In vitro human digestion test to monitor the dissolution of silver nanoparticles

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Abstract. Nanotechnology is a scientific revolution that the food industry has experienced over the last years. Widely employed as food additives and/or food contact materials in consumer products, silver nanoparticles are an example of this innovation. However, their increasing use makes also likely the human ingestion, thus requiring a proper risk analysis. In this framework, a comprehensive characterization of biotransformation of silver nanoparticles in biological fluids is fundamental for the regulatory needs. Herein, we aimed at studying the dissolution behaviour of silver nanoparticles using an \textit{in vitro} test, which simulates the human oral ingestion of NPs during their passage through the gastrointestinal tract. The nanoparticle suspensions were characterized in the different digestion phases using several techniques to follow the changes of key physical properties (e.g., size, surface charge and plasmon peak) and to quantify the biotransformed products arisen by the process, as for example free silver ions.

1. Introduction
Due to their proved antimicrobial activity and to their enhanced spectroscopic properties, silver nanoparticles (AgNPs) are highly employed by industries. They are widely used as health supplements in food industry, as transformed materials in food packaging and appear incorporated in personal care products, inks, electronics, and textile [1]. This increasing industrial production makes very likely the oral exposure of human populations, (e.g., consumers, workers, children) both by voluntary or involuntary actions, thus requiring an urgent identification of the health risk profile [2, 3]. Research, regulatory and policy communities extensively debate this argument [4, 5, 6], highlighting that the quantitative description of NP fate along with the biotransformed species, in real exposure conditions, are related to the concepts of bioavailability/bioaccessibility, which are required by risk assessment.

Given the importance of these parameters, the development of an \textit{in vitro} test using realistic conditions and in standardized manners is key to enable extraction of quantitative information. A classic example of dissolution test is applied in pharmaceutical or food industries. The test provides information on bioavailability (fraction released and adsorbed in the blood) and bioaccessibility (fraction released by a product) of a drug or a food contaminant. In this framework, we evaluated the feasibility of an \textit{in vitro} dissolution test (previously employed for the quantification of bioavailability of food additives [7]) to quantify the dissolution of AgNPs in conditions likely occurring in the human oral ingestion. For the dissolution measurement, we applied UF/ICP-AES, DLS, zeta potential, UV-Vis to characterize size, surface charge and plasmon peak.
2. Methods
An in vitro dynamic model developed by Versantvoort et al. was applied here to digest AgNPs [7]. Briefly, artificial juices were prepared by mixing salt solutions, organic compounds and proteins at pH conditions similar to the human digestive compartments. Suspensions of AgNPs were incubated with the different juices, added in a temporal sequence that simulates the transit of food bolus along the oro-gastrointestinal (OGI) tract.

As reference materials, we used silver nanoparticles referred to as NM300k, obtained from the repository list of the European Commission Joint Research Centre (JRC) [8]. Dynamic light scattering (DLS), zeta potential and Transmission Electron Microscopy (TEM) were employed to evaluate size, morphology and surface charge of NM300k in dispersion medium and synthetic digestive juices. UV-Vis spectrophotometer was used to monitor the plasmon peak of NM300k during the digestion steps. Dissolution was measured by Ultrafiltration (UF) coupled to Inductively Coupled Plasma – Adsorption Emission Spectroscopy (ICP-AES).

3. Results and discussion
Figure 1 reports TEM images and the % of dissolution of NM300k, which are produced during the in vitro digestion test. Consistent with the NM datasheet, the size of NM300k is about 20 nm (Figure 1A) in either the ctrl and in the saliva compartment, although a small population of agglomerates and low level of dissolution are monitored in the saliva (Figure 1B). Along the passage in stomach simulating conditions, TEM analysis evidences a higher degree of particle dissolution, also showing the presence of a population of NPs with size below 5 nm (together with some aggregates). This indicates that most of the particles dissolved. UF/ICP-AES measures a value of about 20% of free ions. This value is not in contrast with the TEM data, as it only refers to the fraction of free ions able to pass though the filter. The remaining fraction is more likely bound to matrix so that it remains into the pellet. In the intestine, the dissolved fraction appeared further reduced to about 2%. Moreover, some aggregates along with nanosized particles of about 20 nm were observed. An in depth characterization of these nano-based salts are currently on going in our laboratories.

Figure 1. NM300k digestion by in vitro dissolution test. (A) TEM images of NM300k in simulated digestive fluids. (B) Percentage of free silver ions by in vitro digestion assay.
These data were confirmed by DLS and UV-Vis characterization (Figure 2). DLS spectra reported a small aggregation in the saliva with a peak size distribution ranging from 14 ± 3 nm to 120 ± 4 nm. This is in line with UV-Vis spectra that confirm the presence of the typical absorption peak at 412 nm for silver nanoparticles, along with a broad band in the red region (which is a typical indicator of agglomerates). In the stomach and in the intestine, DLS detected only big agglomerates whereas the UV-Vis spectra displayed the absence of the peak at 412 nm, further confirming the NP dissolution. Interestingly, by zeta pot analyses, we found out that the nominal surface charge for NM300k progressively changed, mostly acquiring the charge features of the media components (e.g., proteins). We also observed that in the saliva and intestine compartments, NM300k acquired a negative charge (around -35mV), whereas, in the stomach, the surface charge approached quite neutral values (in line with literature data) [8]. Note that, since these NPs partially dissolve in the stomach compartment, it could be possible that the reported zeta pot values in the digestive juices refer to mixed aggregates of biological/nanostructures present in the solutions, which do not relate to the primary NPs. This point deserves future investigations and clarifications.

![Figure 2. DLS, UV-Vis adsorption and zeta potential values of NM300k in the different digestive matrices.](image)

### 4. Conclusions

In conclusions, we performed analytical studies to investigate AgNP fate in synthetic biological fluid, particularly focusing on tracing the changes of size, surface charges and quantifying the dissolution process using a dissolution test. The obtained results show that NM300k did not maintain their primary properties along their passage through the OGI tract due to an almost complete dissolution. In particular, NPs begin to partially agglomerate in the salivary compartment and quite totally dissolve in the stomach compartment. Once they arrive in the intestine compartment, only a small fraction of free ions is quantified to about 2%. Changes in UV-Vis adsorption and surface charge are also monitored at similar conditions.

Overall, these data inform us that, during the digestion process, complex mixtures of bio-transformed products (including different organic, inorganic and ion molecular species) occur. These are the actual species responsible of effective dose, cellular permeability and uptake in vitro/in vivo. In the future, studies on test standardization and characterization of free and bound to matrix ions will provide benefits for the development of a test useful for the nanoregulation needs.

### 5. References

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