Extracellular polymeric substances govern the surface charge of biogenic elemental selenium nanoparticles

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The SI contains 12 pages, 6 figures and 1 table.

ζ-potential measurements for EPS and BSA capped CheSeNPs loaded with Zn

EPS and BSA-capped CheSeNPs (50 mg L^{-1}) were produced and purified by dialysis as described in the manuscript. The pH of the EPS and BSA-capped CheSeNPs was changed to 7.3 using 1 M NaOH. 0.5 mL of $ZnCl_2$ was added to 5.0 mL of EPS and BSA-capped CheSeNPs to vary the Zn concentration from 50 to 1000 mg L^{-1}. The final pH of the EPS and BSA-capped CheSeNPs varied from 5.5 to 6.5. The ζ-potential of EPS and BSA-capped CheSeNPs loaded with Zn were measured in triplicates.

Analytics

SEM-EDXS

To characterize the surface morphology of the BioSeNPs, scanning electron microscopy (SEM) was performed using a S-4800 microscope (Hitachi) operated at an accelerating voltage of 10 kV. For qualitative chemical analysis of the BioSeNPs, energy-dispersive X-ray spectroscopy (EDXS) analysis was carried out by means of a conventional Si(Li) detector with S-UTW window (Oxford Instruments) attached to the SEM. Sample preparation was done by spreading a small amount of BioSeNPs solution over a piece of a silicon wafer, drying it for a few hours at room temperature and mounting the sample on an aluminum holder for SEM analysis.

ζ-potential and hydrodynamic diameter measurements

The ζ-potential and hydrodynamic diameter (HDD) were calculated by DTS software (Malvern Instrument) using electrophoretic mobility and dynamic light scattering measurements carried out at 22 °C by a Nano Zetasizer (Malvern Instruments) at a laser beam of 633 nm and a scattering angle of 173°. The refractive index of 2.6 for selenium was used in the HDD measurement.[1] As the concentration of selenium

nanoparticles in water was low, viscosity of water at 22 °C was used for the measurements. The general purpose algorithm in the DTS software was used for calculating the size distribution.

FT-IR spectroscopy

For IR spectroscopy, KBr pellets were prepared by mixing approximately 1 mg of the samples with 300 mg dried KBr and subsequent pressing for 2 minutes at 145,000 psi until clear pellets were obtained. The FT-IR spectra of BioSeNPs and CheSeNPs were carried out on a Bruker Vertex 70/v spectrometer equipped with a D-LaTGS-detector (L-alanine doped triglycine sulfate), over the range 4000-400 cm^{-1} in the transmittance mode, with a spectral resolution of 4 cm^{-1}. Each spectrum was averaged out over 64 scans.

Acid-base titration

To determine the pKs of BioSeNPs, acid-base titration was carried out using a Metrohm autotitrator unit. 0.1966 mg of BioSeNPs was used in a total volume of 30 mL with a background electrolyte concentration of 1 mM NaCl. The initial pH raised above 9.4 by addition of 0.102 M NaOH. The BioSeNPs were continuously stirred and flushed with nitrogen. The titration was carried by automatic addition of 0.1 mL of HCl (0.01214 M). The change in background ionic strength due to the addition of acid was less than 8%. For the control titration, Milli-Q water (18MΩ cm) at 1 mM of background electrolyte concentration was used.

Total organic carbon analyzer

The extracted EPS was characterized by total organic carbon and total nitrogen measurements using a Shimadzu TOC-VCPN analyzer. Prior to analysis, the samples

were filtered with 0.45 µm filters (Whatman, Dassel, Germany). The determined dissolved organic carbon was considered as the total organic carbon.

Fluorescence excitation and emission matrix spectroscopy

EPS was characterized for various components using a FluoroMax-3 spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ, USA). The samples were diluted to bring the dissolved organic carbon concentration below 1 mg L^{-1}. The fluorometer was operated and stabilized as described in Maeng et al.[2] The measurements were carried out at excitation and emission wavelengths of 200-400 nm and 300-500 nm, respectively.

TEM-EDXS

Transmission electron microscopy investigations were performed to locally analyze the microstructure and in particular the morphology of the EPS and BSA-capped CheSeNPs and CheSeNPs formed in the absence of EPS or BSA. An image-corrected Titan 80-300 microscope (FEI) operated at an accelerating voltage of 300 kV was used. For sample preparation, one droplet of nanoparticles suspended in water was deposited onto a 400 mesh Cu grid coated with a carbon support film. After drying in a desiccator at room temperature and covering with an additional carbon-coated Cu grid, the TEM specimen was placed into a double-tilt analytical holder to perform the TEM analyses.

