Supporting Information

Impact of chemical composition of ecotoxicological test media on the stability and aggregation status of silver nanoparticles

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Silver Nanoparticle Synthesis

1290.3 mg (5 mmol) trisodium citrate $Na_3C_6H_5O_7$ (Sigma-Aldrich) and 169.9 mg (1 mmol) silver nitrate $AgNO_3$ ($AgNO_3$, ≥ 99.9%, p.a., Carl Roth) were dissolved in 950 mL and 50 mL deionised water respectively. The pH values of 11 and 6 were adjusted in trisodium citrate solution using 1 mol L^{-1} NaOH solution (NaOH, 1 N standard solution, Carl Roth) and 1 mmol L^{-1} HNO_3 solution (sub-boiled) for the synthesis of 30 nm and 100 nm Ag-NPs respectively. After pH adjustment, the solution of trisodium citrate was brought to a boil in a 2000 mL beaker glass covered with watch-glass under magnetic stirring using laboratory hotplate magnetic stirrer (RCT basic, IKA Labortechnik). After start of boiling, the solution of silver nitrate was added to the solution of trisodium citrate drop by drop within 5 min. The colour of the reaction solution changed from colourless to yellow and then to turbid grey. The boiling and stirring was continued until no more changes in the colour were observed, for 20 min (30 nm Ag-NPs) and for 2 h (100 nm Ag-NPs). Thereafter, the samples were removed from laboratory hotplate magnetic stirrer and cooled to room temperature. To compensate the water losses through evaporation, the samples were filled up to 1 L in a 1 L volumetric glass flask with deionised water. The samples shoved the final pH values of 8.9 (30 nm Ag-NPs) and 6.1 (100 nm Ag-NPs). The synthesised silver nanoparticle dispersions were stored in the dark at 4°C.

Preparation of SRHA Stock Solution

The SRHA stock solution (200 mg L^{-1}) was prepared by dissolving 10 mg SRHA in 50 mL deionized water. The solution pH was adjusted to 10 using 1 mol L^{-1} NaOH. The solution was then filtrated through 0.1 µm cellulose nitrate membrane filter (Whatman) and stirred with magnetic stirrer for 24 h. The prepared SRHA stock solution was stored in dark at 4-6°C in

refrigerator. The total organic carbon (TOC) concentration of SRHA stock solution was 94.1 ± 2.8 mg L^{-1}. The TOC concentration was determined by TOC analyser (analytikjena multi N/C 2100, Analytik Jena AG).

Calculation of Centrifugation Duration

Centrifugation duration (t, h) for ultracentrifuge was calculated using following equation:

$$t = \frac{K}{S_{20,W}}, \tag{1}$$

where K is the clearing factor for the rotor, and $S_{20,W}$ (S) is the sedimentation coefficient of particles in water at 20°C. K was calculated from maximum (r_{max}) and minimum (r_{min}) rotor radii (m) and rotor speed (ν, rpm) using following equation:

$$K = \frac{253000 \ln \frac{r_{\max}}{r_{\min}}}{\left(\frac{\nu}{1000}\right)^2}. \tag{2}$$

For calculation of $S_{20,W}$ following equation was applied:

$$S_{20,W} = \frac{2r_P^2(\rho_P - \rho_M)10^{13}}{9\eta}, \tag{3}$$

where r_P (m) is the particle radius, ρ_P (kg m^{-3}) is the particle density, ρ_M (kg m^{-3}) is the medium density, and η (kg m^{-1} s^{-1}) is the dynamic viscosity of medium.

Table S1. Chemical composition of ASTM medium.

Component	Manufacturer	Purity	Concentration in mg L^{-1}
$NaHCO_3$	Carl Roth	≥ 99.5%	192
$CaSO_4 \cdot 2H_2O$	Carl Roth	≥ 98.0%	120
$MgSO_4$	Carl Roth	≥ 99.0%	120
KCl	Carl Roth	≥ 99.5%	8
Na_2SeO_3	Sigma-Aldrich	≥ 95.0%	0.00219
Thiamine hydrochloride (B_1)	Sigma-Aldrich	≥ 99.0%	0.075
Biotin (B_7)	Carl Roth	≥ 98.5%	0.00075
Cyanocobalamine (B_{12})	Carl Roth	≥ 96.0%	0.001
Ionic strength			9.25 mmol L^{-1}
pH			8.2

Table S2. Chemical composition of SAM-5S medium.

