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Pilot study on the identification of silver in skin layers and urine after dermal exposure to a functionalized textile

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ABSTRACT

Silver (Ag) is increasingly used in consumer products like functionalized textiles and medical devices owing to its strong antimicrobial activity which is largely assigned to Ag ions released after oxidation of metallic Ag. To increase generation of Ag ions, in various products Ag is often present as nanoparticles. Ideally, Ag ions would remain on the surface of the skin to combat the bacteria and the uptake of Ag into the body should be limited. However, the Ag ions might penetrate across the skin into the body leading to adverse health effects. Data on in vivo uptake of Ag due to dermal exposure are scarce partly caused by the lack of suitable analytical approaches for the determination of Ag in biological matrices, but strongly needed to enable risk assessment of skin exposure to (nano) Ag containing products.

With the developed approach, the presence of Ag in a functionalized textile is confirmed by using scanning electron microscopy (SEM). After in vivo dermal exposure to Ag containing textile material under "in use" exposure scenarios, the outermost layers of the skin (*Stratum Corneum, SC*) were sampled by using adhesive tapes with a size of 3.8 cm^2. Different leaching and dissolution procedures of Ag from biological samples prior analysis by inductively coupled plasma mass spectrometry (ICPMS) have been evaluated. The developed method results in a limit of detection (LOD) of 2 ng Ag per removed *SC* layer. The method allows the measurement of the Ag concentrations at different depths of the *SC* enabling the deduction of the percutaneous penetration kinetics.

Due to the possible bio distribution within the whole body, an indirect exposure matrix (urine) was studied too. The detection power of the method permits measuring the ultra-trace concentrations of Ag in urine before and after dermal exposure; LOD is 0.010 μg Ag/L urine.

1. Introduction

Many engineered, inorganic nanomaterials are used for biomedical applications, consumer and personal care products. Therefore, it is crucial to understand and effectively manage safety and potential human health risks of these products [1]. Silver (Ag) particles are the most common nanomaterials mentioned in product descriptions and as claimed by the Nanotechnology Consumer Products Inventory, they are used in more than 400 consumer products [2,3]. However, nanomaterials may be released from their matrix during its use and especially Ag nanoparticles have a strong release of Ag ions that is responsible for a broad spectrum of antimicrobial activity [4,5]. In close contact with the skin, it is known that various products, e.g. textiles [6], may release Ag that can penetrate the skin, mainly if damaged [7].

Atomic spectrometry leads at the moment to advances in the analysis of nanomaterials; while Ag appears to be the most popular element for investigations [8]. Partly this follows from increased use of Ag nanoparticles and whether there might be any toxicity but also from where it is employed for its antibacterial properties, such as in dressings applied to large exposed wounds and in jug filters for drinking water purification [9]. Hyphenated techniques for the on-line separation of nano Ag of different diameter are dominated by asymmetric flow field flow fraction (AF4) coupled to by inductively coupled plasma mass spectrometry (ICPMS) for an elemental specific detection in aqueous matrices [10]. For the quantification of releasable pharmaceutical relevant nanoparticles in tap water and domestic waste water, analytical assessments were carried out [5].

Various human exposure routes to nano Ag are of interest; e.g. via inhalation [4] and food contact materials [11]. For related risk

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assessments, methods were successfully developed by ICPMS [12]. The bio distribution and kinetics of nano-Ag in in vivo animal test systems is studied after intravenous injection. A number of target tissues like e.g. liver, spleen, kidney, but also organs of special interest like brain, aortic samples are analyzed by ICPMS too [13]. By indirect exposure, skin was a less relevant tissue in these cases [10] and the dermal uptake of e.g. Ag as well as possible health effects are insufficiently known [14]. To assess the risk due to dermal exposure to a (nano) Ag containing product the ability of Ag to penetrate into and across the skin should be known. The in vitro penetration of Ag from a suspension of nanoparticles in artificial sweat has recently been investigated in fresh, cryopreserved, and glycerolized human skin grafts by using diffusion cells [7,15]. Larese et al. determined in vitro penetration of Ag through human skin by ICPMS [16]; however, no depth profiling is possible with this procedure.

Bioimaging of metals in tissue sections by laser ablation (LA)-ICPMS seems to be a powerful imaging (mapping) technique for depth profiling of elements [17]. However, skin samples must be taken by surgery which should not be a method of choice for operational testing of engineered, inorganic nanomaterials in consumer and personal care applications, like sprays and lotions. In addition, the skin sample pre-treatment prior LA-ICPMS can be problematic too.

The outermost skin layer, the so-called *Stratum Corneum* (SC), can be removed sequentially by repeated application of adhesive tape on a site of interest. With this procedure more information about the function of this skin layers as the main barrier for skin penetration will be obtained. The amount of *SC* removed is of relevance in establishing the concentration profile of released and penetration chemicals, e.g. nickel, after topical application [18,19]. With this approach, only a few micrometer of *SC* are removed in a nearly painless manner and no surgery is required for sampling of the outmost skin layer. In this present study, a new method consisting of skin layer sampling, sample pretreatment and analysis by ICPMS is developed for the measurement of the Ag uptake in direct skin contact. In addition, the developed approach for skin depth profiling gives also insights into the relation between the Ag amount and the protein amount per skin layer. Due to the possible bio distribution within the whole body, additionally indirect exposure matrices, which can be reached e.g. via the blood circulation, are of great interest [20]. Therefore, the ultra-trace concentrations of Ag in urine are studied too.

