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Predictability of silver nanoparticle speciation and toxicity in ecotoxicological media

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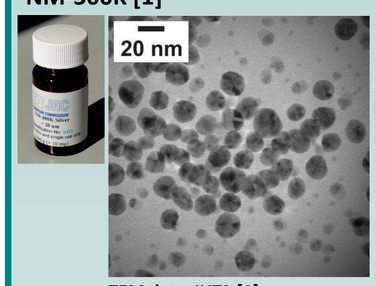
Nanomaterial, testmedia & methods

JRC standard silvernanoparticle NM-300K [1]



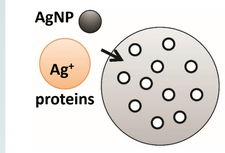
TEM data, IUTA [1]:
bimodal distribution with
d_1 = 5.4 ± 1.7 nm
d_2 = 15.0 ± 1.1 nm

Toxicological test organisms	Test media main components	Ionic strength [mM]
Pseudokirchneriella subcapitata (algae)	$NaHCO_3$, NH_4Cl, $CaCl_2$, $MgSO_4$	1.7
Daphnia magna (water flea, Elendt M7)	$CaCl_2$, $NaHCO_3$, $MgSO_4$, $NaNO_3$	8.3
Lemna minor (duckweed)	KNO_3, potassium phosphate buffer, $Ca(NO_3)_2$	9.3
Arthrobacter globiformis (soil bacterium)	NaCl, glucose, proteins	11.4
Scenedesmus vacuolatus (green algae)	NaCl, KNO_3, sodium phosphate buffer	25.4
HepG2 cells (liver cancer cells, RPMI)	NaCl, sodium phosphate buffer, $NaHCO_3$, glucose, KCl, amino acids, proteins	144.4

Redox glucose NO_3^-
Ligands proteins amino acids Cl^-
Precipitant Cl^-

Experimental speciation

Membrane filtration (MF)

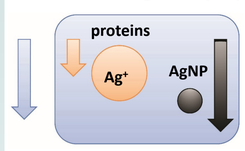


Cutoff: 3 kDa, d << 3 nm
30 min, 14500 rpm = 14000 g

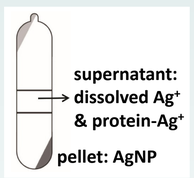
retentate: AgNP & protein-Ag^+

filtrate: dissolved Ag^+

Ultracentrifugation (UC)



Cutoff dependent on particle density:
AgNP: ≈ 3 nm,
proteins: d ≈ 8 nm, ≈ 180 kDa,
40 min, 80000 rpm = 400000 g



supernatant: dissolved Ag^+ & protein-Ag^+

pellet: AgNP

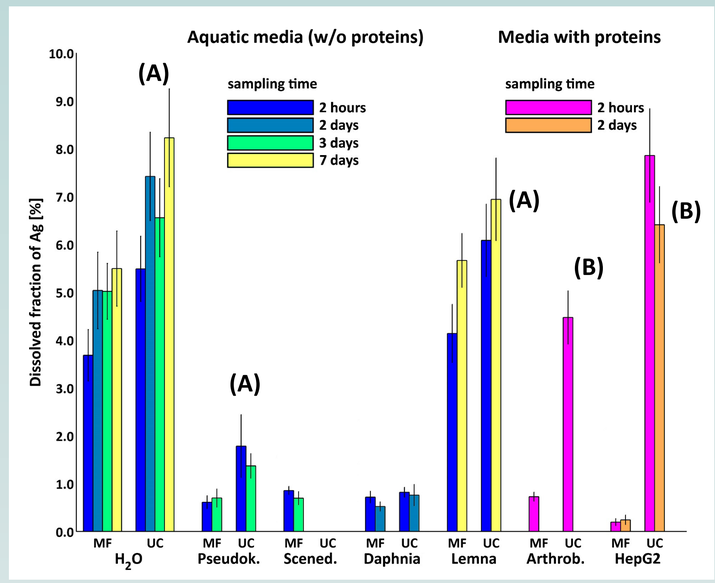
Ag^+ release in biological test media

Measurement of speciation of NM-300K (@ 7.9 mg Ag/L)
in H_2O at 2h, 2d, 3d, 7d;
in media at the beginning and end of the test

