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Detection of silver nanoparticles in seawater at ppb levels using UV-Visible spectrophotometry with long path cells

Pablo Lodeiro^{a,1*}, Eric P. Achterberg^{a,1}, Mohammad S. El-Shahawi^{b,2}

^a*Ocean and Earth Science, University of Southampton, National Oceanography Centre, European Way, SO14 3ZH Southampton, UK.*

^b*Department of Chemistry, Faculty of Science, Damietta University, Damietta, Egypt.*

**Corresponding Author: plodeiro@geomar.de*

Abstract

Silver nanoparticles (AgNPs) are emerging contaminants that are difficult to detect in natural waters. UV-Visible spectrophotometry is a simple technique that allows detection of AgNPs through analysis of their characteristic surface plasmon resonance band. The detection limit for nanoparticles using up to 10 cm path length cuvettes with UV-Visible spectrophotometry is in the 0.1 to 10 ppm range. This detection limit is insufficiently low to observe AgNPs in natural environments. Here we show how the use of capillary cells with an optical path length up to 200 cm, forms an excellent technique for rapid detection and quantification of non-aggregated AgNPs at ppb concentrations in complex natural matrices such as seawater.

¹ Present Addresses: GEOMAR, Helmholtz Centre for Ocean Research Kiel, Wischhofstraße 1-3, 24148 Kiel, Germany.

² Present Addresses: Department of Chemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Kingdom of Saudi Arabia (on sabbatical leave)

Keywords: Silver nanoparticles; seawater; NM300K; surface plasmon resonance band; UV-Visible spectrophotometry; long path cell.

Introduction

The increasing use of silver nanoparticles (AgNPs) in consumer products is raising concerns about their behaviour, fate and toxicological effects following discharge into natural waters [1]. The transformations of AgNPs through aggregation and oxidation processes, and their interactions in the environment, make their detection and quantification challenging.

Furthermore, their low environmental concentrations, typically in the ppt range, forms a major analytical detection problem for the most commonly used analytical techniques, which include Light Scattering and UV-Visible spectrophotometry.

Based on the projected increase in AgNP production and release, the environmental concentrations of AgNPs in river waters by the year 2020 is projected to range between 225 and 1799 ppt total Ag [2]. Currently, the use of mass spectrometric techniques (e.g. ICP-MS, single particle ICP-MS) together with hyphenated systems such as asymmetric flow field-flow fractionation, capillary electrophoresis or liquid chromatography, allows for AgNP quantification in environmental samples at ppt-ppb levels [3-5]. The equipment is however complex and expensive with high running costs, and hence not available in most laboratories. Moreover, the sample analysis is destructive, requires multiple steps, long sample preparation times and well-trained operators.

UV-Visible spectrophotometry is a readily accessible technique that has been widely used to identify, characterize and study the stability of metallic NPs at elemental concentrations in

the 0.1 to 10 ppm range [6, 7]. The surface plasmon resonance band (SPRB) of Ag, Au and Cu NPs is used in these studies, being the result of collective oscillations of the conduction electrons in resonance with the incident light frequency. Therefore, changes to the surface plasmon properties have been extensively utilised to determine NP aggregation processes in different media due to the high sensitivity of SPRB to NP size, shape and chemical composition [8-10].

UV-Visible spectrophotometry allows rapid and high-resolution detection (full spectra within <1s using array-based spectrometers), on-site measurements, and is applicable to complex matrices such as seawater. However, applications have been hampered due to the relatively high detection limit (sub-ppm) for NPs, with the use of 1 or 10 cm path length cuvettes. The absorbance of the analyte (A) is proportional to the optical path length (l), so a decrease in the detection limit is obtained with increasing the cuvette length. In the present work, we report the use of long path cells (100 to 200 cm) in UV-Visible spectrophotometry to enhance the precision and accuracy, and decrease the detection limit for metallic NPs by at least one order of magnitude. Knowledge of the molar attenuation coefficient (ϵ) of the NP under study in the sample matrix is required to obtain NP concentrations from absorbance measurements [11]. Limited information is available on this parameter for specific NPs, so we have determined it for a reference AgNP material NM300K using a calibration in high-purity water. The obtained molar attenuation coefficient was later used to quantify the presence of non-aggregated AgNPs, previously spiked in two different natural seawaters.

