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Chemical characterisation, antibacterial activity, and (nano)silver transformation of commercial personal care products exposed to household greywater

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ARTICLE

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The objective of this study was to test the original speciation of silver (Ag) in eight different commercially available personal care products and investigate the chemical transformation of Ag during exposure to two types of synthetic greywater. The antimicrobial activity of the products was examined to determine the relationship between Ag content and speciation with the antibacterial functionality of the products. The Ag content of each product was quantified and X-ray absorption near-edge structure (XANES) analysis was used to investigate the initial speciation in the products and the changes occurring upon mixture with greywater. The results showed that the total Ag concentration in the products ranged from 17 to 30 mg kg^{-1}, and was usually below the value reported on the label. Analyses revealed the complexity of Ag speciation in these products and highlighted the importance of characterisation studies to help elucidate the potential risks of nano-Ag in the environment. The antibacterial results confirmed that the antibacterial efficacy of the products depends on the concentration, form and speciation of Ag in the products, but is also significantly affected by product formulation. For instance, many of the products contained additional bactericidal ingredients, making it difficult to determine how much of the bactericidal effect was due directly to the Ag content/species. This paper offers some suggestions for standard methodologies to facilitate cross-comparison of potential risks across different studies and nano-enabled products.

Environmental Significance

While a wide range of consumer products containing nanosized silver are on the market, most of the environmental research conducted so far has focussed on the fate of silver-functionalised textiles. Personal care products containing Ag have not been investigated. This work reports on the antibacterial properties of personal care products as related to the chemical species present in these materials. The transformation of silver in greywater, which in some countries is reused/recycled, is also examined. The results show the complexity of these products in terms of Ag speciation pre- and post-usage which have an effect on both antimicrobial properties and fate in the environment. The results provide new information that is needed for modelling nanomaterials fate in the environment.

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Introduction

Due to its antibacterial and antifungal properties [1], silver (Ag) is an increasingly common active antimicrobial ingredient in commercial products [2-4]. Previous research has established that these products contain various forms of Ag, such as ionic Ag (Ag^+), silver nanoparticles (Ag-NPs) and/or their derivatives (e.g. AgCl, Ag_2S), and the use of these products inadvertently releases Ag, whether ionic, nano-particulate or otherwise, to the wider environment [5-7]. Current research estimating the environmental releases of Ag from these products are reliant

on the manufacturers' (largely voluntary) labelling of Ag form and concentration, but the reliability of this data is typically unknown [3, 8]. There is a need for more rigorous testing and independent verification of labelled contents.

The environmental risks associated with nano-products also depend on the chemical, physical, and biological transformations that nanoparticles (NPs) undergo upon use and release. For example, Ag from antibacterial textiles used in socks and sports-shirts is released into laundry greywater during washing [5]. The speciation of Ag and its transformation during washing is complex and depends on the initial form present, the nature of the textile, and the washing conditions [9, 10]. If hypochlorite is present than the Ag released mainly transforms to AgCl during washing [11], and this form of Ag has low water solubility (K_{sp}=1.8×10^{-10}) resulting in lower acute toxicity to terrestrial and aquatic organisms than ionic Ag or Ag-NPs [12, 13]. On the other hand, bathroom greywater is a key release pathway for Ag from antibacterial personal care products (PCPs) such as shampoos, deodorants, and shower gels [14]. This latter release pathway remains largely unexplored but is important for understanding the environmental fate of nano-Ag used in commercial products, particularly in countries where greywater reuse and recycling is common. Keller et al. [15] modelled the release of engineered nanomaterials (ENMs) from PCPs and concluded that they could be an important source of environmental exposure if their incorporation in PCPs increased.

The regulations and guidelines for greywater reuse vary considerably by country [16], and also differ between jurisdictions within a single country [17], with reuse practices ranging from having few or no legal restrictions to being prohibited in all situations [18 19]. Moreover, greywater treatment systems are extremely diverse, including technologies such as constructed wetlands [20, 21], infiltration systems [22], rotating biological contactors [23], membrane bioreactors (MBR) [24, 25], and small scale household systems [26]. In some areas onsite greywater reuse for garden irrigation may be practiced (with or without prior treatment) [27, 28]. This is increasingly common in water-stressed countries (e.g. USA, Israel, Australia, etc.) [17, 29], and especially in times of drought when household water restrictions are in place [30]. In well-serviced urban areas however, greywater is most commonly released into the combined wastewater stream destined for treatment at a municipal wastewater treatment plant [31]. This may occur either directly, in households where the bathroom or laundry greywater is released straight to sewer, or indirectly, as in cases where greywater is reused for toilet flushing and subsequently released to the sewer as part of the combined household blackwater [32]. This pathway (municipal wastewater release) has been subject to intense study in recent years as it has been identified as one of the most significant release points for Ag-NPs to the environment [33, 34]. Many studies have now been conducted to investigate the transformation and fate of Ag-NPs in wastewater sewers [35, 36], wastewater and sludge treatment processes [37-39], and biosolids [40, 41]. By contrast, the initial stage of this process, the release into greywater, remains largely unexplored despite the fact that greywaters could be directly disposed into the environment as explained above. This is mainly due to the significant technical challenge of studying chemical transformations of Ag-NPs from commercial products at realistic concentrations in dilute systems such as greywater. Very few techniques are suitable for detecting and analysing the chemical characteristics of Ag in PCPs and in the receiving water following their use. Indeed, in-depth studies of metals released to greywater such as the Smart Water CSIRO study show that even in cases where a product is known to contain a metal, its concentration within greywater may be below detection due to the large dilution effect occurring during the use of PCPs such as shampoo, hand wash, and shower gels [42].