Component	Manufacturer	Purity	Concentration in mg L^{-1}
$NaHCO_3$	Carl Roth	≥ 99.5%	85.5
$CaCl_2 \cdot 2H_2O$	Carl Roth	≥ 99.0%	147
$MgSO_4 \cdot 7H_2O$	Carl Roth	≥ 99.0%	61.5
KCl	Carl Roth	≥ 99.5%	3.8
NaBr	Carl Roth	≥ 99.0%	1.03
Ionic strength			5.06 mmol L^{-1}
pH			7.8

Table S3. Chemical composition of R2A medium (autoclaved for 20 min at 121°C).

Component	Manufacturer	Purity	Concentration in mg L^{-1}
$MgSO_4 \cdot 7H_2O$	AppliChem	≥ 99.5%	50
K_2HPO_4	Sigma-Aldrich	≥ 98.0%	300
Na-Pyruvat	AppliChem	≥ 99.5%	300
Casein acid hydrolysate (bacteriological, for biochemistry)	Carl Roth	-	500
Yeast extract (powdered, for bacteriology)	Carl Roth	-	500
Proteose peptone (for microbiology)	Carl Roth	-	500
Glucose (α-D(+) Glucose monohydrate for microbiology)	Carl Roth	-	500
Tween-80 (pure, Ph. Eur.)	AppliChem	-	1 mL
Ionic strength			8.75 mmol L^{-1}
pH			6.7

Table S4. Chemical composition of TM. Concentrations of cations and anions and ionic strength are given in mmol L^{-1}. Concentrations of organic compounds are presented in mg L^{-1}.

	ASTM	SAM-5S	R2A
Cations			
Na^+	2.33	1.01	2.7
K^+	0.107	0.05	3.5
Total monovalent cations ($Na^+ + K^+$)	2.437	1.06	6.2
Ca^{2+}	0.694	1	-
Mg^{2+}	1	0.25	0.203
Total divalent cations ($Ca^{2+} + Mg^{2+}$)[a]	1.694	1.25	0.203
Anions			
SO_4^{2-}	1.694	0.25	0.203
HCO_3^-	2.285	1.018	0
Cl^-	0.107	2.051	0
Br^-	0	0.01	0
SeO_3^{2-}	0.00001266	0	0
HPO_4^{2-}	0	0	1.722
Ionic strength	9.25	5.06	8.75
pH	8.2	7.8	6.7
Organic compounds			
Thiamine hydrochloride (B_1)	0.075	-	-
Biotin (B_7)	0.00075	-	-
Cyanocobalamine (B_{12})	0.001	-	-
Na-Pyruvat	-	-	300
Casein acid hydrolysate	-	-	500
Yeast extract	-	-	500
Proteose peptone	-	-	500
Glucose	-	-	500
Tween-80	-	-	1 mL

[a] only Mg^{2+} for R2A medium

Table S5. pH values in ATM after addition of NOM and Ag-NP.

Medium	Long term agglomeration experiments	Early stage agglomeration experiments
ASTM	8.1-8.4	8.0-8.4
SAM-5S	7.8-7.9	7.7-8.1
R2A	6.8	6.7-6.9

Table S6. Stock solutions for the preparation of ASTM medium for early stage agglomeration kinetics experiments

Stock solutions	Component	Concentration in mg L^{-1}
Stock-1	$CaSO_4 \cdot 2H_2O$	1721.4
Stock-2	$MgSO_4$	12037
Stock-3	$NaHCO_3$	9600
	KCl	400
	Na_2SeO_3	0.1095[a]
Stock-4	Thiamine hydrochloride (B_1)	75
	Biotin (B_7)	0.75
	Cyanocobalamine (B_{12})	1

[a] To adjust this concentration in the stock solution 3, a stock solution of Na_2SeO_3 with a concentration of 109.5 mg L^{-1} was prepared

Table S7. Stock solutions for the preparation of SAM-5S medium for early stage agglomeration kinetics experiments

Stock solutions	Component	Concentration in mg L^{-1}
Stock-1	$CaCl_2 \cdot 2H_2O$	1470.1
Stock-2	$MgSO_4 \cdot 7H_2O$	24648
Stock-3	$NaHCO_3$	4275
	KCl	190
	NaBr	51.5

Table S8. Stock solutions for the preparation of R2A medium for early stage agglomeration kinetics experiments

Stock solutions	Component	Concentration in mg L^{-1}
Stock-1	$MgSO_4 \cdot 7H_2O$	123240
Stock-2	R2A medium without $MgSO_4$ (for chemical composition see Table S3)	

Table S9. Input concentration of ions (µmol L^{-1}) and pH values used in the model calculations. In "ASTM with $CaCl_2$" and "ASTM with $CaBr_2$" medium the concentration of $MgSO_4$ was set to 0 mmol L^{-1} and $CaSO_4$ was substituted by an equivalent amount (0.7 mmol L^{-1}) of $CaCl_2$ or $CaBr_2$ respectively.

