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Antimicrobial silver nanoparticles – regulatory situation in the European Union*

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Abstract

Nanosilver is one of the most prominent nanomaterials, resulting from its capability to fight germs like bacteria, fungi and yeasts. Those germs cause nosocomial infections, food poisoning, material deterioration, food and feed spoilage.
In the present review, we give insights into antimicrobial silver nanoparticles from a regulatory point of view. Silver nanoparticles release silver ions, which act as the biocidal substance. This mode of action makes it difficult for regulators to judge the risk effects related to silver nanoparticles. In this article the present situation concerning nanosilver (as a silver ion releasing technology) - state of the art, toxicological effects and risk assessment is discussed. Finally, the benefits of using silver nanoparticles in consumer products are compared to regulatory challenges in bringing such products on the market.

Keywords: Nanosilver, biocide, regulation, safety assessement, nanoparticle, antimicrobial

1. Introduction

Almost ten years ago, several articles published by NGOs raised concerns regarding the safety of nanomaterials. Consequently researches and authorities all over the world requested more data on nanorisks and specific treatment of nanomaterials in all related legislations. One of the most prominent materials is nanosilver or silver nanoparticles. This substance is used in biocidal products for disinfection and microbial inhibition on surfaces. Biocidal products

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are necessary to control organisms that are harmful to human or animal health and to avoid damage to natural or manufactured materials caused by microorganisms.

The use of silver was and still is multiple: Silver was used for many different applications throughout history. Due to its properties it was used in currencies, ornaments, jewellery, electrical contacts and photography. However, one of the most beneficial uses was the antimicrobial effect silver exerts to fungi, viruses, algae and of course bacteria. Therefore, silver has been used as disinfectant for a long time, e.g. in treating wounds and burns. [1]

For many decades nanosilver, formerly known as colloidal silver, has been used for many different purposes (e.g. as medical product, for wound care, water treatment, disinfection, etc.) [2].

Innovations in surface chemistry and process engineering led to a new linne of nanosilver products in the form of concentrates or masterbatches that are usable for active surfaces as well as materials like thermoplastic polymers.

This article explains the technology behind nanosilver and elucidates the legislative framework for bringing a nanostructured biocide on the market. The focus here is on European biocidal products legislation, for which most experience exists.

2. Silver use

2.1 Ancient use of nanosilver

At the end of the 19th century scientists started to produce nanosilver dispersions in a technical way. At that time, the term “nano” was not used, yet particle suspensions were in a “millimicron” scale or colloidal dispersions. Most of the nanosilver dispersions during this time were already used as medical products: “Under the name “Collargol” such a kind of nanosilver has been manufactured commercially since 1897 and has been used for medical applications.” [2]

Those medical products mainly used the antimicrobial effect of nanosilver. Infections have been treated with colloidal silver until the 1930s. After antibiotics had been invented and became widespread, the use of nanosilver declined for decades, but found a renewal when nanotechnology became a scientific discipline.

2.2 Mode of action

The biocidal activity of silver itself has been investigated extensively and is well described: The antimicrobial activity of silver primarily was identified as an oligodynamic effect by Ravelin and Nägeli and described by Russel et al. [3]. In substances showing this oligodynamic effect, only very small portions of the active substance are needed for significant antimicrobial activity [3]. Scientists define nanosilver as particles in a size range between 1 and 100 nm [4]. It is state of the art to incorporate these nanoparticles e.g. into polymers to avoid microbial growth on their surface [5], [6]. The principal mode of action is described in Figure 1.

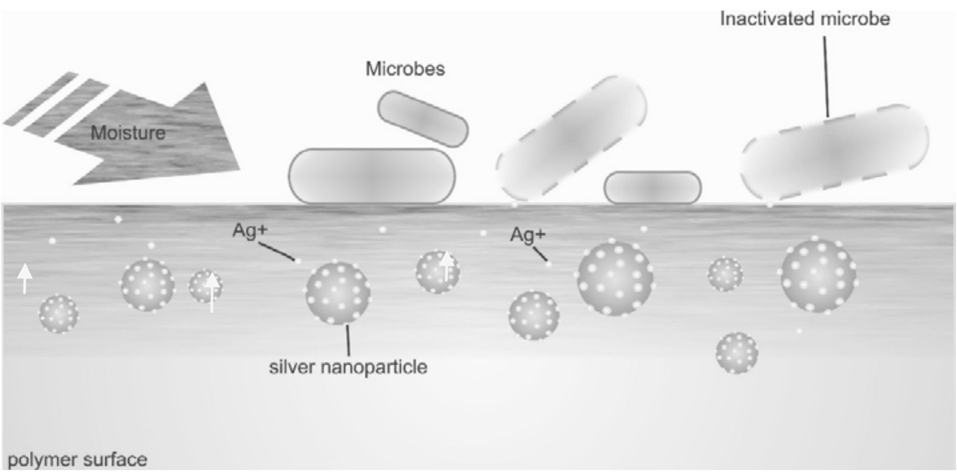

Figure 1: Schematic overview of the nanosilver effect on surfaces (© RAS AG)