Figures and tables

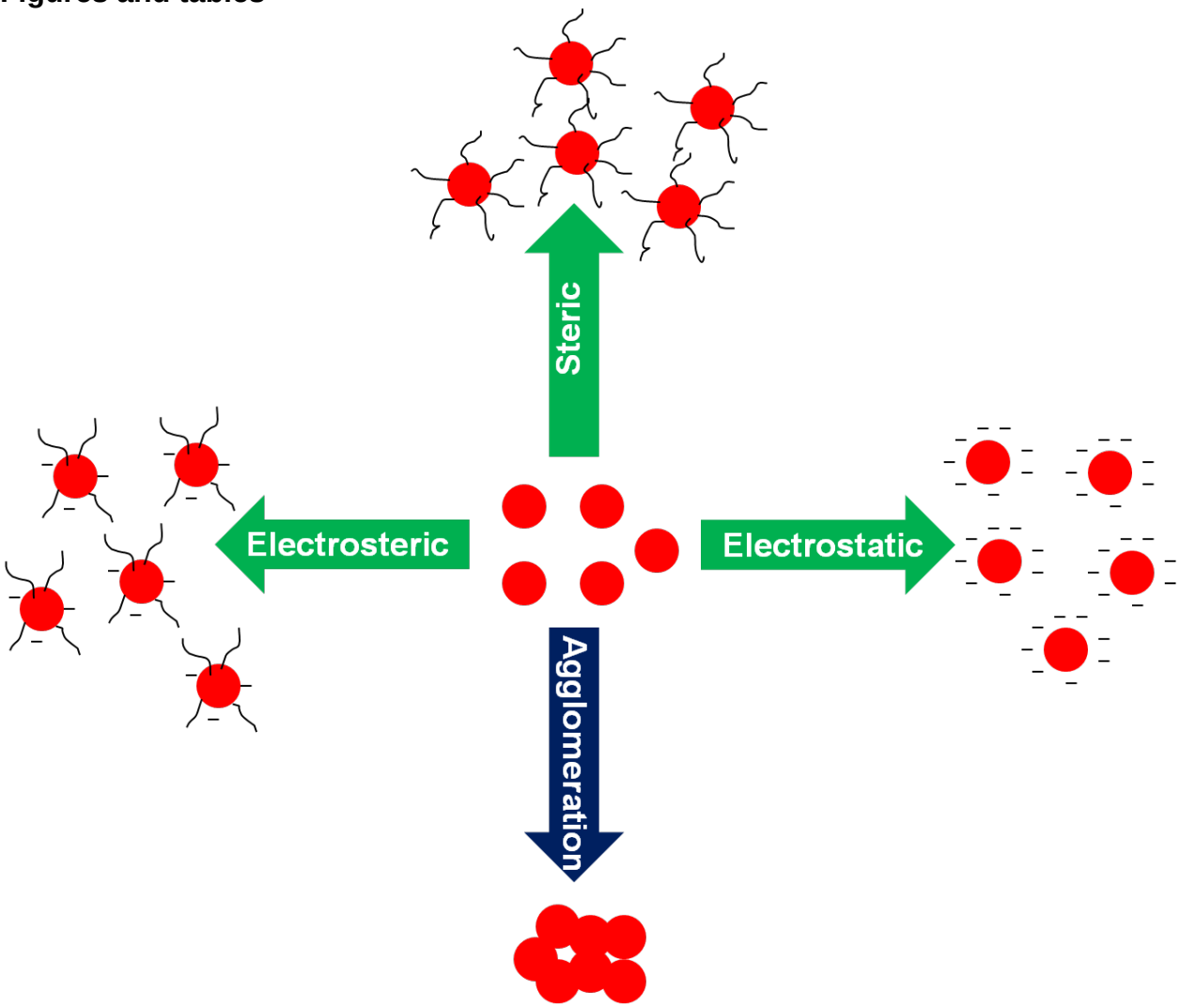

Figure S1. Graphical representation of various stabilization mechanisms of the nanoparticles inhibiting agglomeration.

