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Electronic Supplementary Information

for

What Happens to the Silver Ions? – Nanoparticle Formation in an Artificial Digestion

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1. Experimental details

Materials. $AgNO_3$ was obtained from AppliChem, Ag_2S from Sigma Aldrich and AgCl from JTBaker. Ag_3PO_4, Ag_2SO_4 and AgSCN were produced by mixing stoichiometric amounts of $AgNO_3$ and KH_2PO_4, $NaSO_4$, and NaSCN, respectively. Chemicals for the artificial digestion were purchased from Merck, Sigma-Aldrich, JT Baker or AppliChem in the highest available purity (Table S1). The food components are available supermarket products: Native olive oil from Pietro Coricelli Spa, Sucofin skimmed milk powder from Tsi GmbH & Co. KG and Mondamin starch (corn starch) from Unilever. Ultrapure water was used for all preparations (Milli-Q, 18.2 mΩ at 25°C).

Artificial Digestion. The *in vitro* digestion was adapted from our previous study of silver nanoparticles.[1] Silver nitrate (4.7 mg, 0.028 mmol) was dissolved in water (1 mL). The solution was added to artificial saliva (7.5 mL, pH = 6.4). The mixture was incubated for 5 minutes in a water bath at a temperature of 37 °C. This was followed by the addition of artificial gastric juice (17.5 mL) and the adjustment of the pH to a value of 2 with a solution of 5 wt% HCl. The mixture was incubated at 37 °C for 2 h. After adding artificial intestinal juice (25 mL) the pH was adjusted to 7.5 by adding $NaHCO_3$. The digestive mixture was incubated at a temperature of 37 °C for 2 h. The ingredients of the artificial digestion juices are listed in Table S1. Additionally the *in vitro* digestion was performed in presence of a mixture of food components (starch, oil, skimmed milk powder; 50 mg of each). As a control experiment water without $AgNO_3$ underwent a separate artificial digestion.

Table S1. Composition of the artificial digestion juices for saliva, stomach and intestine.

Compound	Supplier	Amount
	Saliva	
NaCl	NeoLab	12.5 mg
NaSCN	Carl Roth	3.8 mg
$Na_2SO_4 \cdot 10H_2O$	AppliChem	13.7 mg
$NaHCO_3$	NeoLab	3.8 mg
KCl	NeoLab	11.3 mg
KH_2PO_4	AppliChem	15 mg
$CaCl_2 \cdot 2H_2O$	NeoLab	3.8 mg
Uric acid	AppliChem	2.5 mg
Urea	AppliChem	0.3 mg
Mucin	Sigma-Aldrich	18.8 mg
α-Amylase	Sigma-Aldrich	6.3 mg
	Gastric juice	
NaCl	NeoLab	72.5 mg
KCl	NeoLab	17.5 mg
KH_2PO_4	AppliChem	6.7 mg
Mucin	Sigma-Aldrich	75 mg
Pepsin	AppliChem	25 mg
	Intestinal juice	
NaCl	NeoLab	7.5 mg
$CaCl_2 \cdot 2H_2O$	NeoLab	12.5 mg
$MgCl_2 \cdot 6H_2O$	NeoLab	5 mg
$NaHCO_3$	NeoLab	25 mg
Bile extract porcine	Sigma-Aldrich	225 mg
Pancreatin	Sigma-Aldrich	225 mg

Trypsin	AppliChem	7.5 mg
Urea	AppliChem	7.5 mg

Small-angle X-ray scattering (SAXS). SAXS measurements were performed in a flow through capillary with a Kratky-type instrument (SAXSess from Anton Paar, Austria) at 21 ± 1 °C. Samples analyzed with SAXS were used as prepared and measured for 20 minutes (120 measurement frames averaged over 10 s). The measured intensity was converted to absolute scale according to Orthaber et al.[2] The scattering vector q depends on the wavelength λ of the radiation (λ = 0.154 nm): thus $q = 4\pi/\lambda \sin\theta$. Deconvolution (slit length desmearing) of the SAXS curves was performed with the SAXS-Quant software (Anton Paar, Austria). Curve fitting was conducted with the software McSAS (version 1.0.1).