Water molecules are penetrating the upper layers of almost every surface that is based on polymers, lacquers or resins [7]. Nanosilver particles, incorporated in those surfaces, release silver ions (Ag+) by specific corrosion processes [3].

Only silver ions are released, silver nanoparticles remain in the material even when acids are used for migration tests. This was described by Bott [8] when checking silver migration out of LDPE films for food contact. For his research, he used a LDPE film that contains silver nanoparticles. After migration in 3% acetic acid investigated a high silver concentration and concluded, that this only could be explained by migration of silver ions. "The small silver ions (effective ion radius Ag 0.115 nm diffuse in the polymer much faster than the SNP particles of at least 10 nm. Mechanistically this Ag migration is enhanced by the penetration of the small acetic acid molecules into the LDPE film. This leads already in the polymer to Ag formation from the silver particles followed by acetic acid mediated diffusion of the silver ions." [8] His findings shew that the silver nanoparticles themselves remained in the film and the surface. This was supported by other studies with other nanoparticles (e.g. TiN). He concluded that "not the silver particles themselves but dissolved silver ions only are released from the polymer which is the reason for the intended antimicrobial effect of polymers with incorporated silver nanoparticles." [8]

Driven by concentration gradients, the silver ions are „pulled" to the upper layer of the surface, were most of the moisture with less silver ions is present [9]. This liquid layer contains the microbes as well, so the silver ions have reached their target sites [4] where they have different mechanisms to influence microbial vitality.

Silver ions exhibit a broad antimicrobial profile against bacteria, fungi and virus as well. Even bacteria strains, which are resistant against antibiotics, e.g. MRSA can be fought with silver [10]. This makes silver and nanosilver an excellent biocidal substance for applications in medical devices and the food sector. Examples are coated surgical instruments, polymer implants (catheters) or nanosilver incorporated into textiles [11].

3. Nanosilver as a new technology

It is obvious that almost every silver ion releasing substance is principally capable of fighting germs. The use of nanosilver has some benefits, which are a consequence of its unique properties.

The most important reason is the enormous increase of active surface when reaching the scale of nanometers. Nanosilver particles release magnitudes of more silver ions compared to microsilver particle of the same weight [12]. The amount of released silver ions is directly linked to antimicrobial efficacy. This means that nanosilver particles show a much higher biocidal activity while requiring less material compared to microsilver or full silver coating.

The second advantage is the depot effect of nanosilver particles. Other technologies like silver salts release almost all silver ions during the early stage of immersion. The elemental silver in the core of the nanosilver particle and its outer layer of silver oxide serve as a depot which releases just small amounts of silver ions, sufficient for high activity and an antimicrobial effect lasting for years.

Nanosilver particles continuously release silver ions. Nanosilver particles establish a steady state of silver ion concentration that remains constant for a long time without a decrease in antimicrobial activity over time even when the treated product is exposed to UV-light or subjected to harsh cleaning procedures. For technical applications used in the food sector, e.g. for paints, in consumer products or hygienic surfaces for storage of food, nanosilver is incorporated into the substrate material (e.g. polymer or coating) and is therefore irreversibly immobilized.

In polymer fibers, the nanosilver particles protect the textiles (e.g. soaker pads for fresh meat) against the uncontrolled growth of germs. Even food related germs like Salmonella don't have any chance to survive in these textiles. The infection chain is interrupted and food related infectious diseases are avoided.