Materials and methods

A silver reference nanomaterial (NM300K) from the Fraunhofer Institute for Molecular Biology and Applied Ecology was used. The reference material consists of colloidal silver spherical nanoparticles with a particle size ~ 15 nm (90% < 20 nm). The stabilizing capping

agent is an aqueous mixture comprising 4% (w/w) polyoxyethylene glycerol trioleate and 4% (w/w) polyoxyethylene sorbitan monolaurate (Tween-20).

The laboratory chemicals used were purchased from Sigma-Aldrich (Dorset, UK) and Fisher Scientific (Leicestershire, UK). We made use of high-purity water (MilliQ, Millipore, Watford, UK) with a resistivity of $>18.2 \text{ M}\Omega \text{ cm}^{-1}$. The natural surface water samples were collected in a Baltic fjord located in northwest Germany ($54^{\circ}22.1' \text{ N } 10^{\circ}11.7' \text{ E}$) and A Coruña Bay ($43^{\circ}21.8' \text{ N } 8^{\circ}23.4' \text{ W}$) in northwest Spain. The samples were stored in acid-cleaned low-density polyethylene bottles (Nalgene) until use.

The UV-Visible spectra were recorded using a deuterium-halogen light source (DH2000, Ocean Optics), two liquid waveguide capillary cells of 100 and 200 cm path length (LWCC, World Precision Instruments) and a miniature CCD array spectrophotometer (USB-4000, Ocean Optics), connected through two optical fibres (600 μm fibre, P600-025-SR). Using this set-up and an in-house Matlab script we obtained a complete scan from 300 to 650 nm in <1 s. Solutions without AgNPs were used to blank correct the instrument. The solutions were pumped through the capillary cell using a peristaltic pump (Watson-Marlow 400B series) in a recirculation mode at constant flow rate (1.5 mL min^{-1}). The observed absorbance includes surface plasmon resonance band (SPRB) effects and also scattering by disperse NPs, especially at large sizes.

The total Ag content was measured by ICP-MS (Quadrupole Thermo X-Series 2) after dilution and digestion of the samples with 0.3 M HNO_3 (70% Optima, Fisher Scientific). The total Ag concentration measured in the NM300K AgNP solution was 102 ± 1 ppm. This solution was tenfold diluted to obtain our stock AgNP NM300K solution.

The NM300K AgNP solutions (20 to 92 ppb) were prepared for the calibrations by addition of increasing amounts of the stock solution (30, 60, 85, 115 and 135 μL) to high-purity water (15 mL) at $\text{pH } 8.0 \pm 0.2$ fixed using NaOH.

The obtained data was analysed in terms of SPRB evolution (maximum height, area, width at half peak height and position of the absorbance maximum) over time. The SPRB values were determined for at least two independent experiments, as an average of NM300K SPRB evolution with time, after 10 minutes of mixing. The uncertainties in the SPRB measurements were obtained as standard deviation of the different SPRB height values obtained.

Results and discussion

The measurement of the AgNP concentration in solution using UV-Visible spectrophotometry requires the determination of its attenuation coefficient. The AgNP NM300K attenuation coefficient was obtained through calibration using the Lambert-Beer equation ($A = \epsilon \cdot l \cdot c$). The AgNPs NM300K form a monodisperse suspension in high-purity water. Therefore, we consider that the absorbance is proportional to the height of the SPRB. Figure 1 shows the plot of the height of the SPRB vs AgNP concentration (c). The calibration showed a linear relationship with good correlation coefficients.

The molar attenuation coefficient was determined from the slope of the calibration graph using a 100 cm optical path length cell. High quality molar attenuation coefficients determination requires a stable and low noise signal. We obtained an improved signal using a 100 cm cell because the optical transmission is between 36-40% higher in the 325-500 nm range compared with a 200 cm length cell. The obtained value was $\epsilon = 1.14 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$. Based on theoretical Mie calculations, the molar attenuation coefficient for idealised AgNPs should be $2.8 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$ [12]. This value is more than twofold our experimental value, but

it is in close agreement with an experimentally obtained ϵ value for a 15 nm citrate-capped AgNP (between $1.58\text{-}2.27 \times 10^9 \text{ M}^{-1}\text{cm}^{-1}$) [11]. The observed minor discrepancies are explained by the differences in AgNP coatings that result in modifications of their optical properties [13].