	ASTM	SAM-5S	R2A	ASTM with $CaCl_2$	ASTM with $CaBr_2$
Ag^+	0.983 [a]	0.102 [a]	0.037 [a]	0.983 [a]	0.983 [a]
Mg^{2+}	997 [b]	250 [b]	220 [d]	0	0
Ca^{2+}	697 [b]	1000 [b]	9 [c]	697 [b]	697 [b]
Na^+	2285 [b]	1028 [b]	5740 [c]	2285 [b]	2285 [b]
K^+	107 [b]	51 [b]	4364 [d]	107 [b]	107 [b]
SO_4^{2-}	1694 [b]	250 [b]	203 [b]	0	0
HCO_3^-	2285 [b]	1018 [b]	0 [b]	2285 [b]	2285 [b]
Cl^-	107 [b]	2051 [b]	0 [b]	1501	107 [b]
Br^-	0	10 [b]	0 [b]	0	1394
NO_3^-	0.983 [e]	0.102 [e]	6712.037 [e]	0.983 [e]	0.983 [e]
SeO_3^{2-}	0.01266 [b]	0 [b]	0 [b]	0.01266 [b]	0.01266 [b]
HPO_4^{2-}	0 [b]	0 [b]	1722 [b]	0	0
pH	8.2 [b]	7.8 [b]	6.7 [b]	8.2 [b]	8.2 [b]

[a] From Ag^+ release experiments with 1 d shaking duration. [b] From theoretical medium composition. [c] From ion composition determined in organic compounds. [d] From theoretical medium composition and ion composition determined in organic compounds. [e] Added for charge balance between cations and anions. Concentration of HCO_3^-, CO_3^{2-}, and CO_2 was additionally calculated from the CO_2 equilibrium between air and aqueous phase.

Table S10. Speciation of silver (in % of total Ag^+ species concentration) in TM. In "ASTM with $CaCl_2$" and "ASTM with $CaBr_2$" medium the concentration of $MgSO_4$ was set to 0 mmol L^{-1} and $CaSO_4$ was substituted by an equivalent amount (0.7 mmol L^{-1}) of $CaCl_2$ or $CaBr_2$ respectively. As a total concentration of Ag^+ the concentration of $Ag_{<2nm}$ detected after 1 d shaking in Ag^+ release experiments was used. Calculations were performed based on the assumption that the ultracentrifugated samples (< 2 nm) solely contain Ag^+ species. The values in parentheses are saturation indexes for AgCl(s) and AgBr(s).

Species	ASTM	SAM-5S	R2A	ASTM with $CaCl_2$	ASTM with $CaBr_2$
Ag^+	82.12	16.71	99.40	8.82	0.02
AgCl(aq)	16.10	65.43	0	25.59	< 0.01
$AgCl_2^-$	0.15	11.99	0	3.43	< 0.01
$AgCl_3^{2-}$	< 0.01	0.03	0	0.01	< 0.01
AgBr(aq)	0	5.74	0	0	1.13
$AgBr_2^-$	0	0.05	0	0	1.26
$AgBr_3^{2-}$	0	< 0.01	0	0	0.01
$AgBr_4^{3-}$	0	< 0.01	0	0	< 0.01
$AgNO_3$(aq)	< 0.01	< 0.01	0.36	< 0.01	< 0.01
$AgSO_4^-$	1.61	0.05	0.24	0	0
AgOH(aq)	0.01	< 0.01	< 0.01	< 0.01	< 0.01
$Ag(OH)_2^-$	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
$AgSeO_3^-$	< 0.01	0	0	< 0.01	< 0.01
$Ag(SeO_3)_2^{3-}$	< 0.01	0	0	< 0.01	< 0.01
AgCl(s)	0 (-0.201)	0 (-0.576)	0	62.15 (0)	0 (-3.723)
AgBr(s)	0	0 (-0.279)	0	0	97.57 (0)

Table S11. Equilibrium concentration of Ag^+ ions (µg L^{-1}) calculated for equilibrium state between Ag^+ and AgCl(s) or AgBr(s). Calculations were done for Cl^- and Br^- concentrations used in ASTM and SAM-5S.

