BEHAVIOUR OF AG AND SI-GRAPHITE NANOMATERIALS IN ENVIRONMENTAL AND EXTREME CONDITIONS

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Abstract

The growing industrial, health care, pharmaceutical and water purification application of nanomaterials (NMs) has upraised concerns about their effect on the human health and environment. Whether the NM remains stable, dissolves with the release of ions, or forms larger aggregates, is crucial in finding out its possible fate. Herein, the stability of tannic acid/sodium citrate-stabilized Ag (nAg) and Silicon-Graphite flakes produced within the European FP7 project FutureNanoNeeds was investigated at various pH values, ionic strengths (IS) and in natural reservoir water. Furthermore, the effect of these NMs in deionised and reservoir water was assessed on the soil bacterium *Pseudomonas putida*. Three methods were applied in order to determine stability and behaviour of the NMs, namely differential centrifugal sedimentation analysis, UV-Vis spectrophotometry and zeta potential analysis. The kinetics of ion release and/or particle agglomeration was strongly pH- and IS- dependent which resulted in change of UV-Vis spectra, particle size distribution and particle zeta potential. Low pH caused fast dissolution of nAg whereas no significant effect was observed at high pH. In contrary, high pH strongly influenced the stability of Silicon-Graphite flakes. Moreover, it was found that the organic matter and ions present in the reservoir water significantly influenced both NMs causing their rapid agglomeration and/or dissolution. *P. putida* was affected by both NMs at concentration of 50 mg/L after 6h of exposure. Concentrations up to 10 mg/l of Silicon-Graphite did not cause any harm to *P. putida* in both deionised and reservoir water. Interestingly, the negative effect of nAg disappeared in deionised water after 24h and prevailed in reservoir water.

Keywords: Stability, nanoparticle, nAg, Silicon-Graphite, environmental fate, Pseudomonas putida

1. INTRODUCTION

Increasing production and use of nanomaterials in many fields such as industry, pharmaceutical, food, textiles, medicine, agriculture, water/soil remediation and subsequent potential for their release in the environment has been raising concerns about their toxicological and environmental effects that need to be addressed. The most important step is to carefully describe interactions between nanomaterials (NMs) and different environmental compartments considering effect of wide variety of parameters affecting their fate and behaviour, like mobility and bioavailability. These issues lag far behind those of human exposure where the exposure scenarios, though large, are manageable and to some extent characterized. It is necessary to assess the important parameters which affect the environmental behaviour of the NMs in freshwaters, sea water, soil, or other natural environmental compartments. The analysis of nanoparticles in environmental media is a difficult task due to measurements that are highly dependent upon particle size, concentration and physico-chemical characterization [1]. Moreover, nanoparticles in aqueous media are dynamic: agglomeration/aggregation or dissolution is unpredictable and the rates are themselves highly dependent on surrounding conditions. In addition, nanoparticles can be greatly affected by the matrix components. Similarly, size and size distribution

of particles can be affected by ionic strength, pH, or presence of natural organic matter (NOM). When measuring nanoparticles in the environmental media, dynamic changes in nanoparticle aggregation and dissolution must be considered [2].

Engineered silver nanoparticles (nAg) have attracted the attention of scientists for their remarkable properties, such as electrical, thermal conductivity, optical and antimicrobial (explored in consumer products such as cosmetics, textiles, electronics, paints, and water disinfectants) [3]. However, due to the large production and use of nAg in many fields, Ag could be released into the environment either as soluble ions, NPs or NP aggregates, during various human activities (e.g. industrial process, washing of antibacterial textiles, and waste water production) and these release routes raise concerns about potential toxic effects of nAg on the environment and human health [3].

