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Characteristics of silver nanoparticles in vehicles for biological applications

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ABSTRACT

Silver nanoparticles (AgNPs) have been used for decades as anti-bacterial agents in various industrial fields such as cosmetics, health industry, food storage, textile coatings and environmental applications, although their toxicity is not fully recognized yet. Antimicrobial and catalytic activity of AgNPs depends on their size as well as structure, shape, size distribution, and physico-chemical environment. The unique properties of AgNPs require novel or modified toxicological methods for evaluation of their toxic potential combined with robust analytical methods for characterization of nanoparticles applied in relevant vehicles, e.g., culture medium with/without serum and phosphate buffered saline.

1. Introduction

Silver nanoparticles (AgNPs) have been used for decades as anti-bacterial agents in cosmetics, health industry, food storage, textile coatings and a number of environmental applications, although there is still insufficient information on their toxicity and unambiguous opinion on behaviour in vivo. The issues related to synthesis, properties and characterization of AgNPs have been addressed in many publications and reviews (Reidy et al., 2013) where it was clearly stated that antimicrobial and catalytic activity of AgNPs depend on size and size distribution as well as their structure, shape, and physico-chemical environment.

Particle size is one of the most important parameters not only for description of fundamental properties of materials, but also within the biological systems as it can affect a number of key features and processes, such as drug targeting, delivery or distribution. Regarding nanosized particles, ISO standard ISO/TS 27687:2008 (ISO, 2008) provides their definition as an object with a size between 1 and 100 nm. In the EU, the respective Commission Recommendation (EC, 2011) defines a "nanomaterial" to be a "natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm". In an ideal case, the particles that are subject of characterization would be all homogeneous in shape and size with uniform properties. In this situation, any method measuring particle size would provide the same values of their diameters and the same particle size distribution, regardless of the principle of the measurement technique used. In the real world, however, most of the particles are non-spherical with different shapes that would undoubtedly influence their diameter determined using different methods (Merkus, 2009; Barth, 1984). Techniques used for the particle size measurements are based on different principles. Here, visual or microscopic observation, the light scattering, ultrasound absorption, sedimentation velocity or Brownian motion can be named. One of the most relevant methods used is dynamic light scattering (DLS) as it provides measurements of particle sizes from the nanometer up to a few microns. This technique measures scattered light fluctuations caused by the Brownian motion which are then related to the size of the particles via translational diffusion coefficient *D*. Particle diameter thus obtained is referred to as a hydrodynamic diameter and stands for the diameter of a sphere that has the same translational diffusion coefficient as the particle. It is worth mentioning that the hydrodynamic diameter measured by DLS corresponds to the diameter of its dense core

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Table 1
AgNPs used in the study.

Sample	Ag content[a] (ppm)	Date of manufacture
S9	20	03/2013
S11	20	05/2013
S29	20	02/2014

[a] Information given by manufacturer.

increased by the thickness of a layer of molecules adsorbed on its surface (for example surfactants) plus the thickness of the solvation, counter ion layer. The size of particles determined by DLS is z-averaged according to the scattering intensity of each particle fraction present in the sample. In practice relevant to biological applications, volume and even number distribution are more appropriate and they are smaller than z-averages.

Another group of methods commonly applied for AgNPs characterization is a family of microscopic methods. As they facilitate direct observation of the measured objects, they are probably the first-choice techniques to be used. However, the drawback related to their application is the time-consuming sample preparation and necessity to collect sufficient number of images to obtain reliable data. Transmission electron microscopy (TEM) is a technique reporting particle size as an equivalent diameter of a sphere that has the same projected areas as the projected image of the particle. Statistical analysis of the data enables to obtain number based particle distribution as primary data which can be further transformed to volume distribution (Reetz et al., 2000; Rubin, 2004). TEM analysis and light scattering technique DLS are the methods suggested for measurement of size and distribution of nanosized particles in the current study. As the methods work on different principles, their comparison is of interest and is one of the subjects of this study.