Species	ASTM	SAM-5S
AgCl(s)	169	41
AgBr(s)	-	21

Table S12. Metal concentrations (µmol L^{-1}, mean value ± standard deviation from three replicate measurements) in organic compounds used for preparation of R2A medium.

Sample treatment	Organic compounds	Ca	Mg	Na	K	Ca + Mg	Na + K
Unfiltered	Na-Pyruvat	0.19 ± 0.01	0.04 ± 0.01	2267.74 ± 6.78	< LQ [a]	0.23	2267.74
	Casein acid hydrolysate	2.90 ± 0.02	1.16 ± 0.01	3114.71 ± 9.17	2.20 ± 0.02	4.06	3116.91
	Yeast extract	2.79 ± 0.02	13.84 ± 0.02	22.94 ± 0.09	757.07 ± 1.25	16.63	780.01
	Proteose peptone	3.05 ± 0.01	9.69 ± 0.03	304.27 ± 0.75	399.05 ± 1.95	12.74	703.32
	Glucose	0.16 ± 0.01	0.01 ± 0.01	0.32 ± 0.01	< LQ [a]	0.17	0.32
	Tween-80	0.10 ± 0.01	0.03 ± 0.01	13.09 ± 0.23	1.54 ± 0.05	0.13	14.63
	Sum	9.19	24.77	5723.07	1159.86	33.96	6882.93
Filtered (< 3 kDa)	Na-Pyruvat	1.04 ± 0.01	0.21 ± 0.01	2301.33 ± 3.19	< LQ [a]	1.25	2301.33
	Casein acid hydrolysate	3.89 ± 0.01	1.32 ± 0.01	3161.96 ± 21.69	2.15 ± 0.01	5.21	3164.11
	Yeast extract	2.65 ± 0.01	10.93 ± 0.02	22.52 ± 0.02	575.88 ± 1.91	13.58	598.40
	Proteose peptone	1.55 ± 0.01	4.08 ± 0.02	250.22 ± 0.67	344.25 ± 2.96	5.63	594.47
	Sum	9.13	16.54	5736.03	922.28	25.67	6658.31