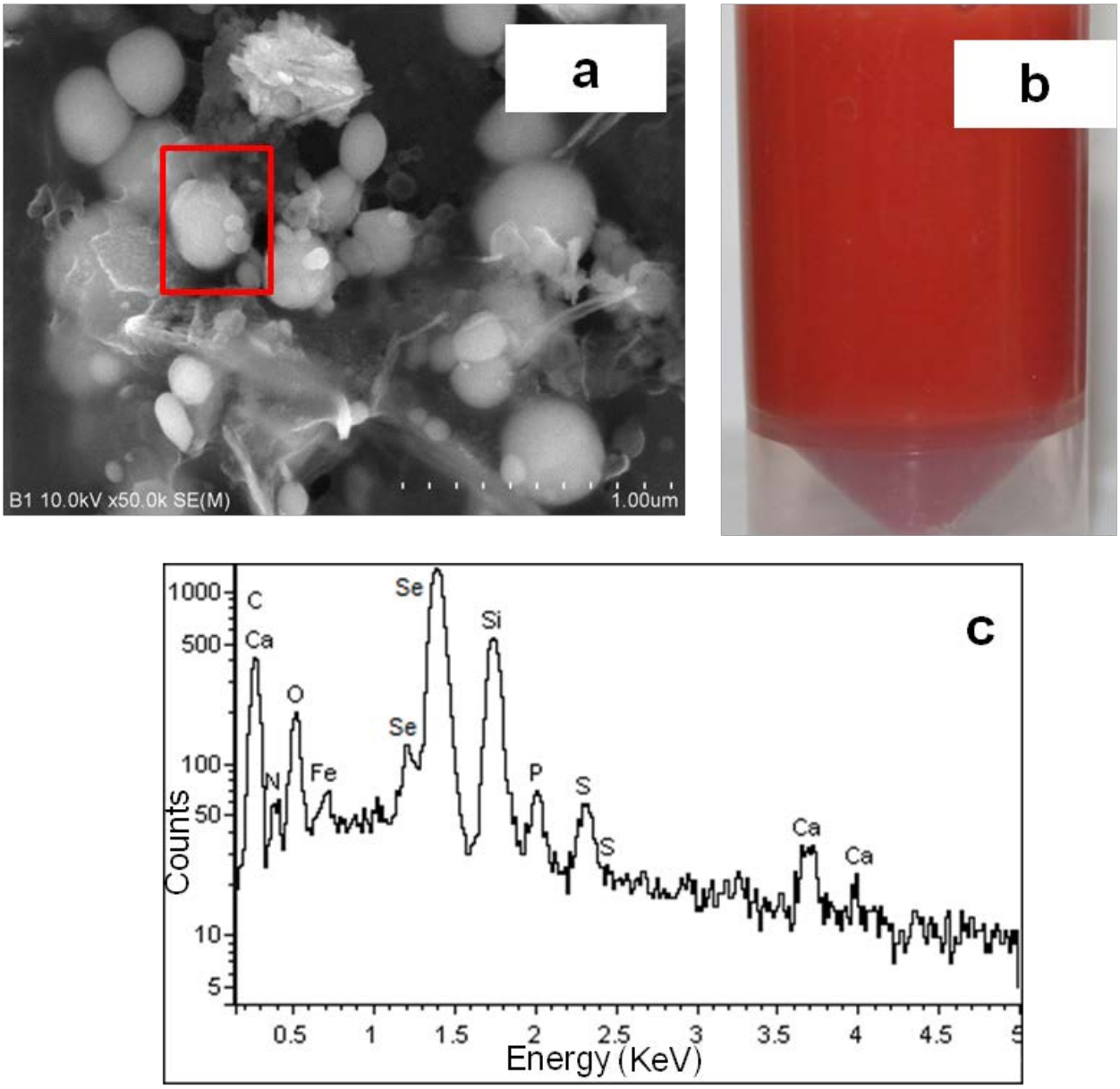

Figure S2. Secondary electron SEM images of (a) BioSeNPs, (b) colloidal suspension of BioSeNPs and (c) representative EDX spectra confirming the presence of selenium in BioSeNPs from the area marked by red square in (a). Please note that the samples were deposited onto a piece of Si wafer.