Amorphous Silicon NMs are nano-sized structures that can be applied in various products, such as sunscreen lotions, cosmetics, or food [4]. Because of their small size, particles have the potential of internalization through different routes to the organisms [5]. The hydrophilic silanol groups (Si-OH) on a silica surface act as binding sites for water. The protonation and deprotonation of these silanol groups determine the surface charge of silica NPs and the extent of the repulsive energy to keep them dispersed in the solution [6]. The mobility of nanoparticles in natural formations is strongly influenced by the dispersion stability of nanoparticles in a continuous phase. Typical subsurface conditions such as temperature, pH, and concentration of salts vary over wide ranges. It is thus difficult to control the stability of nanoparticle dispersion under reservoir conditions, particularly at high salinity and high temperature [7]. Moreover, environmentally relevant extreme conditions like low and high pH, ionic strength, and total organic carbon content are explored to determine effect on the NPs behaviour. To ensure a safe development of nanotechnology, a good understanding of the behaviour and effects of nAg and Silicon NMs on the environment is required.

The present study aims to investigate the effect of extreme and environmental conditions (i.e. low and high pH, various ionic strengths and dissolved organic matter concentration in natural reservoir water) on the stability of nAg and Silicon-Graphite NMs developed within the European FP7 project FutureNanoNeeds. Moreover, the effect of these materials on the soil bacterium *Pseudomonas putida* in deionised and natural reservoir water was determined.

2.   MATERIALS AND METHODS

2.1.   Materials

The tannic acid/sodium citrate-stabilized Ag (VHIR_AgNP_15 nm_060516) were obtained from FutureNanoNeeds partner Vall d'Hebron Research Institute. The mean size determined using TEM was 15.7 nm. Silicon-Graphite flakes (IUTA Si@Gra01) NM was obtained from FutureNanoNeeds partner IUTA. The Si/C content was 55/45 and the specific surface area was of 19.5 m^2/g. Density of bulk silicon was 2.329 g/cm^3 and density of graphite flake was 2.2 g/cm^3. This NM was originally developed for battery materials for Li-ion Batteries. The Gram-negative soil bacterium *Pseudomonas putida* strain CCM 7156 was obtained from the Czech Collection of Microorganisms, Masaryk University Brno, Czech Republic. The bacterial culture was freshly prepared overnight in a Soya nutrient broth (Sigma-Aldrich) at 27 °C before the experiment. The total organic carbon (TOC) of Soya nutrient broth was around 5.6 mg/l.

Deionised water (18.2 MΩ·cm^{-1}) was prepared by an ELGA purelab flex system (ELGA, Veolia Water, Marlow, UK). Natural water was sampled in the Harcov reservoir (Liberec, Czech Republic) with a 1 l fisher bottle and stored in 8 °C. The basic chemical characterisation of Harcov water is presented in **Table 1**.

Table 1 Physico-chemical characterisation of natural water from Harcov reservoir

pH [-]	ORP [mV]	Conductivity [μS·cm^{-1}]	NO$_3^-$ [mg/l]	NO$_2^-$ [mg/l]	PO$_4^{3-}$ [mg/l]	SO$_4^{2-}$ [mg/l]	DOC [mg/l]
7.37	225	245	3.7	<0.5	<0.5	21.8	8.7

2.2. Analytical procedure

The centrifugal particle sedimentation technique, was used for particle size distribution analysis. Measurements were made using CPS Disc Centrifuge (DC24000UHR, CPS Instruments Inc., USA) at a disc rotation speed of 24000 rpm and the particle sedimentation was carried out in a 8–24% (w/w) sucrose density gradient. Prior to each sample measurement, the instrument was calibrated using a PVC nanosphere standard (283 nm). An UV-Vis spectrophotometer (Hach Lange Dr 6000) was used to determine the ultraviolet-visible spectral region (300-700 nm) of the samples. The zeta potential values of NPs were determined in freshly prepared suspensions by means of a high performance Zetasizer ZS particle size analyser (Malvern Instruments Ltd, UK). Each measurement was performed in sequences of ten runs with autocorrelation functions set at 10 seconds. The zeta potential of each sample was obtained from the average of three measurements. The pH and ORP measurements were determined by a WTW pH-meter equipped with SenTix pH electrodes (TMultiLine® Multi 3430 IDS). ORP values are presented as values against a saturated calomel electrode (SCE) and were not corrected to the standard hydrogen electrode (SHE). TOC was analysed by a Multi N/C 3100 analyser (Jena Corporation, Germany).