[a] LQ: Limit of quantification

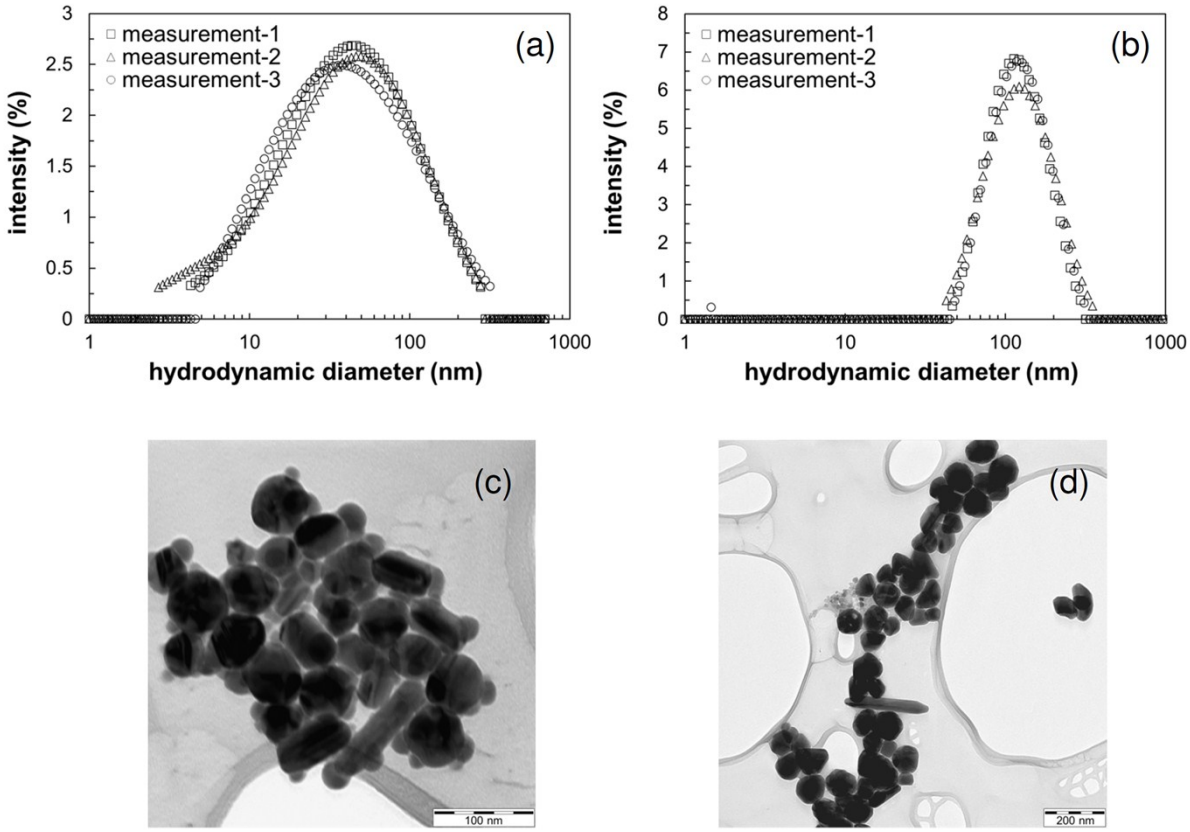

Figure S1. Particle size distribution of 30 nm (a) and 100 nm (b) Ag NP determined by DLS. TEM images of 30 nm (c) and 100 nm (d) Ag NP. The percentage of rod-like and triangle particles was 20% for 30 nm Ag NP and 7% for 100 nm Ag NP.

