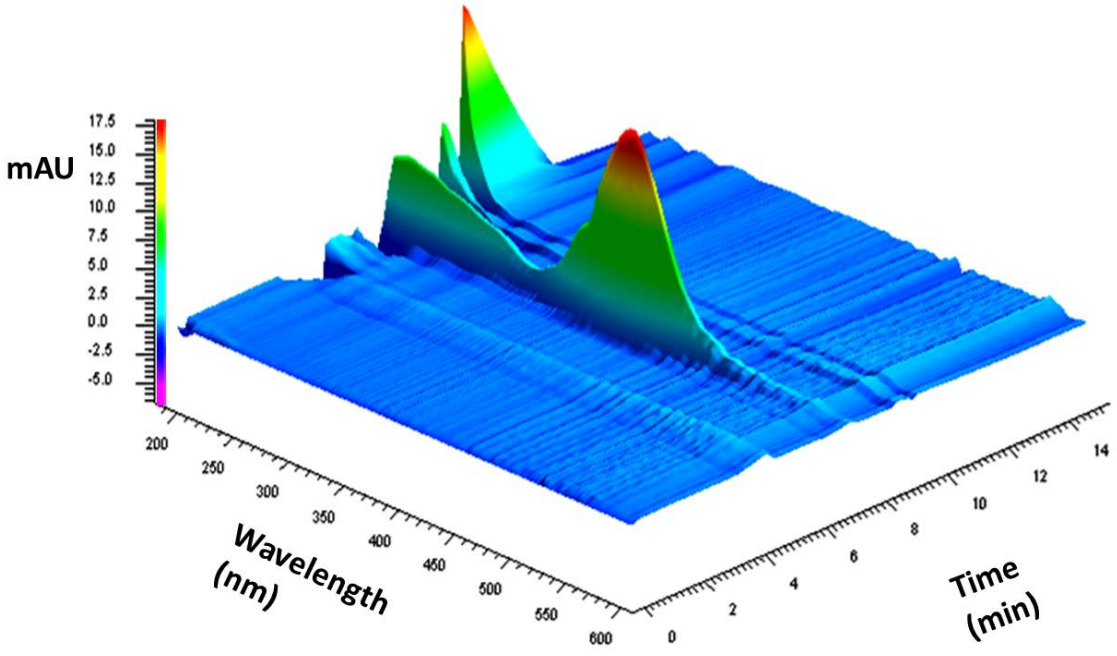
mAU
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
-2.5
-5.0
200
250
300
350
400
450
500
550
600
Wavelength (nm)
0
2
4
6
8
10
12
14
Time (min)

Figure 3.

420 **Table 1.**

421

422

Samples	**Concentration found (µg/mL)**	**Recovery (%)**	**RSD (%)**
Orange juice	3.28	82.0	3.33
	4.91	92.2	6.51
	7.02	90.3	5.92
	9.50	89.3	7.10
Face cream	3.03	75.7	5.53
	4.83	96.6	6.99
	7.22	73.2	10.2

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