2.3. Experimental procedure

The NPs were dispersed in aqueous media of (i) extremely low and high pH conditions (pH 2 and pH 11.5), (ii) lower and higher ionic strength (IS) conditions (NPs dispersed in 0.5 g/l and 1.0 g/l of NaCl solutions) (iii) in deionised water (control), and, furthermore (iv) NPs were dispersed in Harcov reservoir water (unfiltered) collected fresh before the experiment. Final volume of each sample was 100 ml. Samples were taken in selected time points and analysed.

Bacterial tests were described in detail in Ref. [8] and Ref. [9]. Briefly, nanoparticle suspension was added to the freshly prepared bacterial culture adjusted to 0.008 (±0.002) by optical density at 600 (OD$_{600}$) in a range of final concentrations: 0, 0.001, 0.01, 0.1, 1, 10 and 50 (mg/l). From each sample, 1 ml aliquots were transferred into a 24-well plate. Bacteria without NPs were used as negative control (0 mg/l). All samples were incubated at 27 °C for 24 hours. Optical density measurement of the samples in 24-well plate was measured at 600 nm (OD$_{600}$) for every 2 hours using multi-mode reader (Synergy HTX, BioTek, USA). Each sample was prepared in triplicate. The bacterial growth rate (μ) (OD$_{600}$/h) was defined by R linear regression of cell density (OD600) versus incubation time (hour). Effect of nanoparticle concentration at 10% inhibition on bacterial growth rate was also calculated [8, 9].

The results were statistically analysed by ordinary ANOVA, Dunnett's multiple comparison test.

3. RESULTS AND DISCUSSION

To determine the stability of nanoparticles in different pH, ionic strength and natural reservoir water, differential centrifugal sedimentation (DCS) analysis, UV-Vis spectrophotometry and zeta potential analysis were applied.

3.1. UV-Vis characterization

The UV-Vis absorption spectrum of the nAg showed a maximum absorption peak at a wavelength of 401.5 nm (**Figure 1, Figure 2**). The position and width of the UV-Vis absorption spectrum of the nAg depends on the size and polydispersity of the particles as well as the presence of adsorbed substances or an oxidation layer

on the surface of the particles [10]. In general, mono-dispersed silver nanoparticles bellow 100 nm with no adsorbed substances (i.e. ions and/or surfactants) or oxidized surface layer have a narrow peak at a wavelength of 390 to 450 nm that varies with particle size.
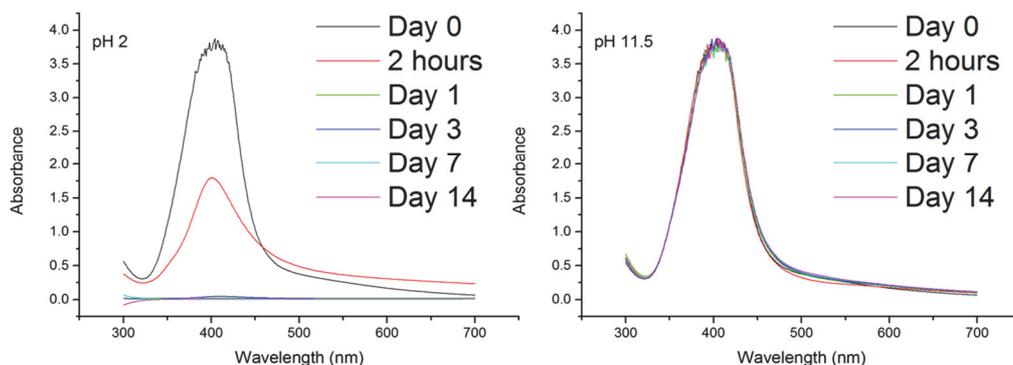