Variations in SPRB height during the calibration experiment were investigated. The height of the SPRB obtained in stable aqueous solutions of AgNPs slowly diminished over time with a decrease in the total Ag concentration. At the lowest Ag concentration (20 ppb), the decrease in SPRB height was ca. 8% over a period between 10 and 90 min. This observation was attributed to slow dissolution kinetics of the AgNPs to Ag ions that occur at very low NP concentrations due to light absorption and a temperature increase inside the capillary cell produced by the light beam. Nevertheless, at Ag concentrations higher than 41 ppb, variations of the SPRB height over 10 to 90 min were only between 0.5-1%. Moreover, the position of the SPRB during the calibration measurements in high-purity water was stable at 413-416 nm, with a width at half height around 72-75 nm (76-80 nm for the highest tested concentration of 92 ppb).

The main aim of our work was to quantify the change in NM300K AgNP concentrations during their aggregation/dissolution in seawaters with different dissolved organic carbon (DOC) contents and salinities. To achieve that objective we used the molar attenuation coefficient obtained from the calibration. This allowed us for rapid detection and quantification of non-aggregated AgNPs in different complex matrices. A 200 cm instead of the 100 cm length cell was used when measuring the AgNP concentration in seawater with UV-Visible spectrophotometry. The 100 cm cell provided a lower noise in the measured signal, but doubling the path length a twofold lower NP detection limit was obtained. We confirmed this approach through analyses of the peak height of the SPRB obtained upon addition of stock solution with a final AgNP concentration of 20 ppb. Therefore, we

calculated the AgNP concentration in high-purity water when using 200 cm cells using the obtained ϵ value. Table 1 shows that the concentration value was in good agreement with the one calculated from the calibration.

The SPRB evolution over time following NM300K AgNP additions (initial concentration 20 ppb) was monitored here for the first time by UV-Visible spectrophotometry using the 200 cm capillary cell. Figure 2 shows the decrease in non-aggregated AgNP concentration observed in the seawater samples.

The Baltic fjord water had an enhanced DOC concentration of $277 \pm 2.20 \mu\text{M}$ and a brackish salinity of 17.19. The Spanish coastal seawater sample contained lower DOC ($83.67 \pm 0.75 \mu\text{M}$) and a higher salinity of 34.65. Interactions between organic compounds and the AgNP surface and its coating are likely to occur, producing small changes observed in the SPRB compared to high-purity water (Figure 2a,b).

Figure 2c shows a faster aggregation/oxidation kinetic (greater slope) of the low organic matter and high salinity Spanish coastal sample compared to the Baltic fjord seawater.

Terrestrial fulvic/humic-like compounds found in the natural fjord waters, together with its low salinity, provide enhanced stabilization against aggregation (a detailed analysis is shown in the supplementary material). The SPRB shape and position values were comparable to the ones observed in the calibration. The SPRB showed only small changes during the experiments in seawater (Figure 2). The SPRB position and width at half height were ca. 415-421 nm and 65-70 nm (Baltic fjord), and 406-410 nm and 70-80 nm (Spanish coastal water). The SPRB changes observed between the different seawaters can be explained by differences in organic matter content. The changes in the refractive index of our solution media are expected to produce no significant variations ($<1.4 \text{ nm}$) in the SPRB position (see supplementary material for details) [14].

The molar attenuation coefficient value used in the calculations can be influenced by the sample matrix, and size and coating of the NPs. The experiments were conducted in seawater, but we consider that the calculated molar attenuation coefficient is similar for the various matrices used, since no significant changes occurred in the SPRB position and shape. The calculated AgNP concentration values in the Baltic fjord seawater were in good agreement with the initial AgNP concentration (20.4 ppb) used in the high-purity water experiments (Table 1). We obtained that concentration value before the start of the aggregation/oxidation process (<10 min) from SPRB height measurements. Nevertheless, the SPRB height detected at identical time in Spanish coastal seawaters was only 16.5 ppb (Table 1). This mismatch was observed only when the NP aggregation/oxidation occurred in the first seconds/minutes, and was likely due to fast reduction in SRPB as the quantity of non-aggregated AgNPs decreased. As NPs aggregate and/or oxidize, the SPRB height will decrease due to a reduction in the number of non-aggregated NPs. If the AgNPs interact to form assemblies of different sizes, new resonance modes will appear [15]. Silver NP aggregates may contribute to the SPRB signal yielding absorption at wavelengths higher than 500 nm. We separated this possible contribution from the signal of the non-aggregated NPs by calculation of the height of the SPRB from a straight line traced from the base of the SPRB between 325 and 500 nm [9].