When applying AgNPs in biological systems, their bioavailability and cytotoxicity are governed by colloidal stability in respective environment, which may be influenced by several variables, such as pH, ionic strength, and the type of electrolyte present (El Badawy et al., 2010; Römer et al., 2011; Prathna et al., 2011). A number of studies dealing with the aggregation of nanoparticles in various environments has been conducted and reported (Cumberland and Lead, 2009; Römer et al., 2011), however, only limited information can be found in literature dealing with the influence of the above listed variables on the changes of particles used for biological studies. The presented study was therefore focused on the determination of size and distribution of AgNPs using the two above mentioned, independent methods, dynamic light scattering and transmission electron microscopy. Behaviour of the particles was assessed in more details after their contact with vehicles simulating human body fluids such as culture medium with/without serum (DMEM) and phosphate buffered saline (PBS).

2. Materials and methods

2.1. Tested materials

Aqueous dispersions of colloidal silver nanoparticles (20 ppm Ag) prepared by electrolysis were kindly provided by Petr Rulc—KC (Děčín, Czech Republic). Specification of the samples used in the study is provided in Table 1.

Dulbecco's Modified Eagle's Medium DMEM containing 4.5 g/L glucose with L-glutamine (LONZA, Cat. No. BE12-604F), new-born calf serum (LONZA, Cat. No. D/14-417F) and phosphate buffered saline PBS (LONZA, Cat. No. D/17-516F) were purchased from P-LAB, Czech Republic.

2.2. Methods for characterisation of the tested materials

2.2.1. Dynamic light scattering (DLS)

Size and distribution of the AgNPs were determined by DLS using a Zetasizer Nano ZS instrument (Malvern Instruments, UK). Measurements of the hydrodynamic radii of colloidal particles, expressed as z-average particle diameter, were performed at 25 °C. The intensity of scattered light ($\lambda = 633$ nm) was observed at a scattering angle of 90°. The polydispersity index (PDI), describing distribution width, was evaluated by assuming log-normal distribution of particle sizes. Prior to measurements,

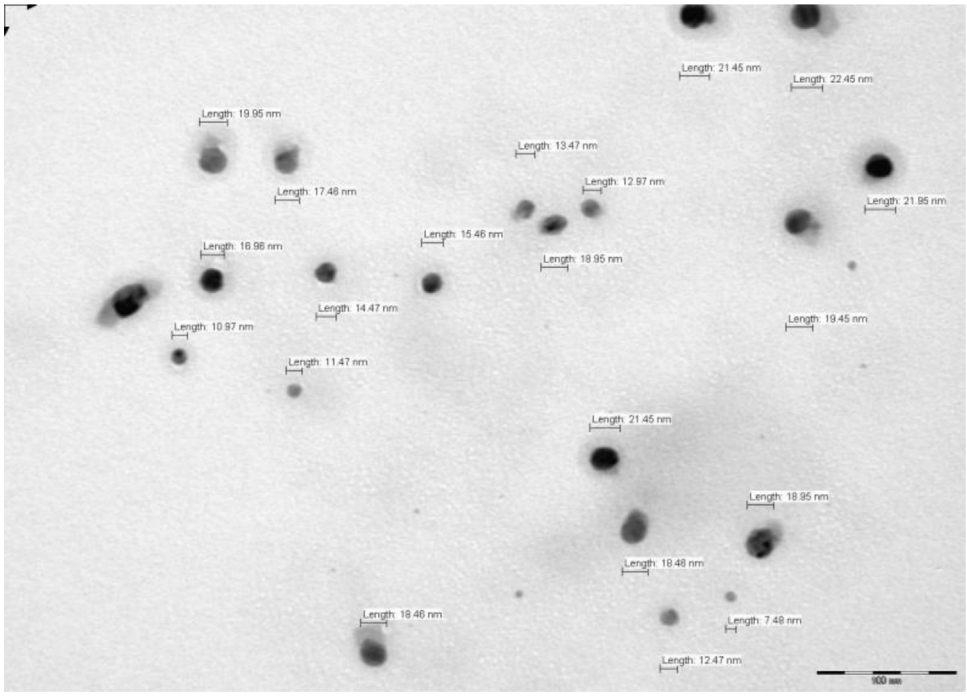

Fig. 1. TEM microphotogram of AgNPs, sample S29.