The risks associated with environmental exposure of nano-Ag through release into greywater may be affected by the initial physicochemical properties of the nano-Ag (size, shape, surface functionality, chemical speciation, etc.), the formulation of the consumer products (type of emulsion, composition of liquids etc.), and the physical and chemical characteristics of the receiving greywater. So far, only a few studies have taken into account the original sources of Ag-NPs in commercial products and directly investigated the Ag leaching from different consumer products such as Ag functionalised textiles [5, 43, 44], toys, and PCPs [6]. All of these studies have reported a substantial release of Ag from these products over their washing procedure. However, a knowledge gap regarding Ag speciation in PCPs and its transformation in different environmental release pathways remains.

The objective of this study was to test the original speciation of Ag in eight different commercially available PCPs and to investigate the subsequent chemical transformation of this Ag following exposure to two different greywater mixtures. The Ag content of each product was quantified by inductively coupled plasma mass spectrometry (ICP-MS), and X-ray absorption near edge spectroscopy (XANES) was used to investigate the initial Ag speciation in the products and the changes occurring upon mixing with greywater. In addition, the antibacterial activity of these Ag PCPs was examined using a standard culture-based method (EN 1276) in an effort to relate Ag content and speciation to the antibacterial functionality of the products.

Materials and methods

Characterisation of Ag in (nano)silver personal care products

The 8 PCPs used in this study (PCP1 – PCP8; Table 1) are commercially available products that, according to their labels, contain Ag as an active antibacterial agent. The Ag content was reported by the manufacturers on 7 of the 8 product labels, but as previous research has revealed discrepancies between the information provided by manufacturers and the actual contents of commercial products [45], the concentration of Ag in each of these products was also measured. Triplicate 300 mg samples of each product were diluted with 3.7 ml of ultrapure water, and acidified by adding 1 ml of reverse aqua regia

(HNO_3:HCl 3:1 ratio) as recommended for improved Ag recovery using US EPA Method 3051A [46]. The samples were left overnight at room temperature in a fume hood for pre-digestion and then microwave digested (ramping to 175°C for 5.5 minutes and holding at 175°C for 10 minutes). The digested samples were diluted, then analysed by ICP-MS (Agilent 8800 Triple Quadrupole). The hydrodynamic size distribution of the NPs in the products with low viscosity (PCP5, PCP6, PCP7 and PCP8) were determined by Dynamic Light Scattering (DLS) using a Nicomp 380 ZLS (Particle Sizing Systems, FL, USA). However, it should be noted heer that all NPs present would have been measured by this methodology, and not only Ag-NPs. These products were analysed directly without any prior matrix manipulation or separation steps. The more viscous product formulations were not suitable for DLS analysis. Liquid-liquid separation to partition the particles from the viscous product formulations into a more suitable DLS sample matrix was attempted using the water-hexane-methanol ultrasonication washing method developed by La Fontaine et al., (2010) [47] but yielded particle concentrations that were too low for reliable DLS analysis. This method did however produce samples suitable for Scanning Electron Microscopy (SEM) and the form and shape (if particulate) of Ag in the products was subsequently investigated using an FEI Quanta 450 FEG-ESEM

Table 1 Description of the personal care products characterized in this study.

Product ID	Product	Ingredients listed on the product label	Advertised form of silver	Labelled silver content (mg kg^{-1})	Measured silver content (mg kg^{-1})	Number weighted DLS particle size (nm)	pH
PCP1	Colloidal silver essential cream	Colloidal silver, organic rose hip oil, organic jojoba oil, organic emulsifier-extract of olives, organic coconut glycerin, calendula extract, aloe vera, geogard	Colloidal silver	-	24.3 (±7.7)	ND	ND
PCP2	Silver face and body cream	Aqua (purified water), triticum vulgare (wheat) germ oil, cetearyl alcohol, glyceryl stearate, butyrosepermum parkii (shea) butter, theobroma cocao (cocoa) seed butter, rosa eglentraria (rosehip) seed oil, aloe baebadensis leaf extract, sodium stearate, glycerin, tocopheryl acetate, sodium hydroxymethylglycinate, active ingredient: silver particles @ 30 ppm.	Silver particles	30	17.9 (±0.4)	ND	ND
PCP3	Colloidal silver gel	silver particles suspended in water (20 ppm), grape fruit seed extract, aloe vera and cellulose	Silver particles	20	34.6 (±1.9)	ND	ND
PCP4	Silver colloid face and body wash	Purified water, decyi glucoside (derived from corn glucose and coconut oil), disodium cocoamphodiacetate, cocamidopropyl betaine (derived using coconut oil source), guar gum (plant source), glycerin (vegetable source), lactic acid, sodium hydroxymethylglycinate, phenoxyethanol (organically approved preservative), 30 ppm silver particles	Silver particles	30	21.4 (±1.0)	ND	ND
PCP5	Colloidal silver spray	Bacteriostatic colloidal silver food grade 50mg/l in a base of activated de-ionised living water	Colloidal silver	50	30.0 (±1.3)	20.2 (±10.0)	5.8 (±0.2)
PCP6	Silver hand sanitiser	Water, silver particles 30 ppm	Silver particles	30	23.8 (±0.7)	17.3 (±0.8)	6.4 (±0.5)
PCP7	Silver colloid antibacterial liquid	Demineralised water, nanosilver particles 30 ppm	Silver particles	30	21.6 (±0.7)	54.2 (±2.7)	6.5 (±0.2)
PCP8	Colloidal silver nasal spray	Filtered pure New Zealand alpine water, silver ions 10 ppm	Silver ions	10	19.0 (±7.7)	8.1 (±0.4)	6.1 (±0.40

