OPTIMIZATION OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR POLYETHYLENE GLYCOL-COATED GOLD AND SILVER NANOPARTICLES

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Abstract

The use of polyethylene glycol modified nanoparticles is becoming an interesting topic since they present a very good stability in biological media. However, the effects of these nanoparticles on organisms are still unclear, so it is necessary to monitor their presence in bodily fluids, such as plasma or urine. Single-particle ICP-MS is a versatile tool to simultaneously detect and characterize nanoparticles in aqueous media, but a previous extraction step is necessary when analyzing complex samples due to the occurrence of matrix effects. In this work, an ultrasound assisted dispersive liquid-liquid microextraction method based on the use of chloroform as extracting solvent has been optimized for the extraction, characterization, and quantification of polyethylene glycol modified gold and silver nanoparticles in aqueous media. So far, we could achieve extraction efficiencies higher than 75% for both types of nanoparticles studied. We believe that with further optimization, the extraction can be made quantitative.

Introduction

In recent years, the synthesis of surface modified nanoparticles (NPs) has become a hot topic. [1-3] One of the most recent and interesting ones are the polyethylene glycol-modified gold and silver nanoparticles (PEG-AuNPs and PEG-AgNPs, respectively), since they are very stable in biological media and can be used in numerous ways [4]. Although these PEG-modified NPs have a good potential in medical, biochemical, and biological applications, their effects on organisms are still unclear [5]. For this reason, it is necessary to monitor these NPs in bodily fluids during application.

Single particle inductively coupled plasma mass spectrometry (spICP-MS) is one of the most flexible techniques for NPs determination, since it allows the simultaneous quantification (i.e., mass and number concentration) and characterization (i.e., size distribution, structure, and composition) of a large variety of NPs. However, these analyses can be affected by matrix effects when introducing complex samples [6].

A classical strategy used to overcome these matrix effects is liquid-liquid extraction. Up to date, and regarding microextractions of NPs, only the cloud-point extraction (CPE) has been studied and optimized [7]. Although CPE can provide quantitative recoveries, this approach is time-consuming, and the heating step can negatively affect the NPs structure and surface molecules.

As an alternative, dispersive liquid-liquid microextraction (DLLME) can be suggested to be used, as it is faster than CPE and requires no heating or aggressive steps that can compromise the integrity of NPs. However, no studies have been published yet with regards to this. The objective of this work was to develop and optimize DLLME for the extraction of PEG-AuNPs or PEG-AgNPs from aqueous samples and quantify and characterize the NPs present in the extracts by means of spICP-MS.

Experimental

Reagents and solutions

All solutions and suspensions were prepared in ultrapure water. A stock suspension of monodisperse PEG-modified AgNPs (nominal diameter 50 nm) was obtained from NanoComposix (San Diego, USA). Chloroform and methanol were obtained from VWR Chemicals (Radnos, USA). Trisodium citrate sesquihydrate was purchased from Alfa Aesar (Ward Hill, USA), and chloroauric acid and thiol-modified polyethylene glycol were obtained from Sigma-Aldrich (Budapest, Hungary). Gold-containing precious metals solution (10 $\mu g \cdot mL^{-1}$) and silver-containing multielemental solution (10 $\mu g \cdot mL^{-1}$) were purchased from Inorganic Ventures (Spetec, Germany).

Synthesis and characterization of PEG-AuNPs

Polyethylene glycol modified AuNPs were synthesized according to the procedure described in [8], used here with slight modifications. Briefly, 735 µL of 250 µM chloroauric acid was added into a flask, mixed with 97 mL of ultrapure water, and heated up to 80°C. Next, 2 mL of 230 mM trisodium citrate was dropped to the mixture under mild stirring to reduce gold and produce citrate-capped AuNPs. After refluxing for 15 min, the suspension was slowly cooled to room temperature. For the surface modification of these AuNPs, 6 mL of the NP suspension was mixed with 6 mL of a 1.5 µM thiol-modified PEG suspension under mild stirring. After 3h of reaction, the resulting suspension was washed to remove any unreacted reagents and was then kept at 4°C until use. PEG-AuNPs were characterized with an Ocean Optics Chem 2000-UV-Vis diode array absorption spectrometer, with a Philips CM-10 transmission electron microscopy (TEM) operating at 100 kV acceleration voltage, and via the dynamic light scattering method (DLS) on a Zetasizer Nano ZS Zen 4003 (Malvern Instrument, UK).

