



# A study on the in vitro percutaneous absorption of silver nanoparticles in combination with aluminum chloride, methyl paraben or di-n-butyl phthalate



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## HIGHLIGHTS

- Citrate, PEG stabilized silver nanoparticles minimally penetrate pig skin in vitro.
- Aluminum chloride, methyl paraben, butyl phthalate do not modify the penetration.
- Laser ablation-ICP-MS is a useful technique for studying skin sections.

## ARTICLE INFO

### Article history:

Received 26 October 2016

Received in revised form 6 March 2017

Accepted 8 March 2017

Available online 14 March 2017

### Keywords:

Silver nanoparticles  
Percutaneous absorption  
Aluminum  
Methyl paraben  
Di-n-butyl phthalate  
Laser ablation ICP-MS

## ABSTRACT

Some reports indicate that the silver released from dermally applied products containing silver nanoparticles (AgNP) (e.g. wound dressings or cosmetics) can penetrate the skin, particularly if damaged. AgNP were also shown to have cytotoxic and genotoxic activity. In the present study percutaneous absorption of AgNP of two different nominal sizes (Ag15 nm or Ag45 nm by STEM) and surface modification, i.e. citrate or PEG stabilized nanoparticles, in combination with cosmetic ingredients, i.e. aluminum chloride (AlCl<sub>3</sub>), methyl paraben (MPB), or di-n-butyl phthalate (DBPH) was assessed using in vitro model based on dermatomed pig skin.

The inductively coupled plasma mass spectrometry (ICP-MS) measurements after 24 h in receptor fluid indicated low, but detectable silver absorption and no statistically significant differences in the penetration between the 4 types of AgNP studied at 47, 470 or 750 μg/ml. Similarly, no significant differences were observed for silver penetration when the AgNP were used in combinations with AlCl<sub>3</sub> (500 μM), MPB (1250 μM) or DBPH (35 μM). The measured highest amount of Ag that penetrated was 0.45 ng/cm<sup>2</sup> (0.365–0.974 ng/cm<sup>2</sup>) for PEG stabilized Ag15 nm + MPB.

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## 1. Introduction

Wide application of nanomaterials in skin cosmetics raises reasonable questions about consumer safety. There is an ongoing

debate on capability of nanomaterials to penetrate through the skin. Some reports indicate such possibility, particularly through damaged skin. Quantum dots QD-565 and QD-655 have been shown to penetrate into the dermis of abraded rat skin (Zhang and Monteiro-Riviere, 2008). Peptide-functionalized fullerenes translocated to the intercellular space of the stratum granulosum layer of flexed but not to unflexed excised porcine skin (Rouse et al., 2007).

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Silver nanoparticles (AgNP) are the most popular advertised nanomaterial present in 438 of the 1814 (24%) products listed in the Nanotechnology Consumer Product Inventory (Vance et al., 2015). Very comprehensive database on products containing nanomaterials, updated daily, “The Nanodatabase” (<http://nanodb.dk/en/search-database>) currently lists 2601 nanoproducts of which 400 are registered as nanosilver containing products (accessed on 2017.02.21). The products are from different categories: health and fitness, cosmetics, personal care, or even food, beverage supplements, cooking. Another inventory maintained by two European consumer organizations, ANEC/BEUC (<http://www.beuc.eu/safety/nanotechnology>; accessed on 2017.02.21) lists nanosilver containing products which come in contact with the skin, like cleansers, crèmes, moisturizers, antiperspirants, washing lotions, soaps, foot balms or shoe deodorants. The majority of AgNP applications results from their strong antibacterial properties (Durán et al., 2015a) primarily linked to release of silver ions.

Available literature data on percutaneous absorption of AgNP mainly concerns studies with medical products, like dressings containing AgNP used to treat wounds, burns, and scars. Some reports strongly indicate that the silver released from these products can penetrate the skin, particularly if damaged (Vlachou et al., 2007; Wang et al., 2009; Moiemien et al., 2011; Pfurtscheller et al., 2014). The studies on percutaneous absorption through normal skin provide evidence for small but detectable penetration of silver nanoparticles (Laresse et al., 2009; Bianco et al., 2014, 2015). When nanosilver dressings were applied to healthy patients with normal intact skin approximately 5 days prior to surgery, AgNP penetrated intact skin and could be identified as deep as the reticular dermis (George et al., 2014). Zhu et al. (2015) using surface-enhanced Raman scattering (SERS) microscopy observed that the penetration depth of poly-N-vinylpyrrolidone AgNP dispersed in pure water exceeded the stratum corneum (SC) thickness of porcine ear, however it was not clear if it was due to penetration of trace amounts of AgNP through the SC barrier or the silver presence inside the hair follicles.