equipped with an EDAX Apollo X Energy Dispersive X-ray (EDX) spectrometer. Following separation of the products according to this protocol, 1 ml of the separated aqueous phase was filtered through a 30 nm polycarbonate filter membrane and filters were examined with and without 20 nm carbon sputter-coating (Quorum Sputter Coater system Q150T-ES). The less viscous samples were also examined directly after filtering through 30 nm filters without prior separation from the product formulation. All products were also analysed by synchrotron X-ray Absorption Spectroscopy (XAS) to directly determine the dominant species of Ag present in each product. This analysis is described in more detail below.

Preparation and characterisation of greywater

Two different types of artificial greywater (designated GW1 and GW2) were prepared using slightly modified versions of a protocol developed for greywater technology testing in Australia [48]. The two greywaters differed mainly by the inclusion of laundry powder in GW1 only. Thus GW1 was designed to simulate bathroom greywater, and GW2 bathroom + laundry greywater. The addition of laundry powder typically results in higher pH, and is also reported to modify Ag speciation [10]. This synthetic greywater formulation was specifically designed to include market share products used by a large proportion of Australian households and to produce greywater of a similar composition to real Australian greywater as determined in previous survey work [48]. The products and reagents used to prepare the greywater are detailed in Table S1, and the ingredients listed on the product labels are documented in Table S2.

To prepare the synthetic greywater stock solution, all ingredients apart from the clay and recycled water were weighed out according to Table S1 and mixed in a blender with 500 mL of warm water for 1 minute. 5 mL of this stock solution was then mixed with 0.05 g of clay and 20 mL of recycled water, and additional tap water was added to bring the resulting greywater suspension to 1L. This was left to stir overnight at low speed. Both GW1 and GW2 were characterised for temperature, pH, conductivity, chemical oxygen demand (COD), dissolved oxygen, and total dissolved solids at the start and end of all exposure experiments (Table S3).

Exposure of (nano)silver personal care products to greywater mixtures

The products were added to 20 mL of greywater (GW1 and GW2) in polypropylene containers to achieve a consistent final concentration of 0.3 mg L^{-1} of Ag (to ensure that good quality XAS spectra could be collected). The sealed containers were agitated (100 rpm) for 8 hours at room temperature. This exposure period was chosen on the basis that guidelines for greywater reuse recommend that greywater is not stored untreated for more than 24 hours because it can become septic, produce an offensive odour, and provide conditions for microbial growth. After 8 hours, each 20 ml sample, including the transformed nano-Ag, was snap frozen using liquid nitrogen to limit any further chemical transformation. Samples were freeze-dried in a Modulyo D freeze dryer (Thermo Fisher Scientific Inc., MA-USA) and the recovered solids were kept under argon prior to XAS analysis to investigate any changes in Ag speciation following exposure to greywater.

X-ray absorption spectroscopy (XAS)

XAS data were collected at the Materials Research Collaborative Access Team (MRCAT) beamline (10-ID) at the Advanced Photon Source (APS), Argonne National Laboratory, USA[49]. The storage ring was operating at 7 GeV in top-up mode. A liquid N_2 cooled double crystal Si(311) monochromator was used to select the incident photon energies and a platinum-coated mirror was used for harmonic rejection. Calibration was performed by assigning the first derivative inflection point of the absorption K-edge of Ag metal as 25514 eV and a Ag metal foil reference spectrum was collected simultaneously with each sample scan. All data collection took place in the dark because Ag is photo-sensitive. A range of Ag standard materials were used to collect reference spectra, including silver -oxide (Ag_2O), -sulfide (Ag_2S), -chloride (AgCl), -sulfate (Ag_2SO_4), -carbonate (Ag_2CO_3), and -phosphate (Ag_3PO_4), as well as Ag sorbed to humic acids (Sigma Aldrich), cystine, cysteine, glutathione, ferrihydrite, zeolite, kaolinite and acetate. All spectra were normalised over the −100 to +220 eV range relative to the K-edge prior to analysis. The XANES region of the Ag K-edge, defined for this study as −25 to +100 eV relative to the calibration energy, was analysed by linear combination fitting (LCF). LCF analysis was conducted after averaging 3-5 replicate scans per sample. In the first instance, principal component analysis (PCA) of the normalised sample spectra was used to estimate the number of species contributing to the overall spectra, and target transformation (TT) was used to identify the likely species required for LCF. The SixPack program [50] was used to perform PCA and TT, while Athena [51] was used to perform data normalisation and LCF. The Ag-K-edge spectra of the standards used for LCF are presented in Fig. S1.