ICP-MS instrumentation and data evaluation

All spICP-MS measurements were performed employing a 7700x ICP-MS (Agilent, USA) instrument with the conventional sample introduction system. Isotopes monitored were $^{197}Au^{+}$ and $^{107}Ag^{+}$. The data acquisition was done in time-resolved analysis (TRA) mode. The measurement time was set to 120 s, with a dwell time (i.e., integration time) of 6 ms. To avoid occurrence of events associated to two or more nanoparticles reaching the detector at the same time, a sample flow rate of 600 $\mu L \cdot min^{-1}$ was chosen for all measurements. Transport efficiency was determined daily with the aid of the PEG-AgNPs via the particle frequency method [9] and found to be ≈ 2.5% for all nanoparticles under study. Microsoft Office Excel software was employed to integrate event signals manually. Separation of events from background signals was carried out by manually selecting background threshold. The number of particle events found can be related to the NP concentration using the following expression [10]:

$$C_{NP} = \frac{n_{NP} \cdot 60}{\eta_n \cdot Q_l \cdot t_{Scan}}$$

where C_{NP} = particle number concentration (mL^{-1}); n_{NP} = number of particles detected; η_n = nebulization efficiency; Q_l = sample uptake rate (mL min^{-1}); t_{Scan} = measuring time (s^{-1}). The intensity of each individual event can be related to a nanoparticle diameter using the

expression:

$$D_{NP} = 10^4 \cdot \sqrt[3]{\frac{6 \cdot I_{NP} \cdot t_{Dwell} \cdot Q_l \cdot \eta_n \cdot f_a}{\pi \cdot \rho_{NP} \cdot b_{Cal} \cdot 60}}$$

where I_{NP} = neat particle signal intensity (counts); t_{Dwell} = Dwell time (s); f_a = mass fraction of analyte in the NP; ρ_{NP} = nanoparticle density (g mL^{-1}); b_{Cal} = ICP-MS signal for a solution standard (counts L µg^{-1}).

Dispersive liquid-liquid microextraction

In this procedure, 4 mL of ultrapure water containing $7.0 \cdot 10^4$ mL^{-1} PEG-AgNPs or $2.5 \cdot 10^4$ mL^{-1} PEG-AuNPs were placed in glass test tubes. Two different extracting solvents have been tested, namely: *i)* chloroform; and *ii) n*-hexane. These two solvents have been selected based on their different polarity and similar volatility as well as good mixing with methanol. For the extracting solvent's volume we tested four different volumes: *i)* 250 µL; *ii)* 500 µL; *iii)* 750 µL; and *iv)* 1000 µL. In all cases, the extracting solvent was mixed with 1000 µL methanol and injected into the sample. Following this the test tube was immersed into an ultrasonic bath for 5 – 10 min. A cloudy dispersion was formed and, after 30 s of vortex stirring, the extractant droplet aggregated and was transferred into another glass test tube for complete solvent and extractant evaporation at room temperature in a fume hood. The solid residue was then re-suspended in 4 mL of ultrapure water with the aid of an ultrasonic bath and directly introduced into the ICP-MS for NPs quantification and characterization.

Results and discussion

Characterization of the synthesized PEG-AuNPs

For a first observation of the PEG-AuNPs, UV-Vis absorption spectroscopy measurements were performed. The absorbance maximum of the plasmon band was found at 520 nm for both the citrate-capped AuNPs and the PEG-AuNPs, indicating that no aggregation or NP degradation had occurred. The core shape and size of the PEG-AuNPs was checked by TEM. All NPs presented a spherical size - ideal for spICP-MS characterization - and a core diameter of 26 nm with a standard deviation of 6 nm. Additionally, a DLS characterization was also carried out. The results of this characterization showed that the hydrodynamic diameter of the PEG-AuNPs was 42 nm, while for the citrate-capped AuNPs it was 30 nm, thus the AuNPs were successfully pegylated.

Optimization of the extraction parameters

First of all, it was necessary to demonstrate that we could still detect and characterize the pegylated NPs after the extraction, evaporation, and reconstitution steps. To this end, a suspension containing PEG-AgNPs in a concentration of 80,000 mL^{-1} was extracted with 250 µL of chloroform, as described in the corresponding section of the experimental procedure. For achieving the best extraction efficiency for PEG-AgNPs and PEG-AuNPs, the main strategy consists of making the NPs to have more affinity for the extracting solvent that for the aqueous media. Since the polyethylene glycol molecules are very stable in a wide range of pH and ionic strength values (i.e., salt concentration), the main parameters affecting the extraction of these nanoparticles are: *i)* type of extracting solvent, since it should have good affinity to polyethylene glycol; *ii)* extracting solvent volume, as it has to be enough to extract the NPs present in the suspension; and *iii)* the use or absence of sonication since it may help in dispersing the extracting agent thereby increasing the specific surface area of the droplets. In order to optimize these parameters, a series of experiments were carried out. Figure 1 shows the NPs

recovery for PEG-AuNPs and PEG-AgNPs by using different volumes of chloroform and hexane.