Wide-angle X-ray scattering (WAXS). WAXS measurements were performed on a Nano-inXider from Xenocs with a solid sample holder unit. The digestive samples were purified by washing them three times with water, followed by centrifugation (6000 rpm, rotor diameter = 19.8 cm, 5 min). The resulting residue was dried at 40 °C. The digestive samples and silver salts were measured for 20 min at medium resolution. The measurements are background corrected with the respective empty sample holder position.

IR spectroscopy (DRIFTS). IR measurements were performed on a Nicolet 6700 from Thermo Scientific with a Praying Mantis™ diffuse reflection accessory from Harrick. The samples were centrifuged for 5 min (6000 rpm, rotor diameter = 19.8 cm). The supernatant was removed and the residue was washed with 10 mL water. This washing procedure was repeated three times. Afterwards the residue was dried at 40 °C for 24 h. The dried samples were pestled with KBr and filled in the diffuse reflection accessory.

2. SAXS and WAXS data

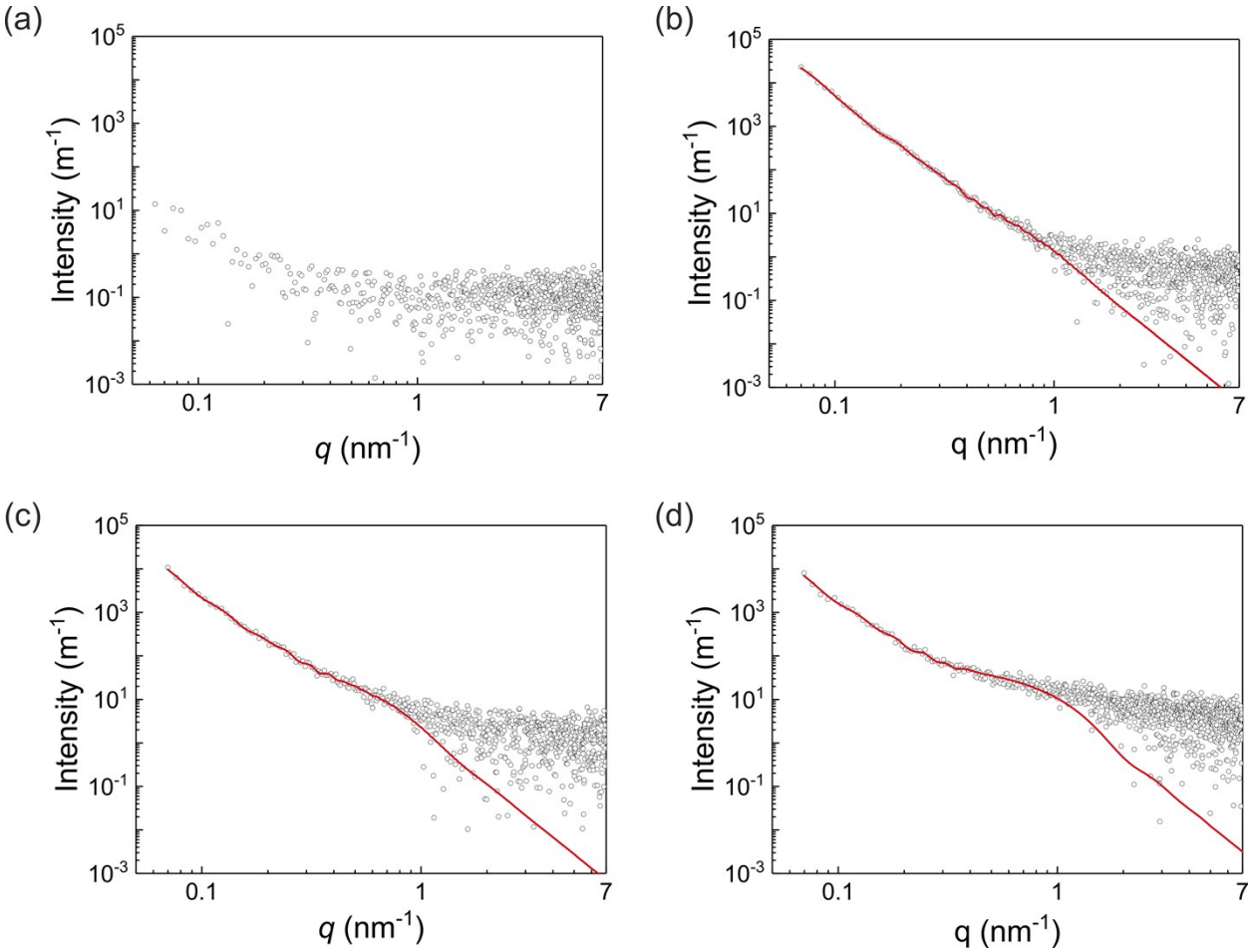

Figure S1. Measured SAXS curves (black symbols) of (a) undigested $AgNO_3$ in water, (b) digestion of $AgNO_3$ in saliva, (c) digestion of $AgNO_3$ in stomach and (d) digestion of $AgNO_3$ in intestine. The measured data are fitted with a Monte Carlo based data evaluation (McSAS[3], red solid lines).