The major advantage of using such textiles is due to their durability, compared to other textiles, which lose antimicrobial activity after a few washing cycles. By incorporating the nanosilver particles (no textile coating is used) into the polymers, the particles cannot be washed out. They continuously release silver ions for a high antimicrobial activity. Studies show, that even after 200 laundries at 60°C the textiles still remain antimicrobial [13]. The nanosilver avoids the growth of germs in the textile. This means it is not necessary to dry the textiles after washing. Wet storage becomes feasible: Nanosilver in textiles therefore minimizes energy costs and CO_2-exhaust by making laundry more efficient. Current studies have shown that the major contribution to the energy savings during a textiles lifetime is caused by washing and drying. Figure 2 shows the numbers of the improved environmental impact: Compared to normal textiles, it is possible to save 50% of electrical energy and have 30% less environmental impact [14]. This is due to a lower consumption of detergents and a reduction of electric energy consumption resulting from fewer washing and drying cycles.

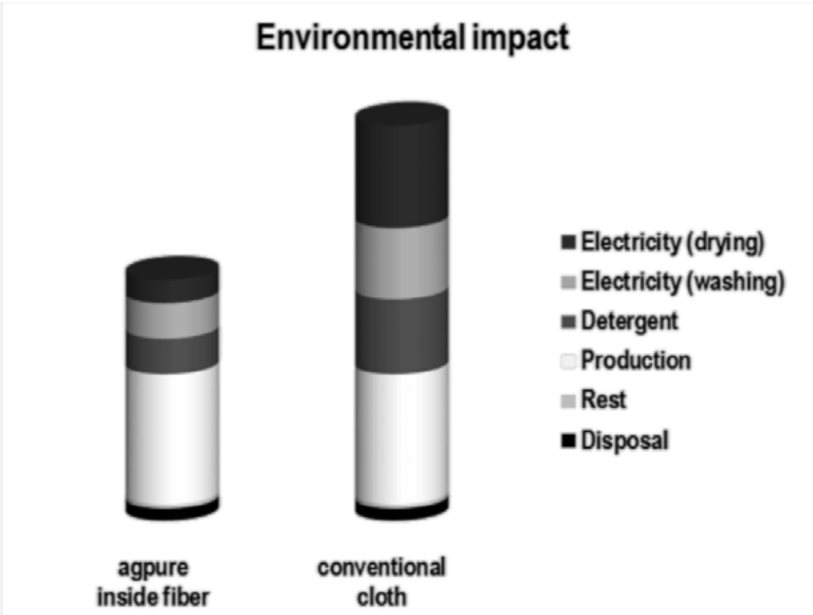

Figure 2: Improved Environmental impact of nanosilver textiles: (Research Project LICARA, funded by European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 315494) [14])

4. Nanospecific regulation and safety assessment

4.1. History

In the last ten years, lots of papers have been published, that talked about a wide availability and distribution of consumer produtcs that contain nanosilver. [15], [1].

Such publications and the apparently abundant availability of so-called nanoproducts in the web gave regulators the impression that thousands of articles treated with nanosilver would already be on the market. Consequently voices for requesting special registration procedures of nano-biocides have been raised. One of the first legislative acts implementing nano-specific regulation was the European Biocidal product regulation (EU-BPR, [16])

4.2. Biocidal product regulation (BPR) and Nanosilver

Depending on the application, the usage of a biocidal substance or product in the EU is subject to several regulatory frameworks, for example the Biocidal Products Regulation (BPR). This regulation (528/2012 EU), applies as of September 1st, 2013 and replaces Directive 98/8/EC.

If materials are treated with silver to avoid the growth of germs (bacteria, fungi, yeast, virus, etc.), this application is in principle inside the scope of the BPR.

Within the authorization process substances owning similar physico-chemical, toxicological and ecotoxicological properties can be grouped together as one “category”. As a result nanosilver is classified as active substance

„silver“(CAS Nr. 7440-22-4). Because the bulk form of silver is a known biocide for a long time, nanosilver is authorized according to the guidelines for “existing substances”, where transitional measures apply.

The EU-Regulation for biocides, who mentions explicitly in § 19: “Where nanomaterials are used in that product, the risk to human health, animal health and the environment has to be assessed separately.” [16], therefore an additional set of tests with nanosilver were necessary. Also other regulatory authorities were struggling with the evaluation of nano-specific risks, to ensure the highest possible level of safety for their citizens.

As a result of this high demand the international “ Organisation for Economic Co-operation and Development’s (OECD) Working Party on Manufactured Nanomaterials (WPMN) is carrying out one of the most comprehensive nanomaterial research programs: the OECD WPMN Sponsorship Program for the Testing of Manufactured Nanomaterials.” [17]. Herein the silver reference nanomaterial in the OECD WPMN international testing program, is the nanosilver product “agpure W10” which is characterized as NM 300K. (agpure W10 is produced by RAS AG)

Consequently the only nanosilver product in Europe, which can provide an additional nano-specific dataset and nano-risk assessments, is the reference material NM 300 K. The results of this extensive research guarantee the safe use of agpureW10 nanosilver and agpureW10 treated articles.