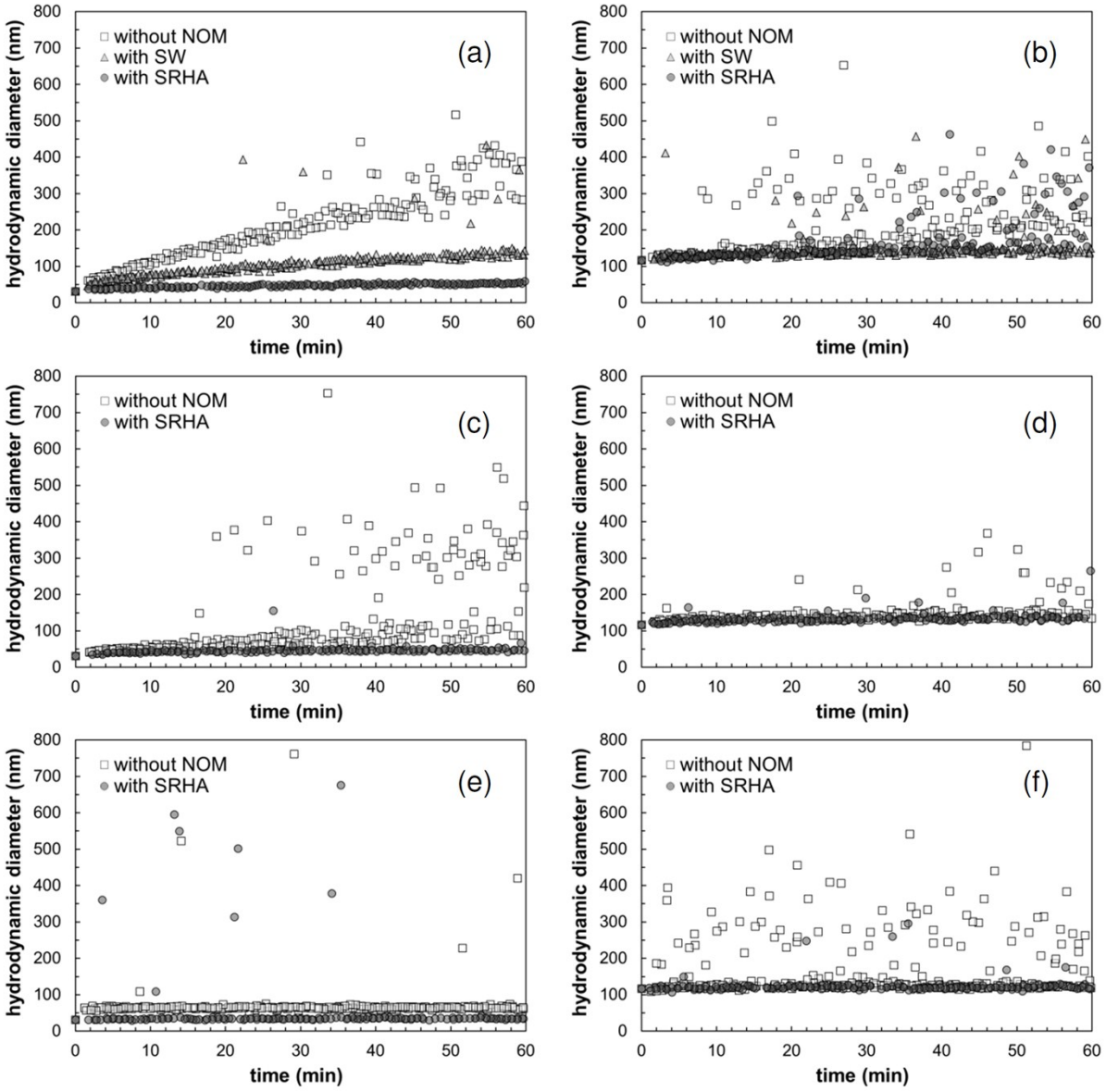

Figure S2. Hydrodynamic diameter of 30 nm (a), (c), (e) and 100 nm (b), (d), (f) Ag NP in ASTM (a), (b), SAM-5S (c), (d), and R2A (e), (f) medium for first 60 min as a function of exposure time. Hydrodynamic diameters are given for two replicate experiments.

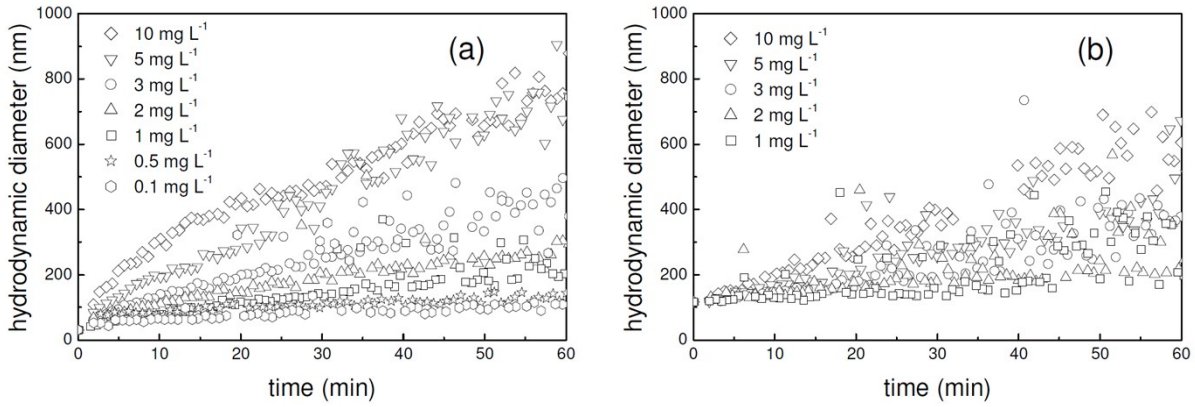

Figure S3. Hydrodynamic diameter of 30 nm (a) and 100 nm (b) Ag NP at different particle concentrations in ASTM medium as a function of time.