Antibacterial efficacy test of PCPs

The antibacterial activities of the PCPs were tested using the Gram-negative bacterium *Escherichia coli* (E.coli) according to European standard method EN 1276 [52]. This method describes a quantitative suspension test for assessing the bactericidal activity of chemical antiseptics and disinfectants. We used it to evaluate how effective the selected (nano)silver products are at reducing the number of viable bacterial cells. This approach can be applied to both the formulated product and the active bactericidal ingredients [53, 54]. As recommended by EN 1276, bovine albumin solution (0.3 g L^{-1}) was used as a standardised interfering substance. One mL of interfering substance was mixed with 1 mL of bacterial test suspension with an initial concentration of 1.5-5 $\times 10^8$ CFU/mL (CFU = colony forming units). The concentration of bacteria in the suspension was measured using an Agilent 8453 UV–Vis Spectrophotometer where the optical density of 1 at 600 nm equals approximately

10^9 CFU/ml and was further validated via colony counting on trypticase soy agar (TSA) plate cultures. After 2 min incubation, 8 mL of the PCP was added to the test suspension and incubated for 5 min (PCPs 1-4 were diluted 2x by mixing 4 mL of the product suspension with 4 mL of MilliQ water). Then, 1 mL of this mixture was transferred into a tube containing 8 mL of neutralizing solution and 1 mL of phosphate buffered saline (PBS), and incubated for 5 min. The neutralizing solution used to neutralize the reactive Ag was based on European standard EN 1276 and contained: Tween 80 (930 g L^{-1}), lecithin (3 g L^{-1}), histidine (1 g L^{-1}), sodium thiosulfate (5 g L^{-1}), and potassium dihydrogen phosphate (34 g L^{-1}). The final mixture was serially diluted in PBS and plated on TSA. The inoculated plates were incubated for 24 h at 37 °C and viable CFUs were then counted to determine the percentage survival of bacteria in the PCP-exposed samples relative to the control plates in which the bacteria were only exposed to PBS and the neutralising solution.

In order to elucidate the contribution of the dissolved Ag (vs. particulate Ag) to the bactericidal effect of the products, Microsep Advanced Centrifugal Devices (1 kDa, Pall Corporation) were used to separate the ionic Ag fraction from the bulk solution in two of the liquid products (PCP7 and PCP8). Other products could not feasibly be filtered to that level due their more viscous formulation. The concentration of Ag in the Microsep filtrates was measured by ICP-MS and the antibacterial activity of the filtrates were also tested. In addition, the antimicrobial potential of the PCP was also compared to that of $AgNO_3$ solutions at two concentrations (17 and 30 mg L^{-1}) to cover the Ag concentration range present in the PCPs.

Results and discussion

Physicochemical characterisation of Ag in commercial PCPs

ICP-MS analysis of the acid-digested PCPs gave mean Ag concentrations ranging from 17 to 35 mg kg^{-1} (Table 1; the results are reported in mg Kg^{-1} as some materials are solid or gels, however in the case of water suspension this is equivalent to mg L^{-1}). For most products, apart from PCPs 3 and 8, the measured concentrations of Ag were less than that indicated by the manufacturer. The concentration of Ag in PCP1 was not reported on the label. The intensity-weighted and number-weighted size distributions of NPs in the PCPs measured by DLS are presented in Fig. S2 of the Supporting Information section and summarised in Table 1. In the water-based colloidal suspensions comprising PCPs 6-8 the average number-weighted NP size ranged from 8 to 54 nm.

SEM examination indicated that the PCPs contained different forms of Ag (Fig. S3), and EDX analysis suggested that all PCPs except for PCP5 contained colloidal elemental Ag (Fig. S4). SEM-EDX analysis of PCP5 indicated that the major form of Ag was cubic AgCl-NPs. Some of the products contained particles that were more or less uniform in size, whereas others varied quite considerably, not only in size but also in structure. For example, PCP2 contained both Ag-NPs and a small fraction of larger microscale AgCl particles (Fig. S3 and Fig. S4). The variation in Ag forms between products is not unexpected given that the products were manufactured by different companies and using different formulations. It is possible that different forms and species of Ag were added to different products to start with during the manufacturing process. It is also possible that the Ag speciation in the final product mix was affected by the formulations due to potential interactions and/or reactions of Ag with the other ingredients. PCP8 for example, whose product name is “colloidal silver nasal spray”, specified the inclusion of silver ions rather than colloids (Table 1). SEM analysis showed the presence of colloidal Ag in the product however, so it is not clear whether the manufacturer added Ag ions with the knowledge that they would form colloidal Ag in the final product, or, that there is a mislabelling in either direction. By comparison all other products were labelled as containing Ag particles (or colloidal Ag) and this was confirmed by SEM.

Regulations for product labelling are complex and country-dependent but the PCPs analysed in this study were purchased in Australia where manufacturers are required to specify ingredient information on the product label. None of the products specified the speciation of the Ag particles added (e.g. Ag-NPs or AgCl-NPs); this could imply that pure Ag-NPs are most commonly added. Overall, information relating to the speciation and structure of NPs in commercial products is generally lacking [3, 4]. This is unfortunate given its central relevance to NP ecotoxicity and fate [55, 56].

Chemical characterisation of Ag in commercial PCPs by XAS

While SEM-EDX can provide indicative information about the chemical speciation of the NPs in the PCPs (based on element co-location and atomic ratios derived from the X-ray intensities), the reliability of these results is affected by the size and form of the NPs and may also be subject to artefacts from the sample preparation steps as the NPs need to be separated from the bulk solution to facilitate imaging and analysis. Synchrotron-based XAS analysis, an ideal method for investigating element speciation in intact samples [57], was thus used to provide an optimal measure of the dominant Ag speciation in each of the products.

The averaged, normalised XANES spectra for each of the 8 PCPs are shown in Fig. 1 and it is evident that a range of different Ag species was present in these commercial PCPs. The normalized XANES spectra of the PCPs were fitted with the reference spectra shown in Fig. S1 using LCF, and the results of this analysis are presented in Fig. 2 and Table S4.