Figure 1 Effect of extreme pH conditions on UV-Vis spectrum of nAg (50 mg/l) during 14-day test. Left pH 2, Right pH 11.5
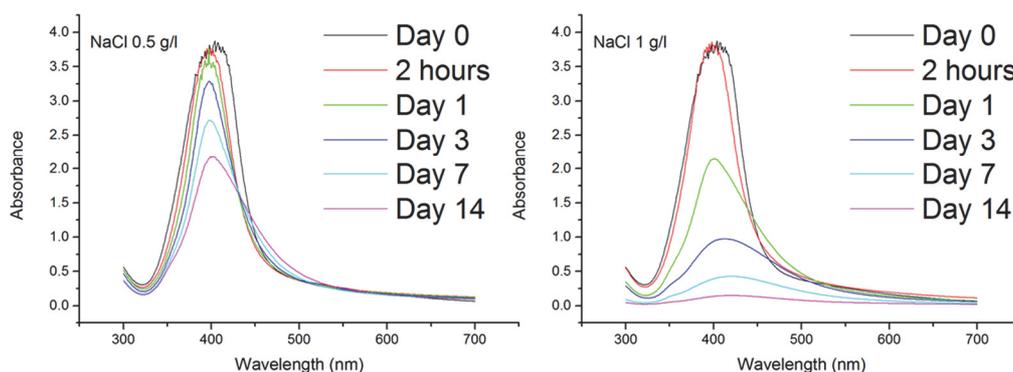

Figure 2 Effect of lower and higher ionic strength on UV-Vis spectrum of nAg (50 mg/l) during 14-day test. Left NaCl 0.5 g/l, Right 1 g/l

Extremely low pH as well as increased ionic strength significantly affected nAg in deionized water which is in good agreement with the work presented by Pinto et al. [11]. Moreover, extremely high pH and different nAg concentrations from 4.63 to 463 μM (data not shown) had little effect on the UV-Vis absorbance spectra. Stability of Silicon-Graphite NPs in extreme pH and different ionic strength conditions are not shown due to non-characteristic UV-Vis absorption spectra in the tested region.

3.2. Size distribution analysis

The size distribution of both NMs changed during the incubation time in the natural water taken from Harcov reservoir is shown in **Figure 3**.

The particle size of nAg increased over time in the reservoir water (**Figure 3a**) comparing to high stability of nAg in deionized water over 14 days. As for Silicon-Graphite NPs, two size distributions were detectable in the beginning of the experiment - smaller nanoparticles of ~ 40-50 nm and larger nanoparticles or aggregates of ~ 250 nm (**Figure 3b**). The smaller fraction was not detectable after 2 days of incubation in Harcov reservoir water. The agglomeration of particles could be caused by lowering the zeta potential (in the beginning of the

experiment) which was insufficient for maintaining colloidal stability and therefore resulted in suspension destabilization and particle aggregation. As for nAg, complexation of dissolved ions with the carboxyl groups on the surface of the citrate-nAg could be the main reason for the reduction in the zeta potential and subsequent particle aggregation as reported by [12].
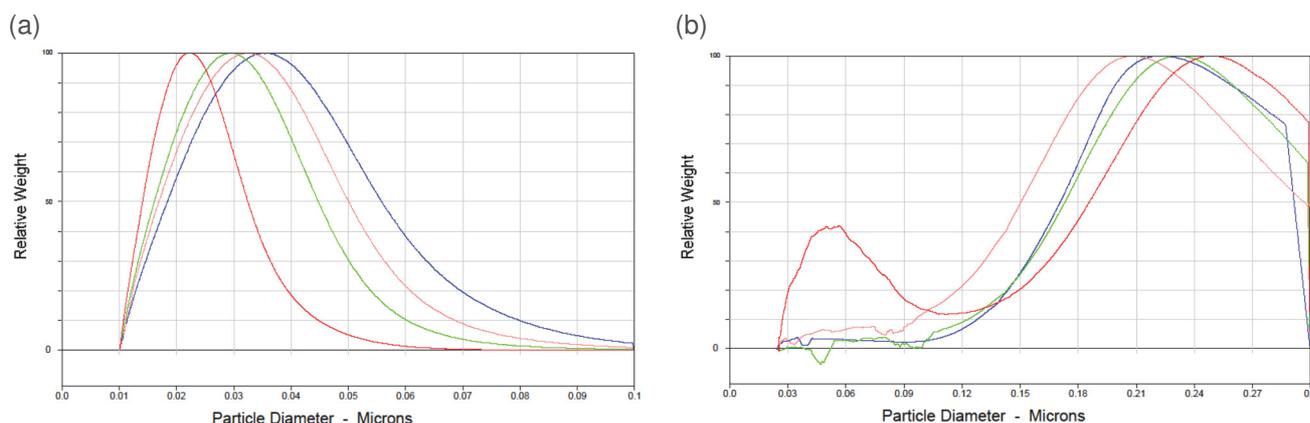
(a)