2. Experimental

2.1. Chemicals

Ultrapure water (H_2O) with a resistivity of $>18\ M\Omega\ cm$ (at 25 °C) was obtained from a Milli-Q Plus system (Millipore, Amsterdam, The Netherlands). Hydrochloric acid (HCl), 30%, ultrapur and nitric acid (HNO_3), 65%, ultrapur, were purchased from Merck, Darmstadt, Germany. Ammonium hydroxide (NH_4OH) (28–30%), analytical grade, was purchased from Sigma Aldrich, Milan, Italy. Calibration standard solutions of Ag and the solution of the internal standard related to the ICPMS measurement (chosen element: rhodium (Rh)) were made of single element stock solutions with a concentration of 1 mg/mL from Inorganic Ventures, Christiansburg, USA.

For the on-line addition of the internal standard, a dilution of 2 ug/L Rh in 2% HNO_3 was prepared.

2.2. Materials and samples

As functionalized textile material, so-called “silver sleeves” were used while two different fittings were tested; skinny fit versus loose fit. The “silver sleeves” are a prototype of an Ag coated medical garment intended for use in the treatment of Atopic Dermatitis. The garment contains 79% modal, 11% polyamide, 7% elastane and 3% silver. Metallic Ag oxidizes in contact with oxygen from the air. Subsequently, Ag ions react with the constituents from the sweat present on the skin surface. The amount of Ag released from the material into synthetic sweat, made of 0.5% sodium chloride, 0.1% urea, and 0.1% lactic acid in H_2O; pH 4.5 adjusted with ammonium hydroxide, was determined in another study, both after 8 and 24 h of soaking. The Ag amount revealed in the bathing solution was (19 ± 3) and (21 ± 3) μg Ag/g fabric [21]. These findings justify the use of Ag garment to combat relapsing superinfection caused by *Staphylococcus Aureus* in Atopic Dermatitis [22–24].

The presence of Ag particles in the silver sleeves was identified by SEM-EDX; see Fig. 1.

Volunteers with healthy skin have worn the “silver sleeves” on the forearm, 8 h a day for five consecutive days. On the other forearm a placebo sleeve was worn as skin blank control. Skin sampling took place after the first and fifth days of exposure. These experiments were carried out under the supervision of the Academic Medical Center (AMC), Coronel Institute of Occupational Health, Amsterdam, The Netherlands.

The human skin removal for the preparation of *SC* layers was taken by round adhesive tape discs (poly acrylate ester adhesive, 3.8 cm^2, D-Squame; CuDerm, Dallas, TX, USA). The tape discs were applied to the skin of the forearm on the sites of interest. Each tape disc was pressed for 10 s with standardized force of 225 $g\ cm^{-2}$ using a disc pressure applicator (CuDerm, Dallas, TX, USA) [25]. From each skin site of interest the entire *SC* was collected by approximately 20–25 consecutive tapes. Each tape disc was gently removed with tweezers and stored in a 1.5 mL Safe-lock tube (Eppendorf, Nijmegen, The Netherlands) at −20 °C until analytical pretreatment. The amount of *SC* proteins on the tape disc was determined by measuring the light absorption of the disc at about 850 nm (infrared radiation) using the D-Squame Scan 850A Instrument (CuDerm, Dallas, TX, USA) [26]. The concentration of Ag on most of the tape discs was determined after leaching and dissolution by ICPMS. Furthermore, a selected number of tape discs were used for the studies by SEM-EDX.

The spot urine samples have been collected from 30 subjects before exposure and after five days of wearing of “silver sleeves”. The urines were stored at −20 °C until analytical pretreatment and quantification.

2.3. Instrumentation

Before leaching Ag, a series of tapes was analyzed with Scanning Electron Microscope Energy Dispersive X-ray (SEM-EDX, type TM-3000 – equipped with Oxford Instruments X-ray Microanalysis Hitachi High-Technologies Europe GmbH, Krefeld, Germany) in order to evaluate the presence of silver nanoparticles.

For the pretreatment by leaching, an ultrasonic bath type Elmasonic S30H from Elma, Singen, Germany, is used. The elemental detection and quantification, is carried out by ICPMS. A quadrupole (Q)–ICPMS type 7500 CE, Agilent Technologies, Santa Clara, CA, USA, with integrated autosampler and a high resolution (HR)-ICPMS type ELEMENT XR, Thermo Fisher Scientific, Bremen, Germany, were utilized. Similar sample introduction systems consist of a concentric nebulizer, a cyclone spray chamber and nickel cones (all from the instrumental suppliers) were used. The operating conditions as well as the method and measuring parameters of the ICPMS instruments are summarized in Table 1.

Sample pretreatment and analysis were carried out in a clean-room facility class 10,000.