The LCF results confirmed that metallic Ag (Ag^0) was the dominant form of Ag in PCP1, PCP4, PCP6, PCP7, and PCP8. In PCP2 and PCP3 both AgCl and Ag^0 were present (approximately in a 2:1 proportion) with AgCl dominating the composition of PCP5. This finding is consistent with the SEM images and EDX spectra for PCP2 and PCP5, which indicated that a significant proportion of Cl was present in the Ag-NPs separated from those same products. Ag bound to reduced

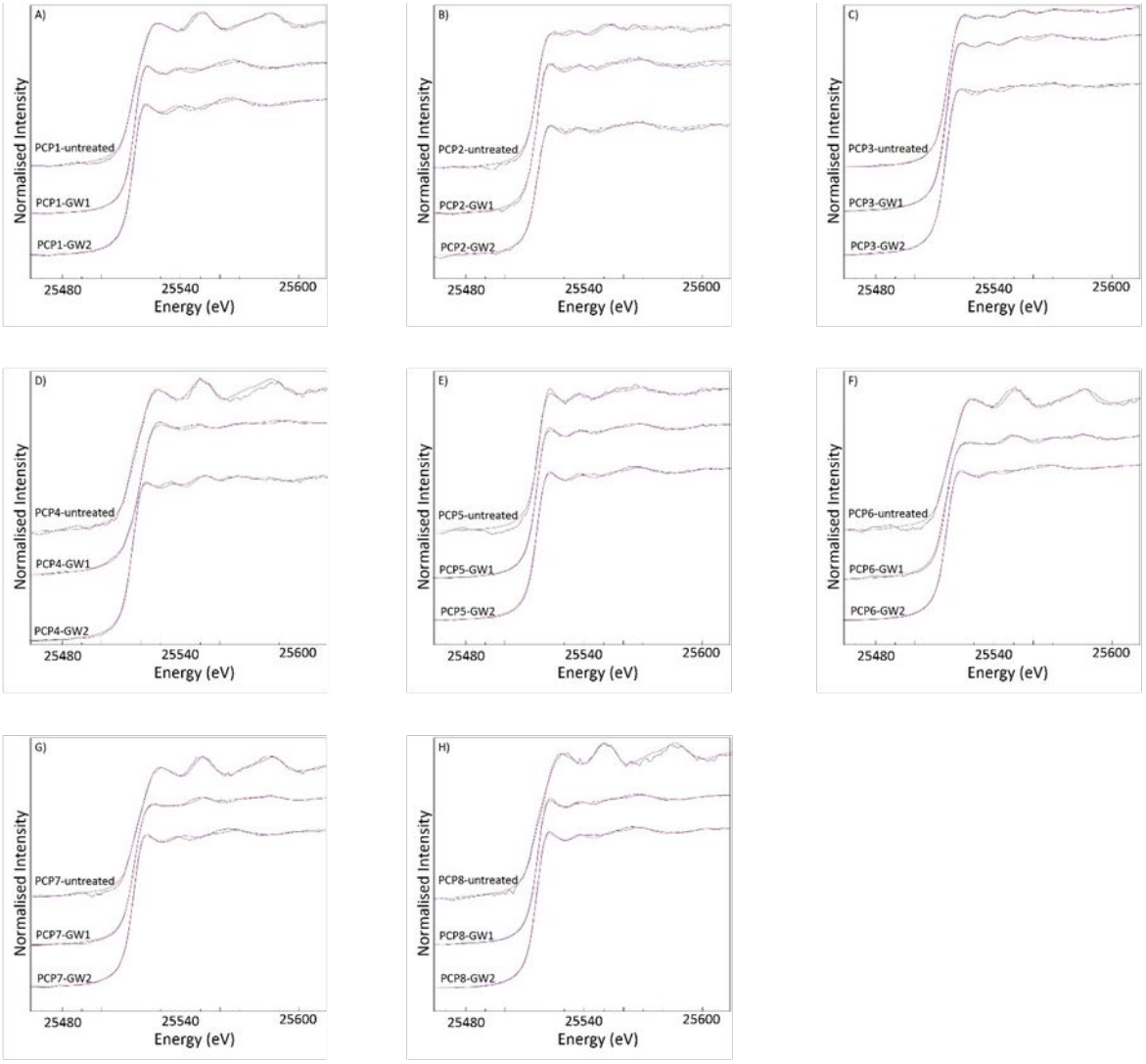

Fig. 1 Measured (blue) and fitted (red) XANES spectra of Ag in personal care products before and after exposure to GW1 and GW2

sulfur (represented by Ag_2S and Ag-cysteine reference spectra) was observed as a minor species for PCP1, PCP6 and PCP7, and the LCF results also revealed the presence of Ag bound to clays in PCP1 and PCP5. Here, the LCF components Ag-kaolinite and Ag-zeolite were combined together as 'Ag bound to clay' for the presentation of results. Both kaolinite and zeolite are natural aluminosilicates that are used extensively in PCP formulations due to their useful rheological properties, high specific surface area, and sorption capacity [58, 59].