(b)

Figure 3 Effect of reservoir water on particle size distribution of (a) nAg (50 mg/l) and particle size distribution of (b) Silicon-Graphite (50 mg/l) in the beginning (red line), and after 2 (green line), 5 (pink line) and 7 (blue line) days

3.3. Zeta potential analysis

Generaly, the electric potential near to the surface of the molecule (ie., ζ-potential) is oftentimes assessed and applied as an indicator of the surface potential. The magnitude of the surface potential regulates the degree of the electrostatic repulsion between molecules therefore accelerating/inhibiting their agglomeration [13, 14]. Zeta potential of nAg in reservoir water was less negative in comparison to that of nAg in deionised water, indicating a reduced electrostatic repulsion.

Table 2 Zeta potential of nAg measured in different conditions after 0, 1, 3, 7 and 14 days

Sample conditions	Zeta potential (mV)				
	Day 0	Day 1	Day 3	Day 7	Day 14
Reservoir water*	-20.07	-21.50	-18.40	-22.25	n.a.
Deionized water	-48.9	-44.9	-40.9	-47.5	-48.4
pH 2	-49.9	-5.11	-0.334	-1.28	-0.087
pH 11.5	-31	-31.9	-47.7	-38.95	-32.5
Lower IS	-35.6	-36.2	-30.7	-33.9	-40.5
Higher IS	-47.8	-38.1	-38.8	-32.1	-19.1

*the time points in test conducted in Harcov reservoir water were: 0, 2, 5, 7

The dispersion surface charge and consequently the hydrodynamic size can be altered by changing the solution pH. At low pH, particles have a positive surface charge. When a particle has highly polar bonds, multiply charged ions may be adsorbed onto the particle in an aqueous environment, leading to an increase in particle surface charge and positive zeta potential.

Zeta potential has sligtly decreased over 7 days in Silicon-Graphite incubated in reservoir water (**Table 3**). At low pH conditions, zeta potential was of positive values and was slowly increasing, while at high pH conditions, it was of clearly negative values.

Table 3 Zeta potential of Silicon-Graphite NPs measured in different conditions after 0, 2, 5 and 7 days

Sample conditions	Zeta potential (mV)			
	Day 0	Day 2	Day 5	Day 7
Reservoir water	-6.57	-13.30	-20.85	-20.15
Deionized water	-11.37	-14.80	-4.90	-28.35
pH 2	5.83	14.70	11.15	14.05
pH 11.5	-31.63	-23.45	-27.85	-36.45
Lower IS	-16.20	-36.55	-38.95	-47.25
Higher IS	-30.67	-29.65	-32.10	-43.05

Effect of nAg and Silicon-Graphite on soil bacterium *Psedomonas putida*

Bacterial growth was significantly reduced ($P < 0.0001$) when *P. putida* grew in presence of nAg (10 mg/l) in deionized water and the reservoir water after 6h of incubation (**Figure 4a**). There was no significant effect of Silicon-Graphite on bacterial growth within the concentration range from 0.001 to 10 mg/l. At 50 mg/l concentration of both NMs, the bacterial growth rate could not be detected after 6h (**Figure 4a**). The bacterial growth in presence of NPs at concentrations up to 10 mg/l was comparable to the growth without NPs (0 mg/l) while it was strongly affected at 50 mg/l ($P < 0.0001$) except for nAg being harmless in deionized water after 24h (**Figure 4b**). The reservoir water contained divers sources of cations, anions and natural organic matter, therefore, it could affect bacterial growth in the presence of nAg (50 mg/L) after 24h.