For food applications, the used biocidal product had to be authorized or had to be notified as existing active substance for product type 4. Being an existing substance, the deadline for authorization respectively the inclusion of silver (nano) into Annex I of the BPR can't be scheduled at the moment. But everybody who is using NM 300 K can therefore benefit from the transitional measures meant before. Examples of the safety assessment, based on public available studies elucidate the quality of existing data for nanosilver.

4.3. Technology and physical-chemical properties

EU Joint Research Centre (JRC) published a report [18] and material information sheet [18] for NM 300 K, which summarizes the physical and chemical properties of the material. Figure 3 shows a TEM image of this nanosilver suspension, which demonstrates the homogenous particle size distribution.

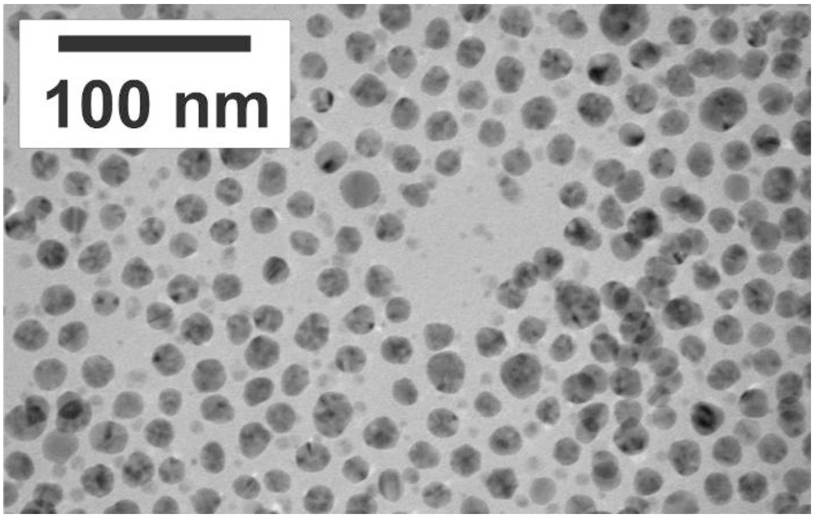

Figure 3: TEM-image of NM 300K (= agpure W10, a product of RAS AG) nanosilver particles. (Klein et al. 2011 [17]).

4.4. Toxicology of Silver nanoparticles and Food contact migration. .

When talking about nanotoxicology it has to be considered, that the increasing number of studies on the safety of nanomaterials was not followed by an increase in quality and reliability of such studies. Because of that Krug and Wick [19] in their review were even forced to describe inadequate methods “together with recommendations how to avoid this in the future, thereby contributing to a sustainable improvement of the available data.”

This means any results of studies on nano-risks have to be used very carefully. As a summary of data on mammalian or ecotoxicity the use of nanosilver can be considered to be safe for humans and the environment, when certain rules are followed. Even the use in food contact materials is feasible.

When talking about migration of silver from silver nanoparticle containing substances it could be shown, that only the silver ion is the relevant species that is released from materials containing nanosilver: "In conclusion, not the silver particles themselves but dissolved silver ions only are released from the polymer which is the reason for the intended antimicrobial effect of polymers with incorporated silver nanoparticles." [8].

Studies show, when using NM 300K incorporated in polymers this release is below the EFSA Substance migration limit of 0.05 mg/kg of food: "The AFC panel also took note of the WHO "Guidelines for drinking-water quality" [20]. According to these Guidelines a total lifetime oral intake of about 10 g of silver (equal to 0.39 mg/person/day) can be considered on the basis of epidemiological and pharmacokinetic knowledge as the human NOAEL. To maintain the bacteriological quality of drinking water, levels of silver up to 0.1 mg/l, could be tolerated without risk to health. On the basis of a daily intake of 2 l of drinking water this concentration is equal to a daily silver intake of 0.2 mg/person and gives a total dose over 70 years of half the human NOAEL. Based on the data above, a Restriction of 0.05 mg/kg of food (as silver) for the substance would limit intake to less than 12.5 % of the human NOAEL" [21]

The highest silver migration rates (6.8 µg/dm²) were found with 3% acetic acid as a food simulant and the plastic film with the highest silver content (11.000 ppm). [22]

Even under food contact related migration conditions that can be found in normative literature like Directive 97/48/EC.: „…silver was neither found in 95% ethanol although the solubility or ability to disperse would have been sufficient nor in isooctane which is known to be very aggressive to LDPE by swelling the polymer. From a migration theoretical view the SNP of at least 10 nm size cannot move anymore at the applied severe test temperatures at a measurable speed in a polymer and therefore are not expected to migrate out of the film. This is supported by other studies with other nanoparticles (e.g. TiN)." [8]

The review of Cushen et al. [23] collected available data on silver migration from nano and non-nanosilver containing products. The migration from the nanosilver containing product was the lowest, not exceeding 0.35 µg/dm².