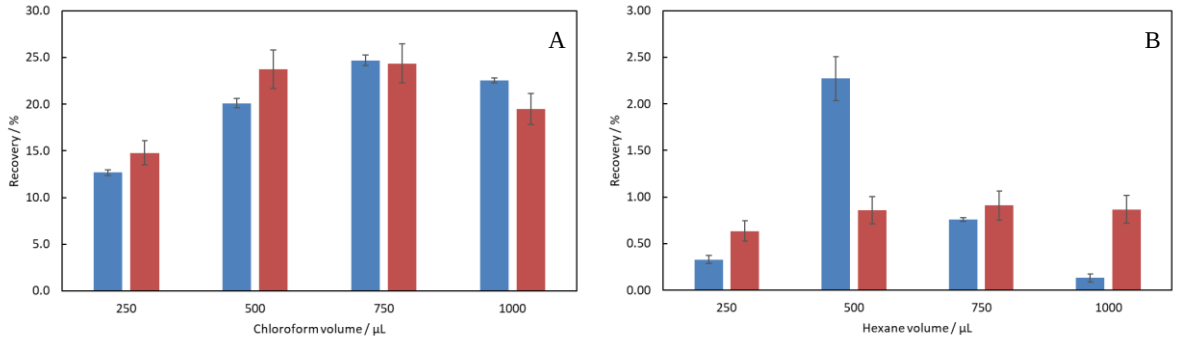

Figure 1. Recovery values for the extraction of (■) PEG-AgNPs and (■) PEG-AuNPs by using different volumes of (A) chloroform and (B) hexane, without sonication. Error bars represent standard deviations based on three replicate measurements.

As can be observed in Figure 1, chloroform provides a NPs extraction recovery of up to 25%, with a maximum efficiency when using 750 µL of extractant. It is interesting to remark that there is a slight decrease of the recovery when using 1000 µL of chloroform, probably due to the difficulty in dispersing such volume of dispersing solvent. With hexane, extraction efficiencies are far from chloroform values, with a maximum of 2.5%. This can be explained by considering that hexane is a purely apolar solvent and PEG is a hydrophilic polymer. In all cases, the mean diameter calculated for all detected nanoparticles is the same that the one obtained for the suspension before the DLLME step, so no dissolution or aggregation occurred during the extraction process. The extraction efficiences were generally quite similar for Au and Ag NPs, in accordance with the fact that their coating should determine their affinity towards the extraction solvent. However, in two hexane cases, the recovery was interestingly significantly different for Au and Ag NPs. It has to be added though that hexane recoveries were so low that these differences in recovery values can be negligible (e.g. ca. 1% as opposed to 2%).

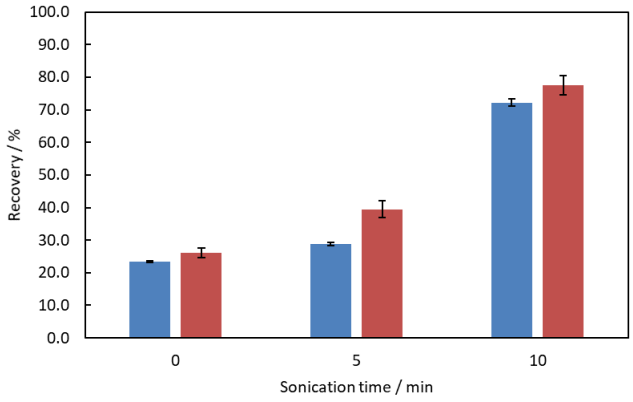

Figure 2. Recovery values for the extraction of (■) PEG-AgNPs and (■) PEG-AuNPs by using sonication. Error bars represent standard deviations based on three replicate measurements.

In order to improve the extraction efficiency of the DLLME using 750 µL of chloroform as extracting solvent, a sonication step was performed after the extractant injection into the sample. The effect of three short sonication times were evaluated: *i)* 0 min, *ii)* 5 min and *iii)* 10

min. Figure 2. shows the recoveries obtained for PEG-AgNPs and PEG-AuNPs operating this way.

As can be observed, NP extraction recoveries rose to 75% for both NPs under study when sonicationwith 10 min duration was employed, thus indicating that ultrasound helps in forming the chloroform suspension. It is important to remark that no NPs aggregation or dissolution was found as a consequence of the sonication step. However, further efforts need to be made in order to improve extraction efficiencies up to quantitative values (i.e., higher than 85% recovery). For this purpose, higher sonication times (e.g., 15 min) or frequency, or alternative extracting solvents with more polarity than chloroform (e.g., dichloromethane, tetrahydrofuran-decanoic acid micelles, ionic liquids, etc.) could be tested.

Acknowledgements

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