Antibacterial activity of Ag PCPs

Silver is added to these products to impart antibacterial properties to the PCPs. As Ag speciation plays an important role in determining antibacterial efficacy it is of interest here to investigate the relative efficacy of these PCPs in light of the above information on speciation and form. Although there is some debate in the literature about Ag-NP specific antibacterial effects, the majority of evidence suggests that the release of free Ag ions from Ag-NPs is particularly important in terms of antibacterial properties (even though direct mechanisms related to Ag-NPs have also been reported [60]). Therefore, the potential antibacterial effect of the products containing AgCl and Ag bound to reduced sulfur may be expected to be less than that of the products containing mainly elemental Ag-NP due to the lower reactivity and solubility of AgCl and sulfidized Ag (e.g. $K_{sp}=6\times10^{-51}$ for Ag_2S, $K_{sp}=1.8\times10^{-10}$ for AgCl) [61, 62]. For example, Lorenz et al. [43], found that the antibacterial effects of some commercial Ag functionalised textiles was negligible as a result of the very slow dissolution rate of the Ag_2S or AgCl NPs incorporated into the textile.

The bactericidal effects of all PCPs as well as two selected concentrations of $AgNO_3$ (equivalent to the minimum and maximum concentrations of Ag in the tested PCPs) against the gram-negative bacterium *E. coli* are presented in Fig. 3. The data show that all PCPs, with the exception of PCP3, had lower antimicrobial potential than the two $AgNO_3$ treatments, even when the ionic Ag concentration was 17 mg kg^{-1}. This result is

in line with the reports showing Ag ions being more toxic than Ag-NPs at the same concentration [63].

antibacterial efficiency. However, the bactericidal effect of these two products was much greater (37% and 0% *E. coli*

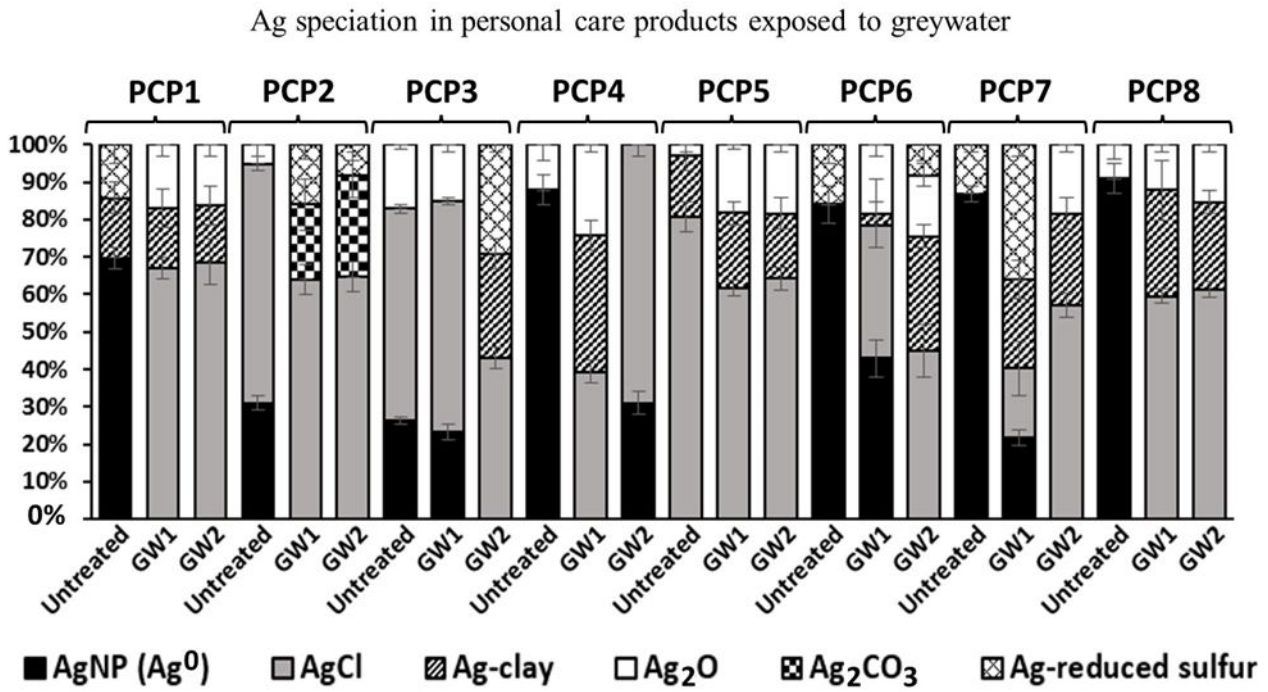

Fig. 2 Ag speciation in personal care products before and after exposure to GW1 and GW2 for 8 hours as identified by Linear Combination Fitting (LCF) of Ag K-edge XANES spectra. Species proportions are presented as percentages of their contribution in the overall spectra of sample.

PCP5 showed the lowest bactericidal efficacy despite the relatively high measured Ag content of 30 mg L^{-1}. This is in line with the Ag speciation results based on XANES analysis which revealed that AgCl was the major form of Ag in PCP5 (higher than in any other product tested, Table S4). Thus, the limited bactericidal effect of PCP5 could be attributed to the low solubility of AgCl limiting the contribution of dissolved Ag and hence limiting the product's antibacterial activity [61].

