Exposure of the bivalve *RUDITAPES PHILIPPINARUM* to gold nanoparticles: Location study by electron microscopy

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Abstract

Gold nanoparticles (AuNPs) are introduced into a growing number of commercial products, eventually leading to their release to the different environmental compartments. Ecotoxicological risks in non target organisms associated with NPs are showing increasing consideration in the literature [1-3]. Thus in this study, the marine bivalve *Ruditapes philippinarum* was chosen as ecotoxicological model considering also its important filtering activity for nutritional and respiratory purposes. In this paper electron microscopy studies, both in transmission (TEM) and scanning (SEM) modes, will be presented to figure out first, the evolution of Au-citrate NPs in sea water media; and secondly, the location of the NPs in the tissues.

In the first part of this work investigations are presented regarding the final presentation of the nanoparticles in the marine media after delivery. The degree of aggregation and/or coalescence has been investigated by TEM for citrate stabilized gold NPs colloidal solutions, in a comparative study between milli-Q and simulated marine water. In ecotoxicological relevant concentrations (few to tens of ppb), the expected behavior of salt-induced aggregation of these dipole stabilized NPs was not found. Figure 1 shows how at the ppb level the degree of aggregation is very similar at fresh or sea water. Not remarkable coalescence of particles was found. This indicates that ecotoxicological impact may be considered at the coastal ecosystems even for the so called “non- resistant to salt-induced aggregation” NPs as in the case of the citrate stabilized gold nanoparticles.

In the second part of this work the organisms were exposed in the laboratory to gold added to natural filtered seawater either in the form of citrate reduced AuNPs (approximately 6 and 30 $\mu g \cdot L^{-1}$) in the range of 20 – 30 nm or as soluble gold, $H(AuCl_4)$ (50 $\mu g \cdot L^{-1}$) for 28 days. Samples (digestive gland and gills) were taken throughout the exposure period in order to monitor the location and evolution of gold. New results are presented in this paper in relation to Au location and distribution at the clam tissues by SEM and TEM coupled to microanalysis (EDX). Dissection, fixation (including osmium tetroxyde solution), drying, embedding in resine, staining (uranil salts) and ultramicrotom cutting were used for preparation of tissue slices. It is worth of mention here that in addition to TEM analysis with thin specimens (ca. 80 nm slices), the SEM analysis in a FEG microscope with a transmission mode detector allowed to work with thicker samples (300 nm slices) at 30 kV. For big particles (diameter >20 nm) the development of very thin slices can lead to the displacement of the NPs to the edges of the specimen by the cutting tool. In these cases better results can be obtained from thicker slides investigated at SEM-FEG. In both cases EDX is mandatory to unequivocally ascribe electron dense features to Au NPs as Os and U features (from the preparation procedure) could in some case be misinterpreted as NPs. Figures 2 and 3 show respectively TEM and SEM-FEG images from the digestive tissues as well as the corresponding EDX microanalysis. Our results show that accumulation of the NPs is much more important at the digestive gland while chemical analysis indicates a major concentration of dissolved gold in gills. Fig. 2 shows the presence of Au NPs in a heterolisosome from the digestive gland tissue. Also dissolved gold showed in this work to produce a stronger toxicological response as compared to Au NPs for similar levels of gold concentration.

References

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Figures

Fig.1.- TEM micrographs for Au NPs at 60 ppb: (A) in milli-Q water; (B) in simulated marine water

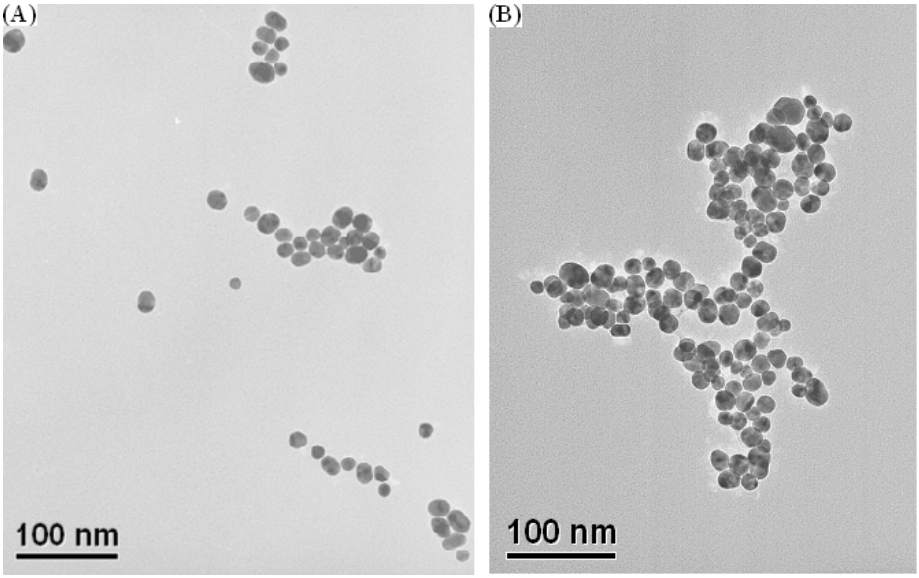

Fig.2.- Left: TEM image of a 80 nm slice of digestive gland tissue. Right: EDX spectrum from the area containing the Au NPs.

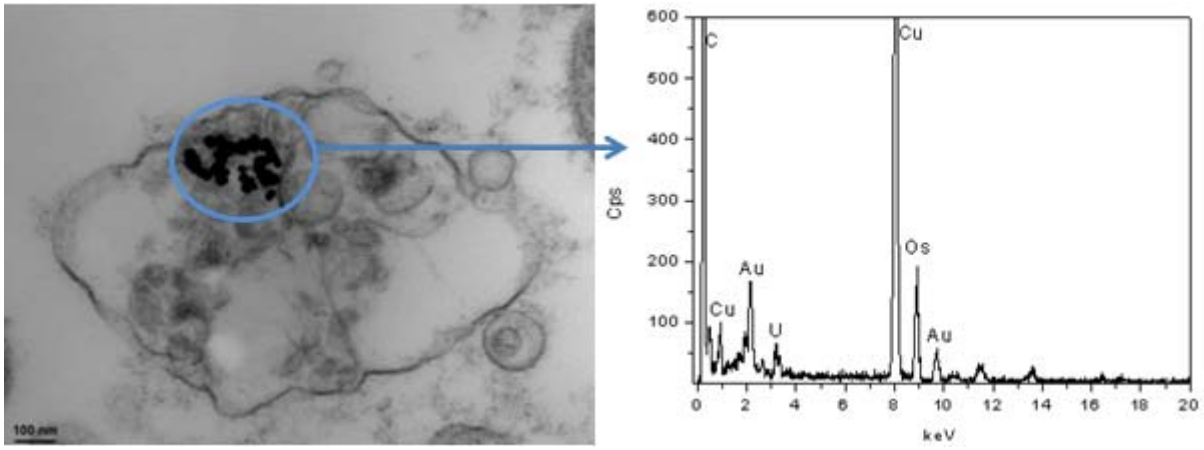

Fig.3.- Left: SEM-FEG image (transmission mode) of a 300 nm slice of digestive gland tissue. Right: EDX spectrum from the area containing the Au NPs.