As the cosmetics are usually mixtures of many ingredients, there is a possibility of modification of percutaneous absorption of AgNP by the ingredients. This question becomes more relevant when some of the formulations are applied on micro-abraded skin, like underarm roll-on deodorants or antiperspirants, where the region of the skin may be regularly shaved. For example, it is not known how the presence of aluminum salts in antiperspirants can influence AgNP absorption. Many effective antiperspirants currently available on the market such as NIVEA, Dove or AXE contain aluminum salts (e.g. aluminum chloride, chlorohydrate or aluminum zirconium tetrachlorohydrate gly) at concentrations reaching 20%. Similarly, no data is available on a potential modification of AgNP absorption by other popular cosmetic ingredients, like parabens or phthalates. Although, cosmetic companies try to diminish content of parabens in their products, many formulations applied on skin still contain one or more of them. For example, NIVEA uses parabens in around 30% of its products (<https://www.nivea.co.uk/advice/nice-skin/parabens-in-cosmetics>), primarily in body care, sun protection and face care products. Methyl paraben (MPB) is the most frequently used antimicrobial preservative (Soni et al., 2002) and shows the highest solubility in water compared to other parabens. According to the Scientific Committee on Consumer Safety (SCCS), MPB used as preservative in cosmetics at the maximum authorized concentrations (0.4% for one ester or 0.8% when used in combination) is considered safe for human health (SCCS, 2010, 2013). Phthalates have been banned in the European Union under the Cosmetics Directive as CMR substances, however they are allowed as traces and/or impurities, not used intentionally in the perfumes at up to 100 ppm total or per

substance (SCCP, 2007). Dibutyl phthalate (DBPH) was found in a variety of consumer products (Koniecki et al., 2011).

As in the open literature there is no information on potential influence of different cosmetic ingredients on percutaneous absorption of AgNP, in the present study we conducted a series of such measurements using in vitro model based on isolated pig skin. Porcine skin is a very good model representing penetration of human skin because it has thickness and absorption rates comparable to those of human skin (Monteiro-Riviere and Riviere, 1996). We used AgNP of different nominal size (15 nm or 45 nm) and surface modification, i.e. citrate or PEG stabilized nanoparticles, in combination with aluminum chloride (AlCl<sub>3</sub>), methyl paraben (MPB), or dibutyl phthalate (DBPH). For detection of silver in different matrices (receptor fluid and histopathological skin sections) powerful techniques based on ICP-MS and laser ablation ICP-MS were applied (Becker et al., 2014).

## 2. Materials and methods

### 2.1. Chemicals and reagents

Nitric acid (65%, pure P.A. #529603115) was purchased from POCH (Poland). Aluminum chloride (AlCl<sub>3</sub>, #06220), methyl paraben (MPB, #H6654), di-n-butyl phthalate (DBPH, #524980), Nile Red (#72485), 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, #32670), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide #M5655), Hanks' Balanced Salt Solution (HBSS without phenol red, pH 7.4, #H9269; KCl 0.4, KH<sub>2</sub>PO<sub>4</sub> 0.06, NaCl 8.0, NaHCO<sub>3</sub> 0.35, Na<sub>2</sub>HPO<sub>4</sub> 0.04788 g/L) were from Sigma-Aldrich, Co.

Silver nitrate (AgNO<sub>3</sub>, 99.999% metals basis), PEG [poly(ethylene glycol) methyl ether thiol, Mw=6000] were purchased from Aldrich. Sodium citrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>·2H<sub>2</sub>O, ≥99%) was bought from Sigma-Aldrich. Tannic acid (C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>, pure), sodium borohydride (NaBH<sub>4</sub>, ≥99%) were purchased from Fluka.

### 2.2. Synthesis and physico-chemical characterization of silver nanoparticles

#### 2.2.1. Citrate stabilized Ag 45 nm

##### 2.2.1.1. Citrate stabilized Ag 45 nm colloid synthesis.

To the aqueous solution of silver nitrate (94.5 g, 0.017 wt%), stirred and heated to the boiling point under reflux, a warm mixture of tannic acid (1.3 g, 5 wt%) and sodium citrate (4.2 g 1 wt%) was added. After a few seconds the solution colored to brown (formation of AgNP). The mixture was stirred for additional 15 min under reflux and next cooled down to room temperature. The silver colloid was stored in darkness. The concentration of silver was 100 ppm.

##### 2.2.1.2. Citrate stabilized Ag 45 nm colloid purification.

The synthesized colloid was purified to remove tannins by centrifugation (three times; rotation centrifugal force RCF = 18,944 g; 30 min). Twice removed supernatant was replaced with the aqueous solution of sodium citrate (0.03 wt%). In the last step of centrifugation the supernatant was removed and the colloid was concentrated to 750 ppm (concentration determined by the atomic adsorption spectroscopy (AAS); silver nanoparticles solubilized with 70% of nitric acid before measurement). Before the AAS measurements the colloid was filtered through a 0.1 μm polyvinylidene fluoride (PVDF) membrane. The final concentration of sodium citrate and tannic acid in the colloid was 2950 ppm and 8 ppm, respectively.

#### 2.2.2. PEG stabilized Ag 45 nm

AgNP were synthesized according to procedure described in citrate stabilized Ag 45 nm colloid synthesis. AgNP with the size of about 45 nm were modified with PEG-ligand [poly(ethylene glycol) methyl ether thiol, Mw=6000]. Briefly, the modification of NPs was carried out during the colloid purification stage (double centrifugation and next removed supernatant was replaced with deionized water). After the first step of purification process the colloid was modified with PEG ligand. The amount of PEG used for modification corresponds to 10 ligand molecules per 1 nm<sup>2</sup> of NP surface (shape of NPs – spherical; size of NPs – 45 nm; NPs concentration – 100 ppm). After the modification the colloid was left for 24 h. Next, the colloid was purified by double centrifugation (RCF = 18,944 g, 30 min) from the excess of unbound PEG-ligand and concentrated to 750 ppm. After the first centrifugation the supernatant was replaced with deionized water and after the second centrifugation the supernatant was removed. In the next step the colloid was filtered through a 0.1 μm polyvinylidene fluoride (PVDF) membrane. The final

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