Given that PCP2 and PCP3 also contain AgCl as the major form of Ag content, they could also have been expected to have low

survival for PCP2 and PCP3, respectively). According to the labelled ingredients, PCP2 and PCP3 contain cetearyl alcohol and grapefruit seed extract (GSE) respectively, both of which reportedly have antimicrobial potential [64-67], although some studies have reported that the anti-microbial activity associated with GSE can actually be attributed to the contamination of commercial GSE with synthetic antibacterial agents and preservatives (e.g. benzethonium chloride) incorporated during its preparation [68]. Cvetnic et al. [69] showed that the antibacterial efficiency of GSE is due to ethanol

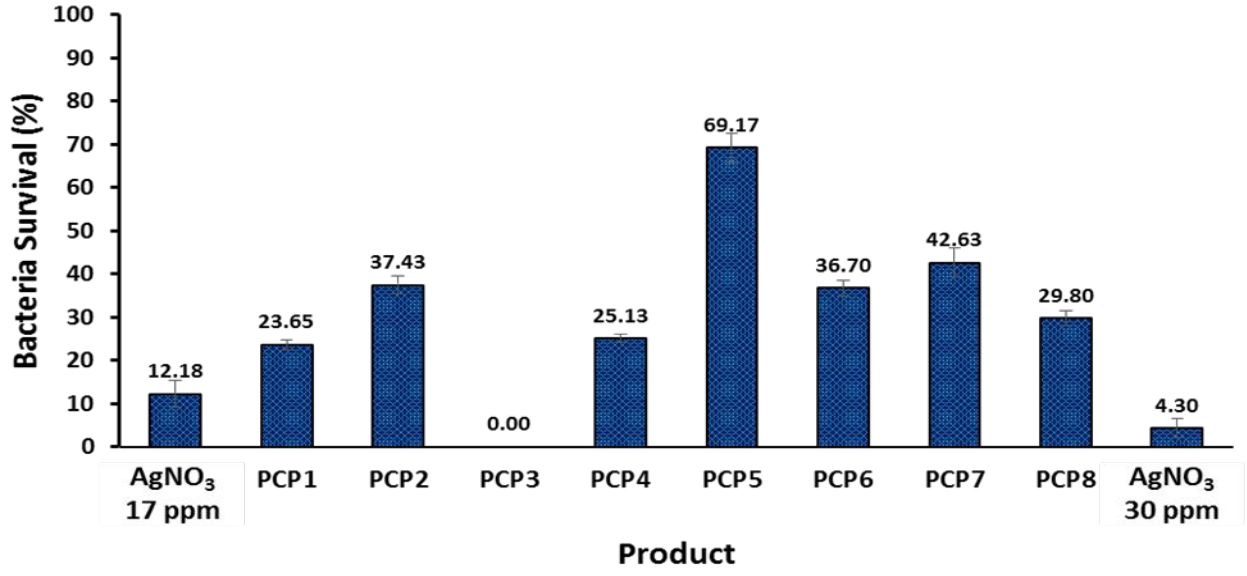

Fig. 3 The bactericidal effect of all products, 17 and 30 mg kg-1 AgNO3 against gram-negative bacterium Escherichia coli; bacteria survival percentage after 5 min exposure to PCPs.

contamination used in the extraction process. Thus the antibacterial activity of other ingredients or contaminants in the PCP formulations may also have a direct impact on the overall efficacy of the product.

To support this, PCPs 6-8 share similar product formulations (all are comparatively simple aqueous Ag suspensions of similar concentrations with XANES-based speciation of >80% metallic Ag-NPs) and their antibacterial efficacies are evidently similar. The small differences in the efficacies may be rationalised, at a simple level, by the Ag-NP size (Table 1): PCP7 has the largest nano-Ag size according to DLS (54.2 nm) followed by PCP6 (17.3 nm) and PCP8 (8.1 nm) [70]. Furthermore, ICP-MS analysis of the filtrates of PCP7 and PCP8 showed that these products contain 2.2 ± 0.9 and 12.9 ± 0.7 mg kg^{-1} ionic Ag, respectively. This indicates that 10.2 and 67.8 % of total Ag in the two products were dissolved. The filtrates were also tested for their antimicrobial potential: the results showed a 77.2 ± 2.7 % bacteria survival for PCP7 filtrate relative to control (compared to 42.6 % by the original PCP7) and 31.1 ± 3.3 % bacteria survival for PCP8 filtrate (compared to 29.8 % with the original product). This reveals that for PCP8, the bactericidal effect is mostly attributed to the Ag ions and indeed has the highest efficacy between the three similar products [63].

For the other products that have more complex formulations, it is clear that the antibacterial efficacy of the PCPs cannot be accounted for by the Ag concentration and speciation alone. As noted above, the direct antibacterial activity of some of the other ingredients may add to the overall efficacy. However, perhaps more importantly, the other ingredients may also affect the efficacy of nano-Ag antibacterial activity. For example, since macromolecules such as those occurring in natural organic matter (NOM) can reduce the dissolution of Ag-NPs even without direct surface interactions, macromolecular ingredients such as oils and gums may impart a similar effect, thereby reducing the antibacterial efficacy. Finally, the efficacy of the products may undergo changes over the storage time due to the potential chemical transformation of the Ag-NPs and dissolution of Ag-NPs. Kittler et.al. [71] investigated the dissolution rate of citrate stabilized and poly(vinylpyrrolidone) stabilized Ag-NPs in water over 125 days of storage at different temperatures. The results revealed that the dissolution rate of Ag-NPs and consequently their toxicity is dependent on the surface functionalization of NPs and the storage temperature. However, information regarding product shelf life, production date, and storage conditions for PCPs is generally not available.