The effective concentration (EC10) showed that nAg inhibited 10% of *P. putida* growth at the dose of 3.36 and 2.86 mg/l in deionized and reservoir water, respectively. EC10 for Silicon-Graphite was determined to be 10.84 mg/l in deionized water and 12.30 mg/l in the reservoir water (**Table 4**).
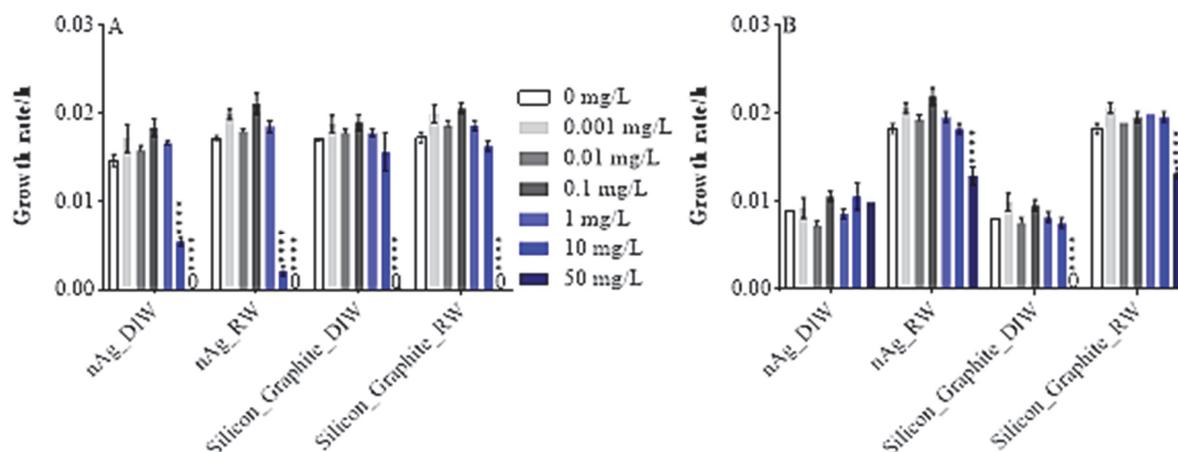

Figure 4 Effect of nAg and Silicon-Graphite on *P. putida* growth rate after 6h (A) and 24h (B) exposure. DIW - Deionised water, RW - reservoir water. The significance level: ****$P < 0.0001$.

Table 4 The effective concentration at 10% inhibition, EC10 (mg/l) of nAg and Silicon-Graphite determined for *Pseudomonas putida* growth in deionized water (DIW) and reservoir water (RW)

	nAg (mg/l)	Silicon-Graphite (mg/l)
DIW	3.36	10.84
RW	2.86	12.30

4.   CONCLUSIONS

The nAg and Silicon-Graphite particles were characterized in natural reservoir water, at low and high pH, and at different ionic strengths (0.5 and 1 g/l of NaCl) conditions in order to determine their stability. The effect of NMs in deionised and natural reservoir water was assessed on the soil bacterium *Pseudomonas putida*. Particles were found to be unstable in extreme and environmental conditions rapidly agglomerating especially at low pH. High pH appeared to affect stability of nAg not as much, and there could be found connection between the ionic strength (amount of NaCl added) and the stability of the materials. Our study shows that various cations, anions and natural organic matter present in the reservoir water had strong impact on the nanoparticle stability, leading to the aggregation of the particles. Moreover, the growth rate test of soil bacterium *Psedomonas putida* revealed that nAg were more toxic in the reservoir water than in the deionised water after 24h, in contrary to the Silicon-Graphite which had shown an oposite tendency. The reason why remains unclear and will be subjected further investigation.

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