The lowest SPRB height that was quantified (0.12 ± 0.02 A.U.) corresponded to an AgNP concentration of 4 ppb in Spanish coastal seawater. Therefore, we showed here that a simple and fast spectroscopy technique allows detection of non-aggregated AgNPs in seawaters at low ppb levels, close to the projected future environmental concentrations.

In order to contribute to future improvements in the methodology presented here, the use of complementary techniques, such as single particle ICP-MS, to verify the silver concentration present in NPs and ionic forms in solution, will be important. This approach will allow us to

verify that the Ag present in solution during the calibration is mostly in NP form and therefore contributes entirely to the SPRB.

Conclusions

This study demonstrates for the first time the quantification of AgNPs in environmental samples at low ppb levels by UV-Visible spectrophotometry using capillary cells with an optical path length up to 200 cm. The heights of the SPRB have been used to obtain a linear calibration with an AgNP concentration range between 20-92 ppb. The molar attenuation coefficient obtained from the calibration has been successfully used to calculate the AgNP concentrations in natural seawater samples at low ppb levels.

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Supplementary material. Details of experimental procedures and supplementary results.

References

- [1] A. Bour, F. Mouchet, J. Silvestre, L. Gauthier, E. Pinelli, Environmentally relevant approaches to assess nanoparticles ecotoxicity: A review, *J Hazard Mater* 283(0) (2015) 764-777.
- [2] A. Massarsky, V.L. Trudeau, T.W. Moon, Predicting the environmental impact of nanosilver, *Environ Toxicol Pharmacol* 38(3) (2014) 861-873.
- [3] F. Laborda, E. Bolea, G. Cepriá, M.T. Gómez, M.S. Jiménez, J. Pérez-Arantegui, J.R. Castillo, Detection, characterization and quantification of inorganic engineered nanomaterials: A review of techniques and methodological approaches for the analysis of complex samples, *Anal Chim Acta* 904 (2016) 10-32.
- [4] F. Laborda, J. Jimenez-Lamana, E. Bolea, J.R. Castillo, Selective identification, characterization and determination of dissolved silver(I) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry, *J Anal Atom Spectrom* 26(7) (2011) 1362-1371.
- [5] S.M. Majedi, H.K. Lee, Recent advances in the separation and quantification of metallic nanoparticles and ions in the environment, *Trac Trend Anal Chem* 75 (2016) 183-196.
- [6] O. Geiss, C. Cascio, D. Gilliland, F. Franchini, J. Barrero-Moreno, Size and mass determination of silver nanoparticles in an aqueous matrix using asymmetric flow field flow fractionation coupled to inductively coupled plasma mass spectrometer and ultraviolet-visible detectors, *J Chromatogr A* 1321(0) (2013) 100-108.

- [7] F. Piccapietra, L. Sigg, R. Behra, Colloidal Stability of Carbonate-Coated Silver Nanoparticles in Synthetic and Natural Freshwater, *Environ Sci Technol* 46(2) (2012) 818-825.
- [8] M. Baalousha, Y. Nur, I. Römer, M. Tejamaya, J.R. Lead, Effect of monovalent and divalent cations, anions and fulvic acid on aggregation of citrate-coated silver nanoparticles, *Sci Total Environ* 454–455(0) (2013) 119-131.
- [9] P. Lodeiro, E.P. Achterberg, J. Pampín, A. Affatati, M.S. El-Shahawi, Silver nanoparticles coated with natural polysaccharides as models to study AgNP aggregation kinetics using UV-Visible spectrophotometry upon discharge in complex environments, *Sci Total Environ* 539 (2016) 7-16.
- [10] L. Zhang, X. Li, R. He, L. Wu, L. Zhang, J. Zeng, Chloride-induced shape transformation of silver nanoparticles in a water environment, *Environ Pollut* 204 (2015) 145-151.
- [11] D. Paramelle, A. Sadovoy, S. Gorelik, P. Free, J. Hopley, D.G. Fernig, A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra, *Analyst* 139(19) (2014) 4855-4861.
- [12] J.R.G. Navarro, M.H.V. Werts, Resonant light scattering spectroscopy of gold, silver and gold-silver alloy nanoparticles and optical detection in microfluidic channels, *Analyst* 138(2) (2013) 583-592.
- [13] M.E. Stewart, C.R. Anderton, L.B. Thompson, J. Maria, S.K. Gray, J.A. Rogers, R.G. Nuzzo, Nanostructured plasmonic sensors, *Chem Rev* 108(2) (2008) 494-521.