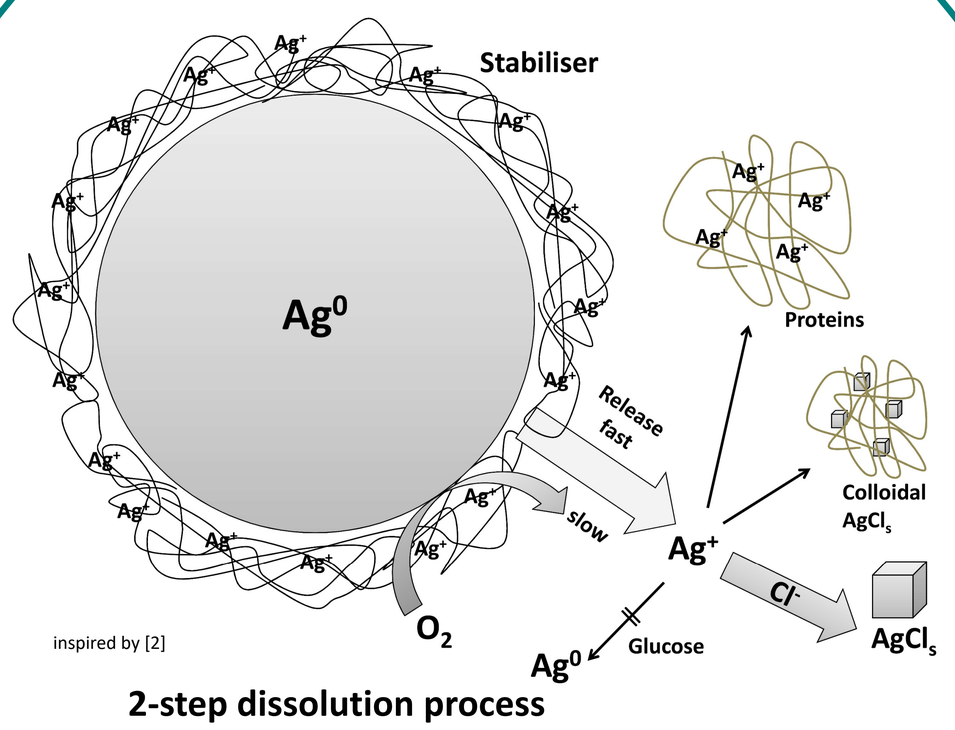
Ultracentrifugation (UC)
slightly overestimates dissolved silver content (A)

Membrane filtration (MF)
retains silver bound to proteins in media (B)

RPMI contains 8 % FCS
Main component of FCS is albumin (BSA, 69 kD)



Controlled speciation in environmental media



2-step dissolution process

1. Fast ion release from outer shell (stabiliser)
2. Slow oxidative release from elemental silver (hindered by stabiliser)

Conclusions from experimental speciation

- Increase of silver release due to oxidation (O_2, NO_3) is very slow in water and aqueous media
- Precipitation of silver chloride is controlling the released silver in the chloride containing aqueous media
- Influence of reducing components ammonium and glucose can be neglected
- Proteins and amino acids have a strong influence on the silver release due to binding effects

Speciation calculations PHREEQCi [3]

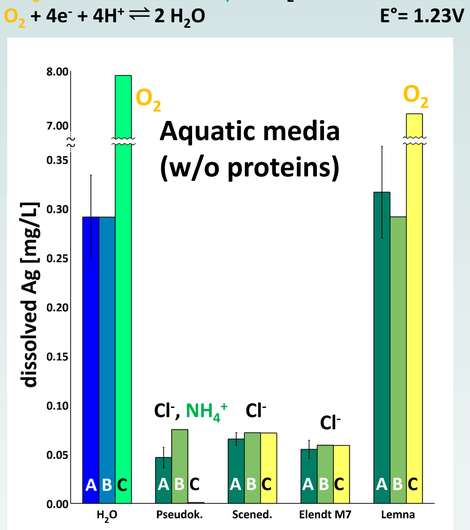
Initial silver speciation for calculations is experimental first time point of AgNP/H_2O by MF:
Ag^0 7.623 mg/L & Ag^+ 0.291 mg/L

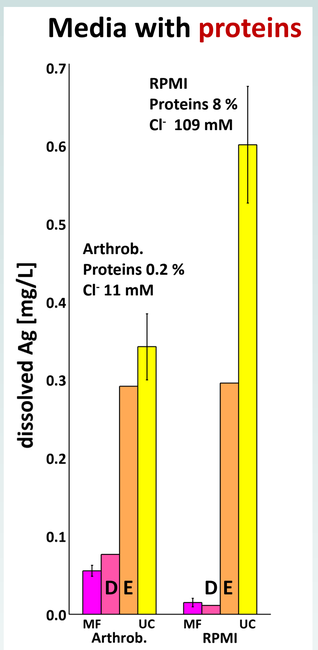
A) Experimental data for MF

B) First step calculations only allowing for complexation and precipitation, e.g.:
Precipitation:
$Ag^+_{(aq)} + Cl^-_{(aq)} \rightleftharpoons AgCl_{(s)}$
Complexation:
$Ag^+_{(aq)} + 2\,Cl^-_{(aq)} \rightleftharpoons [AgCl_2]^-_{(aq)}$

C) Second step calculations allowing for redox reactions, e.g.:

$Ag^+ + e^- \rightleftharpoons Ag^0$	E°= 0.80V
$Gluconate^- + 3\,H^+ + 2\,e^- \rightleftharpoons Glucose + H_2O$	E°= 0.135V
$NO_3^- + 3\,H^+ + 2\,e^- \rightleftharpoons HNO_2 + H_2O$	E°= 0.93V
$NO_3^- + 10\,H^+ + 8\,e^- \rightleftharpoons NH_4^+ + 3\,H_2O$	E°= 0.96V
$O_2 + 4e^- + 4H^+ \rightleftharpoons 2\,H_2O$	E°= 1.23V





