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Monitoring the fate of small silver nanoparticles during artificial digestion

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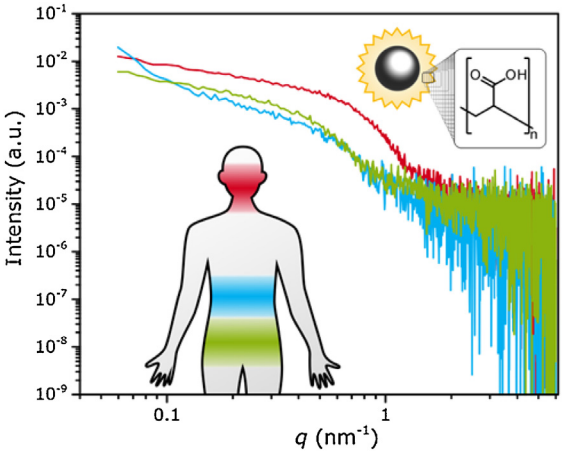
HIGHLIGHTS

- SAXS is useful for determination of particle size distributions in digestion fluids.
- Silver nanoparticles without food components aggregate strongly during digestion.
- Sizes of silver nanoparticles are constant in saliva and stomach but particles were etched in the intestine.
- Milk powder is an excellent colloidal stabilizer and prevents aggregation and etching during digestion.

GRAPHICAL ABSTRACT



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ABSTRACT

We report on the results of an *in vitro* digestion study of silver nanoparticles in presence and absence of food. The particles were poly(acrylic acid) stabilized ultra-small silver nanoparticles with a radius of 3.1 nm and a relative size distribution width of 0.2. As food components oil, starch, skimmed milk powder and a mixture thereof were chosen. Aggregation of the particles was quantified with small-angle X-ray scattering in terms of log-normal radii distributions. Complete aggregation of the primary particles was determined in the absence of food. In contrast, the presence of oil and starch initiates a disaggregation in the intestine. Only small aggregates of 6 nm radii and aggregation numbers of 7 were found in the presence of milk powder. It prevents primary particles from etching in the gastric and intestinal juice. Our results indicate that the silver nanoparticles can pass the digestion process in a nanoscale form but undergo a strong and food-dependent transformation in their state of aggregation.

1. Introduction

The use of silver nanoparticles in consumer related products has significantly increased over the last decade due to their special antimicrobial properties [1]. The estimated global production

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Table 1
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

amounts to 55 tons per year [2]. Today silver nanoparticles are contained in a high variety of products from cosmetics like tooth pastes, to textiles, to children's toys and to dietary supplements [3]. The estimated humans' dietary intake of silver is 70–90 μg per day [5] which can be individually substantially higher due to the widespread application of silver nanoparticles. The unique properties of the nanoparticles are strongly depending on the surrounding conditions. Temperature, pH and salts can alter the particle characteristics by changing the size and size distribution through aggregation [6,7]. Additionally the surface chemistry and the interaction with biomolecules are crucial factors in determining the properties of nanoparticles [8]. For the different uptake mechanisms this is especially important for orally ingested particles. Thereby, the particles pass different digestion steps where physicochemical parameters like the pH are shifted over a wide range. The steps of an *in vivo* digestion contain the introduction of the nanoparticles in the saliva which is followed by the exposure to the gastric and intestinal juices. Hence, different mechanisms are possible, which include aggregation, enhanced ion release or *de novo* particle formation [9,10].

Most studies regarding the toxicological potential of silver nanoparticles are focused on their intestinal uptake and interaction with different cell types like M-cells or Caco-2-cells [11]. But considering different altering mechanisms during the digestion process the question arises whether the silver particles can pass the different digestion steps in a nanoscale form. These complex interactions between particles and the surrounding conditions can only be investigated in an *in vitro* model. Therefore, as previously reported [12], we used an artificial digestion procedure, which is based on the German standard DIN 19378 [13]. We adjusted this procedure in the present study for the digestion of silver nanoparticles (Fig. 1). It simulates the three steps of the gastro-intestinal passage by mimicking the oral, gastric and small intestinal conditions. These contain all relevant factors for a realistic environment such as pH changes, transit times between the different digestion steps, enzymes and digestive juices. Furthermore, the addition of food components completes the simulation of a realistic digestion process in a human body. Previous studies only considered the digestion without food components [10]. Detailed investigations regarding the influence of food components on changes of the size distribution of silver nanoparticles are still lacking [14].

We synthesized ultra-small silver nanoparticles with a core radius of 3 nm and poly(acrylic acid) as stabilizer for their application in the artificial digestion process. To evaluate possible changes in their characteristics, including core sizes, shapes and size distributions, we used small-angle X-ray scattering (SAXS). The advantages of this technique are (i.) an easy sample preparation, (ii.) a non-destructive measurement and (iii.) a statistically representative particle ensemble average. The silver particles can be measured directly in the saliva, in the gastric or the intestinal juice without purification or separation from the juices [10]. The measurement time amounts to 20 min. Additionally due to the use of X-rays with a wavelength of 0.154 nm the characterization of dispersed nanoparticles with radii from 1 nm to 60 nm is possible [15].

The aim of this study is to reveal the impact of digestion on the colloidal stability of very small silver nanoparticles for two different scenarios: Digestion with and without food additives. As representative food components we chose oil, starch, skimmed milk powder and a mixture of these three components in order to mimic realistic food surroundings.

2. Materials and methods

2.1. Materials

For the synthesis of nanoparticles silver nitrate was obtained from AppliChem, poly(acrylic acid) (M_w = 1800 g/mol) from Sigma Aldrich, ethylene glycol from Acros and sodium hydroxide from Fisher Scientific. Chemicals for the artificial digestion were purchased from Merck, Sigma-Aldrich, JT Baker or AppliChem in the highest available purity (Table 1). 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).

2.2. Preparation of nanoparticles

For the synthesis of silver nanoparticles silver nitrate and poly(acrylic acid) (M_w = 1800 g/mol) were used in a modified polyol process according to Hu et al. [16]. For the work-up phase the procedure was changed. Therefore no centrifugation was necessary and the particles were washed three times with excessive slight acidic water (pH 5). After washing the particles they settled over night and the supernatant was separated by decantation. The particles were dispersed in water and the pH was adjusted to a value of 11 with a solution of 5 wt% NaOH. The particles were characterized by SAXS and used in an artificial digestion process.

2.3. Artificial digestion

The *in vitro* digestion was applied as described previously by Lichtenstein et al. [12]. Firstly 1 mL of nanoparticle dispersion (3 g/L) was added to 7.5 mL artificial saliva with a pH of 6.4. The mixture was incubated under stirring for 5 min in a water bath at a temperature of 37 °C. After adding 17.5 mL of artificial gastric juice the pH was adjusted to a value of 2 with a solution of 10 wt% HCl and the suspension was incubated at 37 °C for 2 h. In the last step the addition of 25 mL of artificial intestinal juice was followed by adjusting the pH to 7.5 by adding $NaHCO_3$. The suspension was incubated under stirring at a temperature of 37 °C for 2 h. The ingredients of the artificial digestion juices are listed in Table 1. The *in vitro* digestion was additionally conducted in presence of the food