[14] A.D. McFarland, R.P. Van Duyne, Single Silver Nanoparticles as Real-Time Optical Sensors with Zeptomole Sensitivity, *Nano Lett* 3(8) (2003) 1057-1062.

[15] D.D. Evanoff, G. Chumanov, Synthesis and Optical Properties of Silver Nanoparticles and Arrays, *ChemPhysChem* 6(7) (2005) 1221-1231.

Figure 1. Calibration for NM300K in aqueous solution at fixed pH 8.0 ± 0.2 . The height of the SPRB was determined from UV-Visible spectra obtained using a capillary cell with an optical path length of 100 cm.

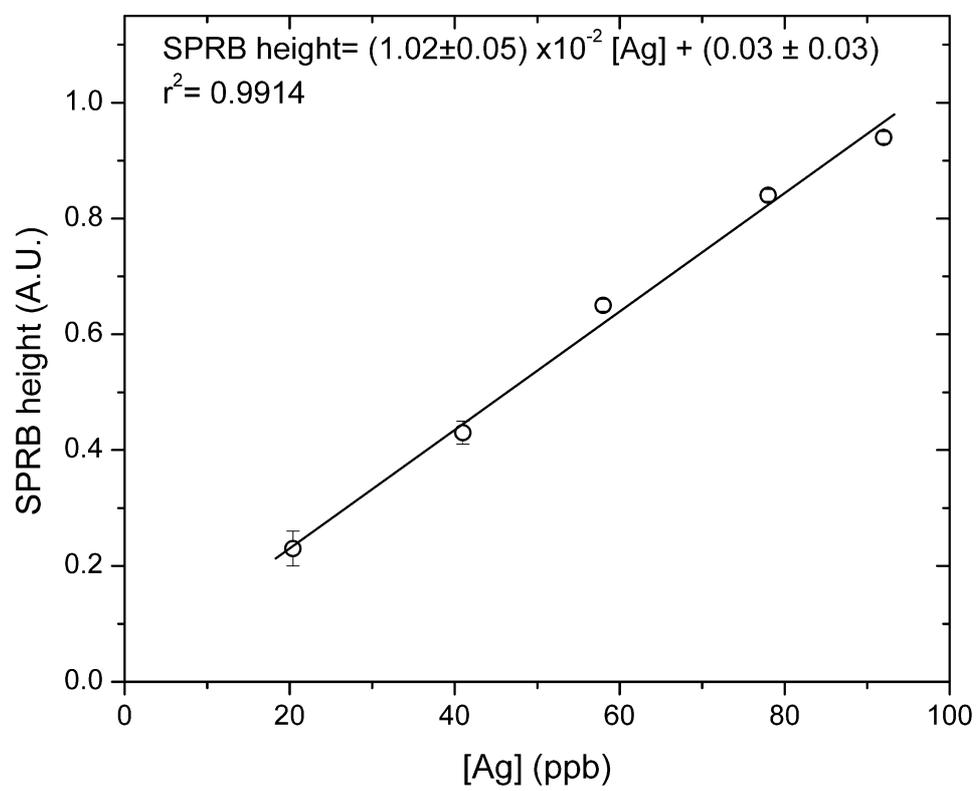
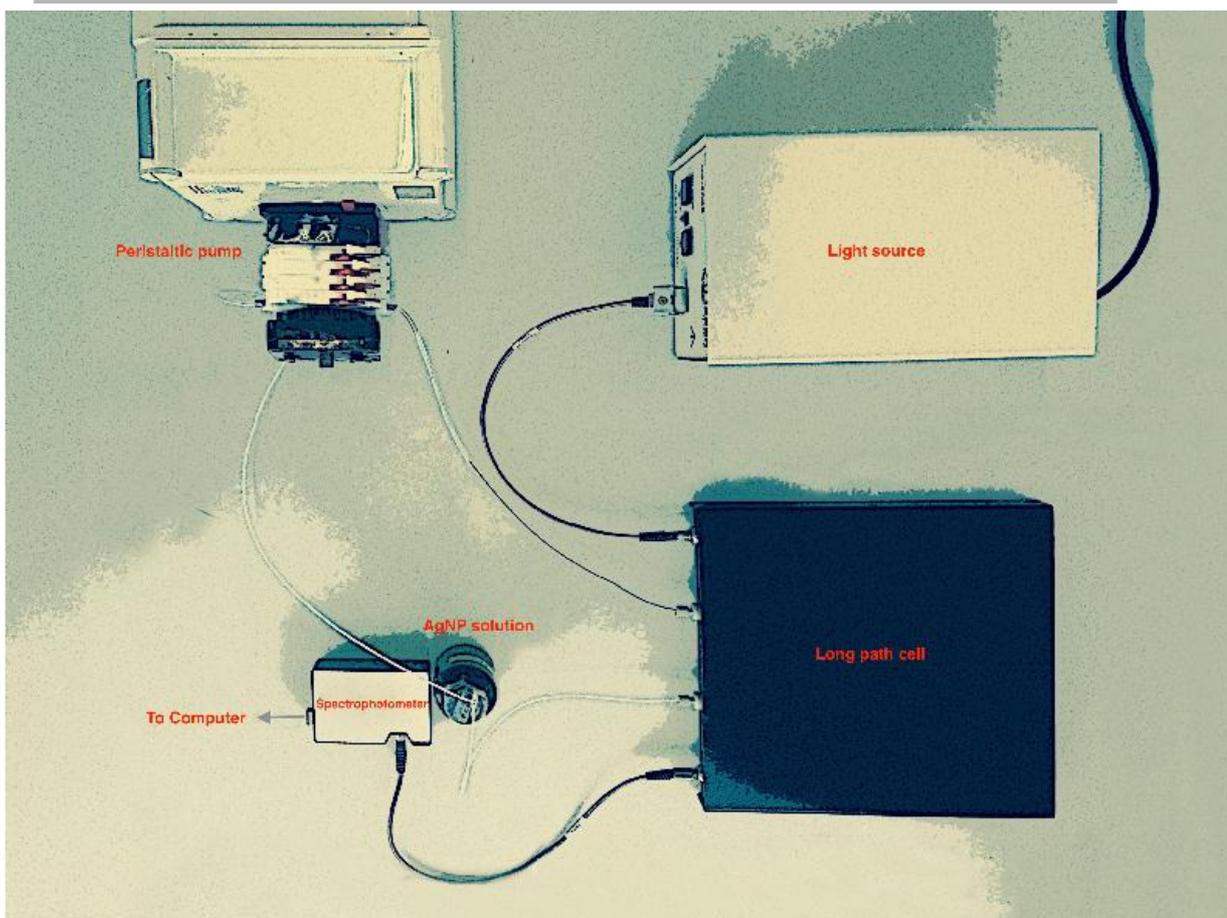
Figure 2. a) Normalized spectra (Abs/Abs_0 , Abs_0 = Absorbance at $t= 12$ min) for NM300K recorded at different times (black= 12, red= 22, green= 44, blue= 92 and grey= 197 min) in Baltic fjord seawater. The magenta curve represents the normalized spectra in high-purity water after 10 min. b) Normalized spectra (Abs/Abs_0 , Abs_0 = Absorbance at $t= 11$ min) for NM300K recorded at different times (black= 11, red= 22, green= 44, blue= 56 and grey= 71 min) in Spanish coastal seawater. The magenta curve represents the normalized spectra in high-purity water after 10 min. c) Normalized SPRB height ($SPRB \text{ height} / SPRB \text{ height}_{t_{max}}$) evolution over time for NM300K AgNPs in Baltic fjord (black line) and Spanish (red line) seawaters. Spectra were obtained using capillary cells with an optical path length of 200 cm.