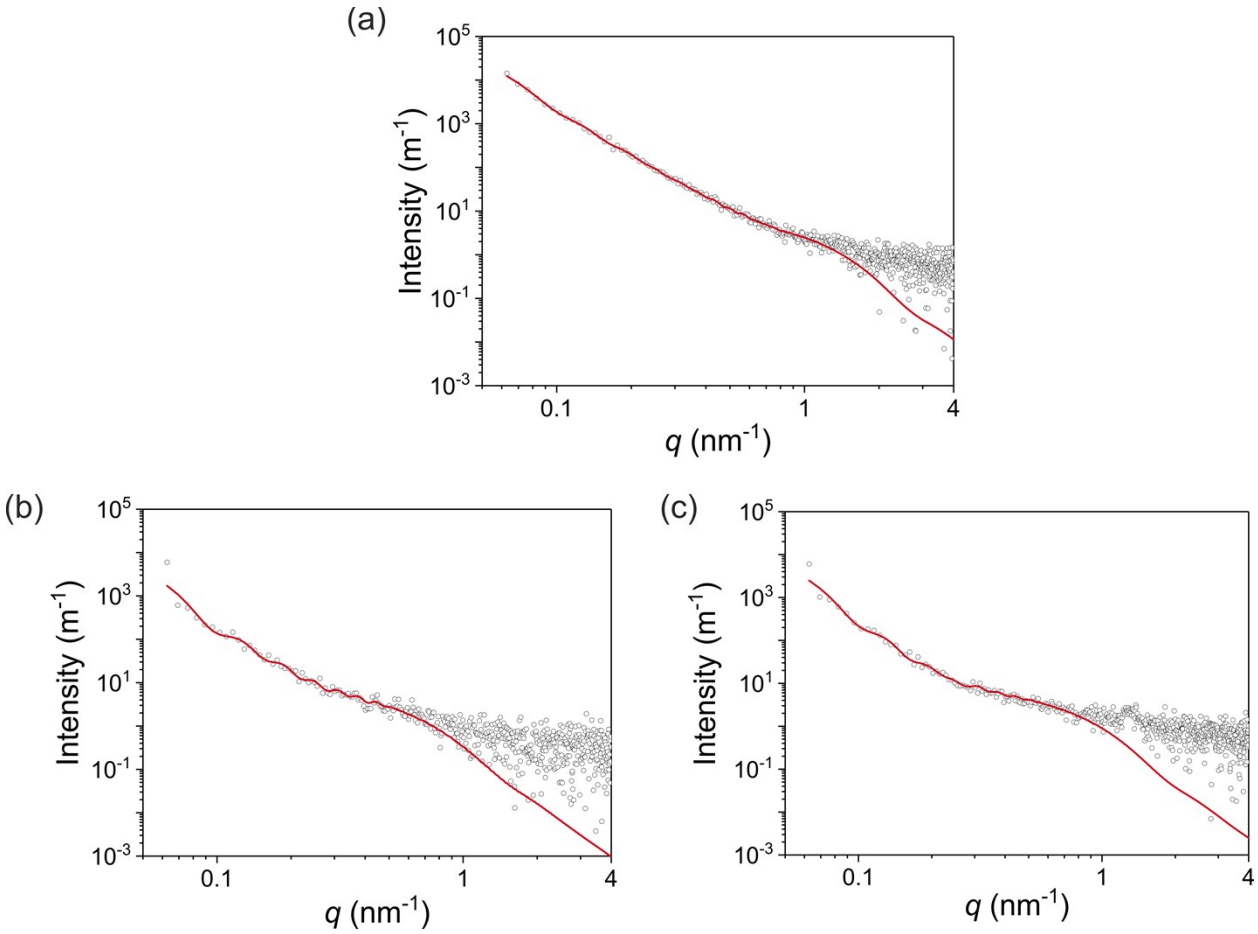

Figure S2. Measured SAXS curves (black symbols) of (a) digestion of $AgNO_3$ + food components (starch, oil, skimmed milk powder) in saliva, (b) digestion of $AgNO_3$ + food components in stomach and (c) digestion of $AgNO_3$ + food components in intestine. The measured data are fitted with a Monte Carlo based data evaluation (McSAS[3], red solid lines).

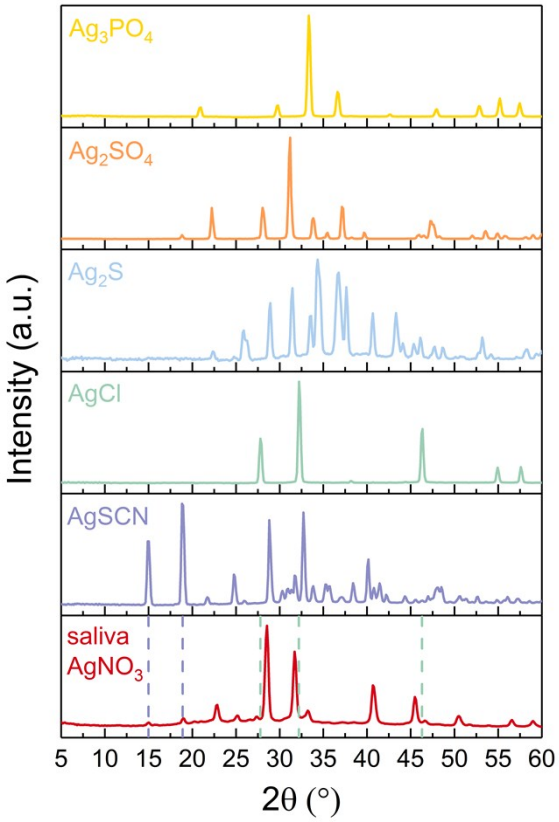

Figure S3. Comparison of the WAXS curves of the particles formed in saliva from $AgNO_3$ (red) with the silver salts AgSCN (violet), AgCl (green), Ag_2S (blue), Ag_2SO_4 (orange) and Ag_3PO_4 (yellow). The positions of the most intensive peaks of AgSCN and AgCl are marked with dashed lines (violet and green, respectively) in the WAXS curve of saliva.

3. References

1. C. Kästner, D. Lichtenstein, A. Lampen and A. F. Thünemann, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2017, **526**, 76-81.
2. D. Orthaber, A. Bergmann and O. Glatter, *Journal of Applied Crystallography*, 2000, **33**, 218-225.
3. I. Bressler, B. R. Pauw and A. F. Thunemann, *Journal of Applied Crystallography*, 2015, **48**, 962-969.