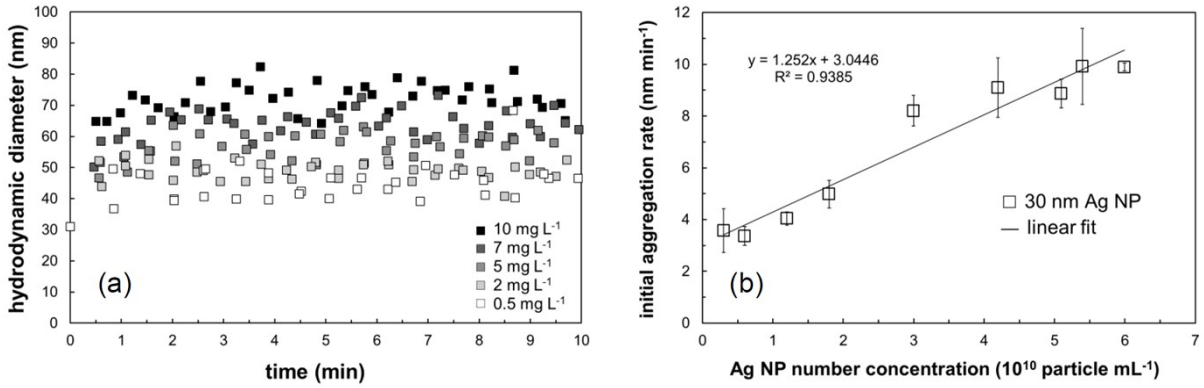

Figure S4. Hydrodynamic diameter of 30 nm Ag NP in Mg^{2+} free R2A medium at different particle concentration as a function of time (a). Mean initial aggregation rate of 30 nm Ag NP in Mg^{2+} free R2A medium as a function of particle number concentration (b). Hydrodynamic diameters and initial aggregation rates are given for two replicates. The error bars represent minimal and maximal values of replicates.

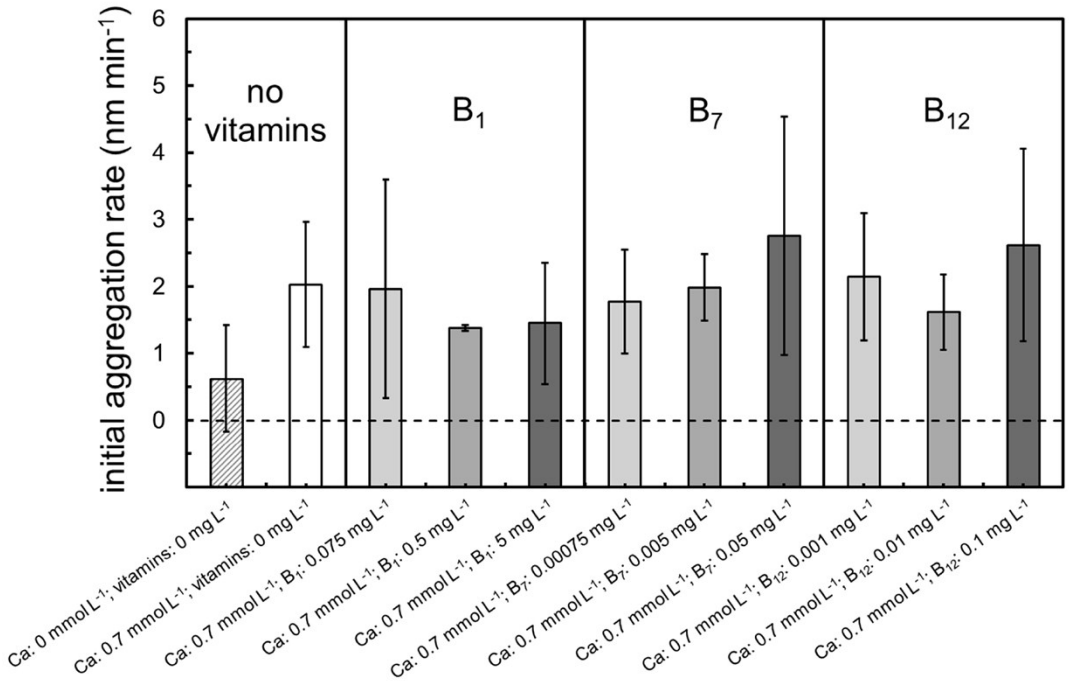

Figure S5. Mean initial aggregation rates of 30 nm Ag NP in modified ASTM medium in the absence and presence of Ca^{2+} (0.7 mmol L^{-1}) as well as in the absence and presence of vitamins (B_1, B_7 and B_{12}) at different concentrations. The lowest concentrations of vitamins are typical concentrations used in ASTM medium. The modified ASTM medium did not contain $MgSO_4$. In modified ASTM medium Ca^{2+} was added as a sulfate. The horizontal dashed line shows the initial aggregation rate when no aggregation occurs. The error bars represent the standard deviations from three replicates.