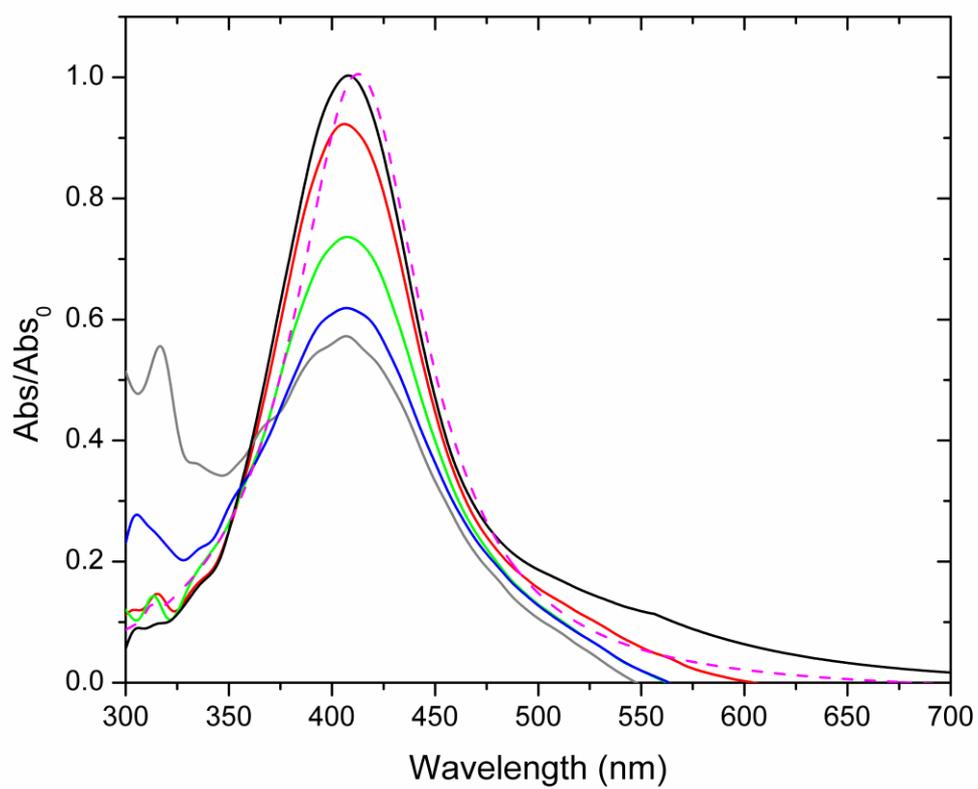
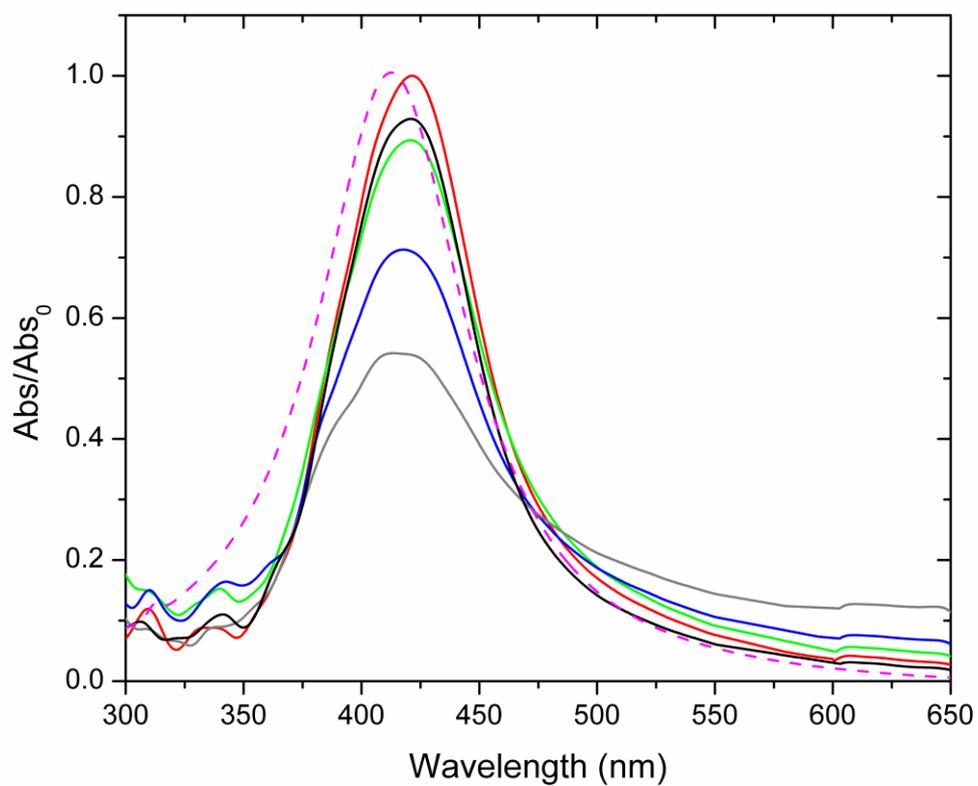
Table 1. Initial height of the SPRB and calculated concentrations ($c = \text{height SPRB} / \epsilon \cdot l$) for AgNP NM300K in high-purity water and seawaters obtained using different lengths of capillary cells. Initial AgNPs concentration was 20.4 ± 0.9 ppb.