Echegoyen and Nerin [24] concluded, that "in all cases the total silver migration is far below the maximum migration limits stated by the European legislation…".

These results demonstrate that the even for food contact applications a daily use of nanosilver containing products is safe for humans and the environment. Because these products with integrated nanosilver particles wound not lead to a considerable silver migration into the foodstuff.

5. Conclusion

Silver is known to be used since the 4th millennium BC. Nanosilver dispersions were used as medical products already in the 19th century without showing adverse effects on patients. Additionally, silver has been authorized by EU EFSA as E174 for coloring food.

The antimicrobial effect of silver is well understood. Silver ions exhibit a broad antimicrobial profile against bacteria, fungi and virus as well. Even bacteria strains that are resistant against antibiotics, e.g. MRSA, can be fought with silver [10]. This makes silver and nanosilver an excellent biocidal substance for applications in medical devices and in the food sector.

Increased surface area and silver ion release combined with a silver depot effect makes nanosilver the ideal additive to be used as a biocidal substance for any type of surfaces.

For technical applications used in the food sector, e.g. for paints, in consumer products or hygienic surfaces for storage of food, nanosilver is incorporated into the substrate material (e.g. polymer or coating) and is therefore irreversibly immobilized.

Compared to normal textiles, nanosilver textiles save 50% of electrical energy and result in a 30% lower environmental impact. This is due to a reduced consumption of detergents and a benefit of electric energy resulting from fewer washing and drying cycles.

Silver is used in the food area to fight microorganisms that cause food spoilage or even diseases like food poisoning. Especially animal stalls are a major source of multidrug-resistant organisms such as the dreaded MRSA wound and pus germ or dangerous intestinal bacteria like 3,4MRGN (Multi Resistant Gram Negatives). All relevant bacterial strains are sensitive against silver, even the multiresistant strains that will increasingly cause hygienic problems.

The regulation of nanosilver for food contact materials is a complex issue. One topic, which is discussed at the moment within European authorities, is the approach on how to deal with applications for BPR product type 4 to avoid legal uncertainty and dual approval processes (EU-BPR 528/2012 vs. (EU) No 10/2011 positive list). But it seems obvious that nanosilver products that wanted to be placed on the EU-market have to be authorized compliant to existing law and their nano-specific risk has to be assessed additionally.

Becoming the silver reference nanomaterial within the OECD WPMN international testing program, the nanosilver product “agpure W10” (produced by RAS AG) was characterised as NM 300K. It is the only nanosilver in Europe which provides extensive research data on nanosafety. Summarizing the data on mammalian and ecotoxicit, using nanosilver can be considered to be safe for humans and the environment, as long as certain rules are followed.

The results of the nanorisk assessment and silver migration studies show that the use of a product for food contact applications which contains NM 300K nanosilver as antimicrobial additive is safe for humans and the environment. It will not lead to a considerable silver migration into the food.

Overall, nanosilver for food contact materials is a safe material that can be used to face new challenges in our society. Besides the conservation of resources, more hygiene will be demanded, to guarantee safe food in a growing population. Even new threads like multiresistant bacteria, as a consequence of factory farming, can be stopped by using nanosilver [25].

Nomenclature

BPR	Biocidal Products Regulation
CAS	Chemical Abstracts Service
DNA/RNA	Deoxyribonucleic acid/Ribonucleic acid
EC	European Commission or European Community
EFSA	European Food Safety Authority
ESBL	Extended-spectrum beta-lactamase
EU	European Union
FCM	Food contact material
JRC	Joint Research Centre
MRGN	Multi Resistant Gram Negatives
MRSA	Methicillin-resistant Staphylococcus aureus
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PIM	Plastics Implementation Measure
TEM	Transmission electron microscopy
UV-VIS	Ultraviolet- visible
WHO	World Health Organization
WPMN	Working Party on Manufactured Nanomaterials

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