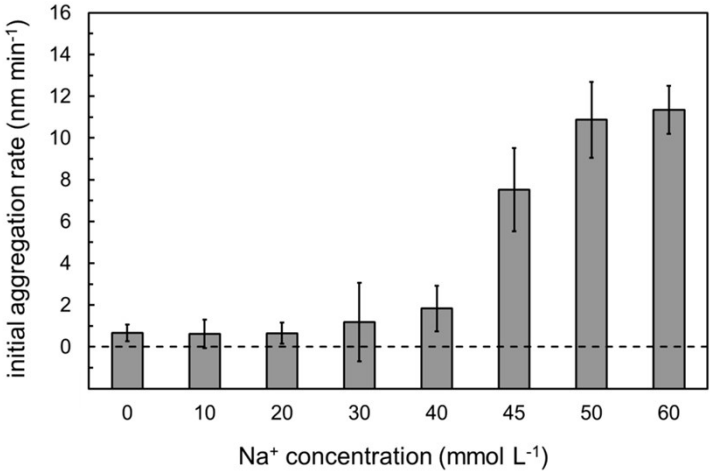

Figure S6. Mean initial aggregation rates of 30 nm Ag NP in deionized water in the presence of 0.03 mmol L^{-1} Ca^{2+} and at different Na^{+} concentrations. The horizontal dashed line shows the initial aggregation rate when no aggregation occurs. The error bars represent the standard deviations from three replicates.

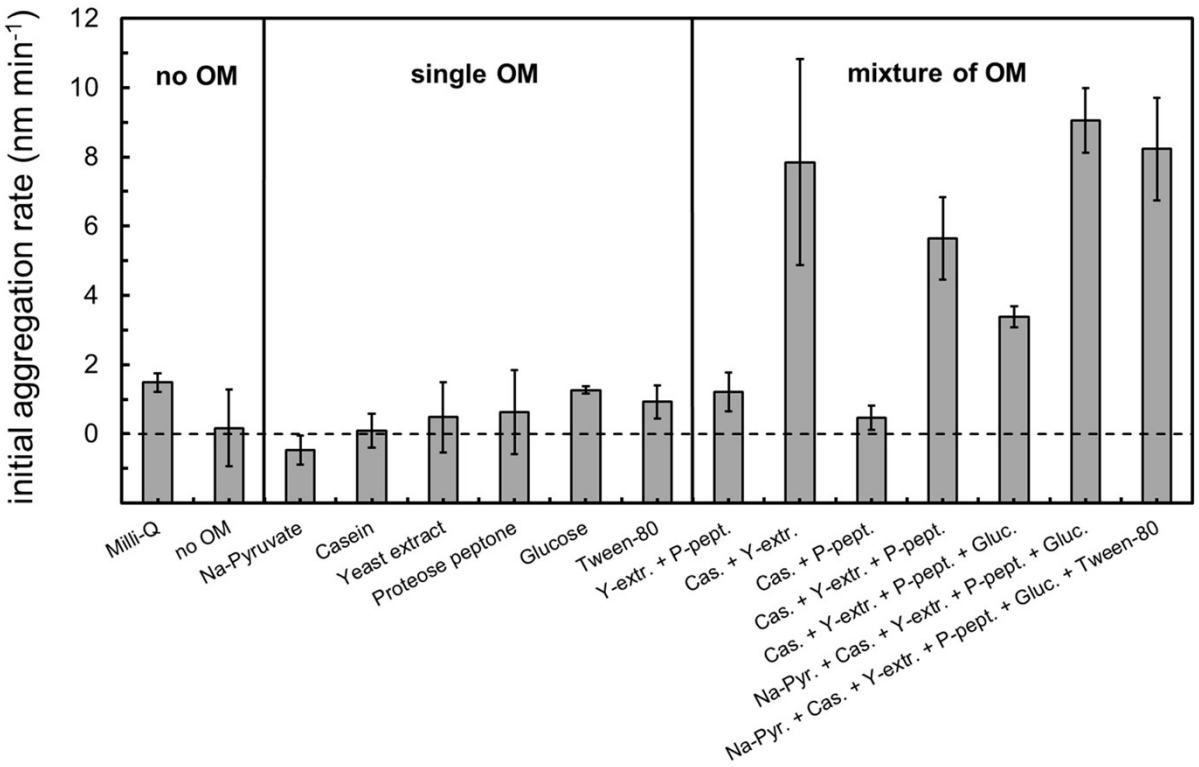

Figure S7. Mean initial aggregation rates of 30 nm Ag NP in deionized water and in Mg^{2+}-free R2A medium in absence and presence of individual organic constituents of R2A and their mixtures. The horizontal dashed line shows the initial aggregation rate when no aggregation occurs. The error bars represent the standard deviations from three replicates. In the Figure following abbreviations are used: OM: organic matter, Y-Extr.: yeast extract, P-pept.: proteose peptone, Cas.: casein, Gluc.: glucose, Na-Pyr.: Na-Pyruvate.