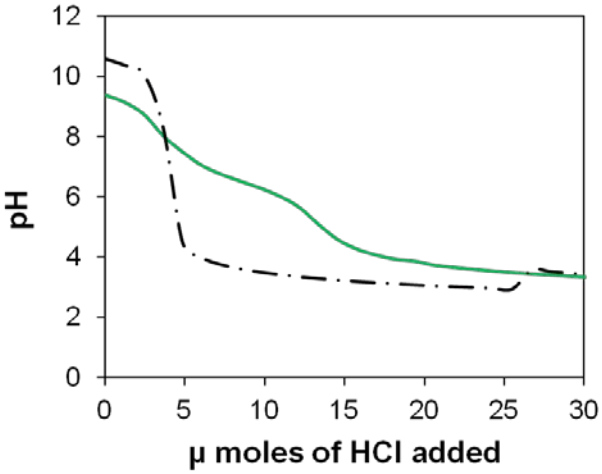

Figure S3. Acid-base titration of BioSeNPs produced at 30 °C (—) and MQ water as control (—·—·—).

Figure S4. 3D fluorescence spectra of extracted EPS: (a) confirming the presence of aromatic proteins and soluble microbial byproduct by observing a maxima at excitation and emission wavelength of 230/370 nm and excitation and emission wavelength of 300/370 nm, respectively; and (b) further aromatic proteins by observing a maxima at excitation and emission wavelength of 230/330 nm.

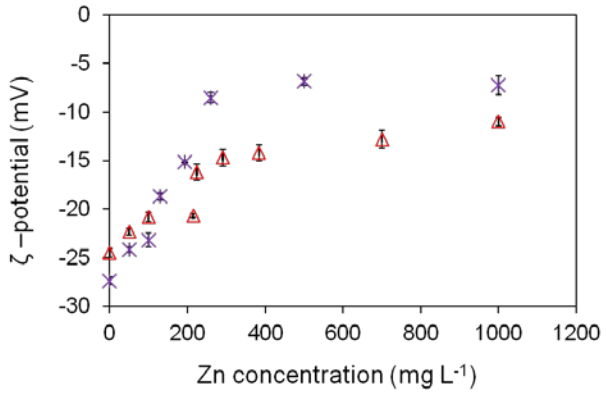

Figure S5. ζ-potential variation of BSA (∗) and EPS (Δ) capped CheSeNPs with increasing Zn concentrations.

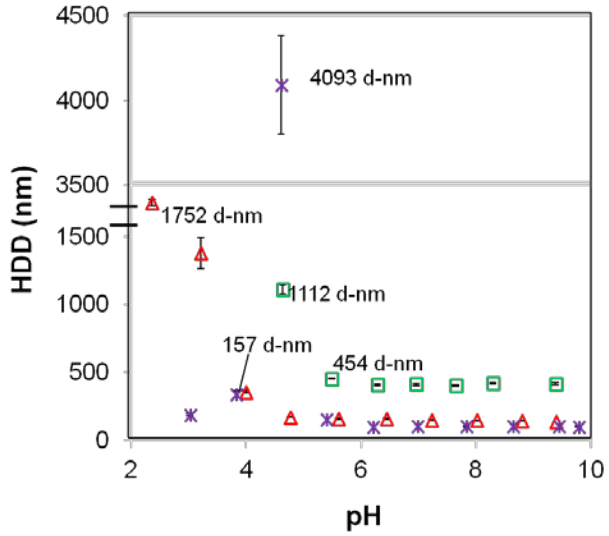

Figure S6. Hydrodynamic measurements were carried out for BioSeNP (□), EPS capped CheSeNPs (Δ) and BSA capped CheSeNPs (∗) versus pH at 10 mM NaCl background electrolyte concentrations.

Table S1. Assignments of various functional groups to different features (cm^{-1}) in the FT-IR spectra of BioSeNPs, EPS, EPS capped CheSeNPs, BSA and BSA capped CheSeNPs.

Functional groups	BioSeNPs	EPS	EPS capped CheSeNPs	BSA	BSA capped CheSeNPs	Ref.
-O-H, -N-H	3404-3270	3400, 3062	3420, 3062	3315, 3063	3283, 3063	3
-C-H	2959, 2928, 2866	2962, 2932	2928	2959, 2932, 2866	2955, 2934, 2865	4
-COOH	1720	1720	1720	–	1720	
-C=O Amide-I	1646	1653	1646	1654	1654	3
-N-H Amide-II	1542	1530	1537	1532	1540	4
$-CH_3$/$-COO^-$ antisymmetric	1460	1452	-	1451	-	5
$-COO^-$ symmetric	1394	1388	1404	1390	1401	5
-C-N, -N-H,	1242	1236	1243	1242	1238	4

P=O						
-P–O	1151	1153	1151	1166	-	4
-C-O-C, -C-H	1073 - 1038	1077-1040	1077-1040	-	-	4, 5

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