Speciation of PCP-derived Ag after exposure to greywater

Silver speciation determined by XANES LCF analysis before and after greywater exposure is summarised in Table S4 and Fig. 1 and 2, where it can also be compared with the initial Ag speciation in the PCPs prior to greywater exposure. Changes in Ag speciation as a result of greywater exposure are clearly evident. The two greywaters used for the exposure experiment (GW1 and GW2), contained a variety of components (Table S2) that may chemically interact with the Ag in the PCPs upon mixing.

The LCF results indicated that AgCl was the dominant form of Ag in the majority of products after exposure to GW1 and GW2, reflecting its attributes as a common, insoluble Ag species. For products with high initial AgCl proportions (PCP2, PCP3 and PCP5), there was only a small change in their speciation due to greywater exposure, with the majority of the changes occurring in the proportional distribution of Ag species other than AgCl. In contrast, a greater extent of Ag transformation was observed for the other Ag based products (PCP1, PCP4, PCP6, PCP7 and PCP8) with Ag mainly transforming to AgCl after exposure. As discussed above, it is therefore very likely that the antibacterial potential of these products is greatly reduced once they have been in contact with greywater, which would in turn decrease their toxicity when released to the environment.

At the same, time, a substantial proportion of Ag_2O was also observed for some products after exposure, as was a small proportion of sulfur-bound Ag. Similar variability in Ag speciation in antibacterial textiles has been reported by Lombi et al. [9] where XANES spectroscopy was used to investigate the Ag speciation in Ag containing textiles upon washing. A wide range of Ag species (e.g. Ag^0, AgCl, Ag_2S, Ag-phosphate, Ag^+, etc.) was found to coexist in the textiles before and after laboratory and machine washing. Lorenz et al. [43] also suggested the presence of Ag_2S and AgCl in some of the same commercial antibacterial textiles after washing on the basis of STEM (scanning transmission electron microscopy) and EDX results. Impellitteri et al. [11] used XAS to identify the Ag speciation in antimicrobial sock fabric before and after washing with hypochlorite/detergent solution and identified Ag^0 in the textile before washing but a significant conversion (more than 50%) to AgCl occurred as a result of washing. Our observations are therefore consistent with these results.

Greywater characterisation (Table S3) showed that GW1 had a higher pH value than GW2. This could be expected to result in greater oxidation of Ag in the products over the course of exposure, and as observed in the LCF results, greater formation of Ag_2O occurred for some products (PCP3 and PCP4) during their exposure to GW1. This oxidation process may be attributed to the possible generation of hydrogen peroxide (H_2O_2) and its interactions with Ag ions; He et al. [72] reported greater likelihood of H_2O_2-mediated oxidation of Ag-NPs at high pH values. While bleaching agents were not specifically added to either GW1 or GW2 (in keeping with the standard Australian protocol by Diaper et al [48]), their presence in greywater is plausible and can be linked to the oxidation and dissolution of NPs [73].

It should also be noted that the differences observed in the behaviour of Ag from different PCPs in the same GW type may be attributed to more than the initial Ag speciation and the GW properties. For example, size distribution of the nano-Ag, the relative proportions of particulate, adsorbed or dissolved Ag, the nano-Ag surface functionality and the other ingredients in the PCP formulation may also impact their behaviours. Importantly, surface interactions with these

ingredients, and also with GW components may have an impact on their short term speciation. Surface functionalization of NPs with polymers and biological ligands is being actively explored as a means to impart desired surface chemical and physical properties for specific commercial applications [74, 75], even though previous studies have not shown a strong influence of surface functionalisation on the environmental transformation of Ag-NPs [76, 77]. These factors need to be taken into account to elucidate the fate and behaviour of Ag in products exposed to different environmental systems, and more systematic approaches to their study are therefore recommended. For example, the effect of these physicochemical properties on Ag-NP fate and environmental impact could be investigated by embedding a number of well characterised Ag-NPs into different personal care products with well-known ingredients and formulation and conducting XANES analysis upon their exposure to different environmental matrices; this approach has been used by Mitrano et al. for textiles [10].

Conclusions

Overall, this study shows that the initial Ag speciation in untreated products can be generally categorised either as "Ag^0 based" or "AgCl based" products. The Ag in "Ag^0 based" products was mainly transformed to AgCl after exposure to greywater whereas "AgCl based products" were more stable over the course of exposure. Some contribution of other species such as Ag_2O, Ag-reduced sulfur, Ag-zeolite and Ag-kaolinite was also observed for the products exposed to greywater. The antibacterial susceptibility testing of the products indicated that their bactericidal efficacy depends partly on the Ag speciation within the products but is also affected by the inclusion of other biocidal agents in their formulation. The significant transformation of Ag^0 to AgCl upon exposure to greywater is likely to reduce the toxicity of PCP-derived Ag and therefore reduce the risk to the environment.

This study highlights the importance of knowledge gaps that exist in Ag nanotechnology product inventories, such as the size of Ag-NPs and of the chemical speciation of Ag incorporated in the products. Provision of such data is not a regulatory requirement and complex analysis is required to obtain it, but it is extremely useful to scientists aiming to elucidate the behaviour and fate of these particles upon their release from real commercial products into different environmental systems. Currently, the limited studies on this subject exist in isolation and are not necessarily comparable to one another. A final comment for the planning of new studies to address some of the knowledge gaps identified here is to establish a unique standard methodology with complete detailed experimental descriptions to investigate the potential antibacterial efficiency of these products as well as their environmental fate and impact in the compartments they are likely to be released to. This would allow cross-comparison of potential risks across different studies and products.

Conflicts of interest

There are no conflicts to declare.

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