Data of binding of Ag^+ to albumin (BSA) was used as model for Ag^+-protein interaction based on 9 cysteine sites per BSA [4,5]

D) Dissolved Ag^+ not bound to proteins

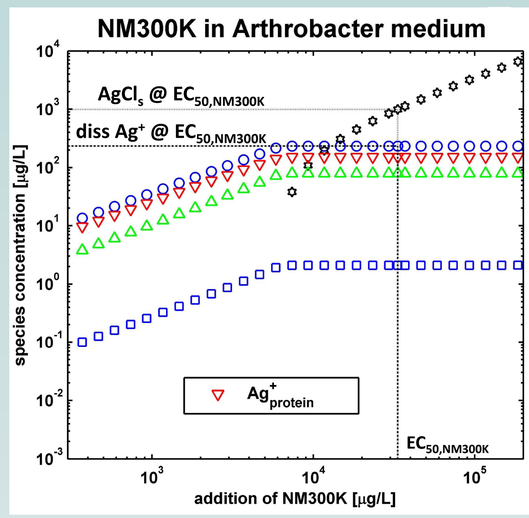
E) Total dissolved Ag^+ (protein bound Ag^+ included)

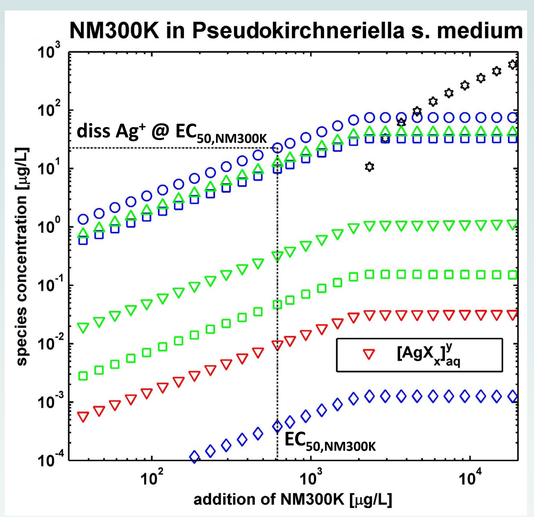
Elevated release caused by accelerated oxidative dissolution due to very high chloride content [6]

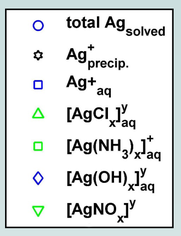
Redox: Glucose content leads to full reduction of Ag^+

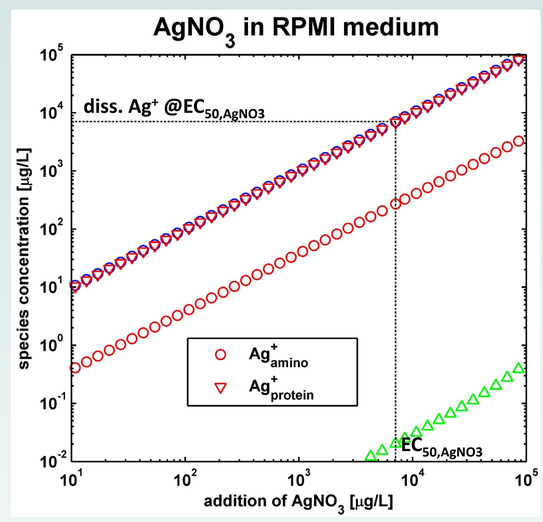
INFLUENCE OF DISSOLUTION

Speciation calculation in the range of the EC_{50}









Prediction of AgNP toxicity based on dissolved Ag^+

Test organism	$EC_{50,NM300K}$ [µg Ag/L]		Ag^+_{diss} at $EC_{50,NM300K}$ [µg Ag/L]	$EC_{50,AgNO_3}$ [µg Ag/L]		$EC_{50,NM300K}$ predicted [µg Ag/L]
Pseudok.	617 ± 367	[7]	22.7 ± 13.5	16.1 ± 4.9	[7]	436 ± 134
Scened.	1399 ± 540	[7]	51.4 ± 19.9	8.4 ± 3.2	[7]	228 ± 86
Daphnia m.	41 ± 14	[8]	1.5 ± 0.5	2.3 ± 0.3	[9]	63.1 ± 8.4
Lemna m.	496 (192-1105)	[10]	18.2 (7.1-41)	31 (26-37)	[11]	843 (707-1006)
Arthrob.	33380 (29940-38370)	[12]	233 (233 233)	1430 (1210-1710)	[12]	*38890 (32907-46505)
HepG2	≫50000	[7]	≫1839	7080 ± 2410	[7]	192550 ± 65542

Conclusions

- **Predictability** of EC_{50} for NM-300K is demonstrated
- The **deviation** of prediction is mostly in the range of experimental standard deviation
- Exception: Scenedesmus (twofold standard deviation)
- The underestimation of EC_{50} in case of Scenedesmus cannot be explained by water chemistry and is probably attributed to test organisms
- ***NM-300K EC_{50} for Arthrobacter** is correctly predicted including $AgCl_s$, suggesting **toxic effect of colloidal $AgCl_s$**

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