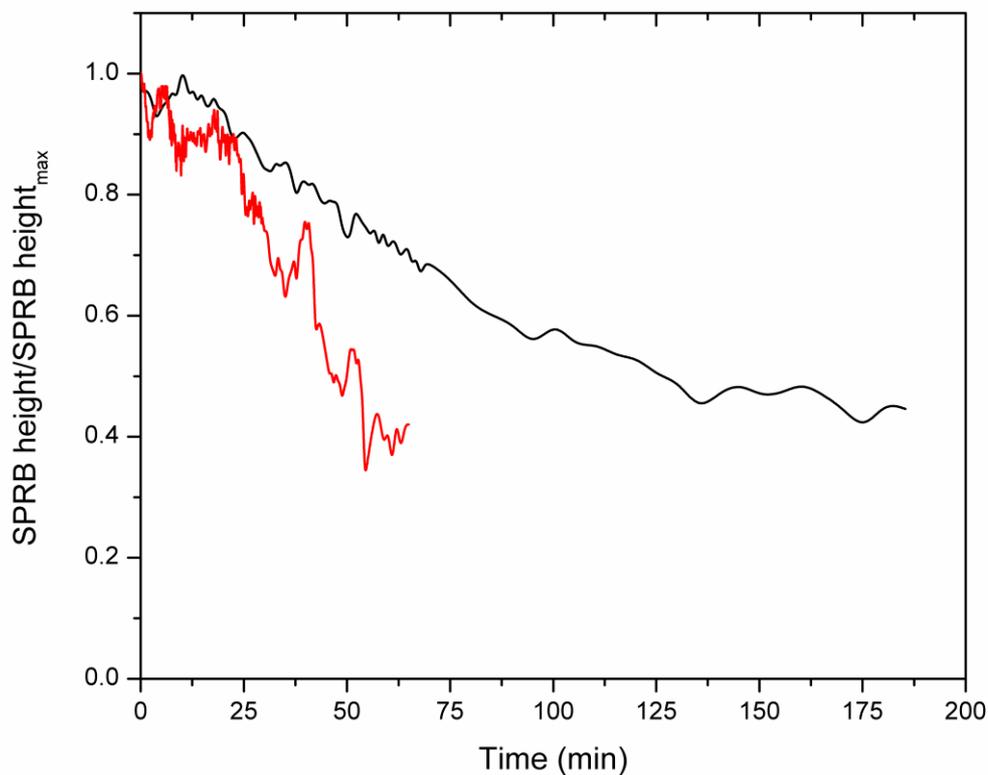
	High-purity water 100 cm	High-purity water 200 cm	Baltic fjord water 200 cm	Spanish coastal water 200 cm
SPRB height	0.23 ± 0.02	0.50 ± 0.02	0.51 ± 0.02	0.37 ± 0.01
[Ag] ppb	20 ± 2	23 ± 2	24 ± 2	16.5 ± 0.9

GRAPHICAL ABSTRACT

Accepted manuscript







Highlights

- Detection and quantification of AgNP at ppb levels in natural seawater samples
- The use of long path cells (up to 200 cm) in UV-Visible spectrophotometry is proposed
- Knowledge of the molar attenuation coefficient of the NP under study in the sample matrix is required