



REVIEW ARTICLE

Silver nanoparticles in polymeric matrices for fresh food packaging



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Abstract The growing demand for increased fresh food shelf life as well as the need of protection against foodborne diseases urged the development of antimicrobial food packaging. Among the most efficient methods, the combination of organic–inorganic, packaging, i.e. polymer embedded metal nanoparticles proved to be highly effective. Silver nanoparticles (AgNPs), in particular, have antimicrobial, anti-fungi, anti-yeasts and anti-viral activities and can be combined with both non-degradable and edible polymers for active food packaging. The actual application of AgNPs in food packaging is regulated by EU and USA food safety authorities in a prudent way, due to the inability to make conclusive statements on their toxicity. Therefore, their use is evaluated in terms of Ag⁺ migration into the packed food.

In this mini review, the most recent studies are reported on protection of meat, fruit and dairy products against the most common food pathogens by AgNPs-doped non-degradable and edible polymers and oils are reported.

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1. Introduction

In the last few decades, the demand for “ready to eat”, “ready to cook” and “ready to use” food dramatically increased with a consequent increased necessity of the manufacturers to produce minimally processed food, in an attractive and hygienic way.

One of the main issues in food processing is the protection against foodborne diseases which still represent a global problem of public health. The Center for Disease Control and prevention (CDC) estimates that the impact of foodborne diseases in countries such as the United States results each year in 76 million sick people, 325,000 of which are hospitalized and 5000 die (Morris, 2011).

The urgency of preventing foodborne diseases required acceleration in the development of antimicrobial food packaging, a special packaging that releases active biocide substances in order to improve the quality of the food, extend shelf life, and prevent or delay the spoilage. The antimicrobial action may be obtained by releasing the biocide directly into the food or in the space around the food (Vermeiren et al., 2002) and can be exerted by both organic and inorganic materials (Malhotra et al., 2015). The former ones are mostly organic acids, enzymes and polymers, the latter ones are nanoparticles of metals or metal oxides. The organic antimicrobial materials are less stable at high temperatures compared to inorganic ones, whereas metal and metal oxide nanoparticles withstand harsher processing conditions (Metak, 2015; Metak and Ajaal, 2013).

The use of nanomaterials in several fields is growing (Carbone et al., 2015) and also for food packaging it largely increased over the past decade (Bumbudsanpharoke and Ko, 2015; Nasr, 2015). Nanotechnology-enabled food packaging can be divided into two different key points: (i) improved packaging, where nanomaterials are mixed into the polymer matrix to improve the gas barrier properties such as polymer/clay nanocomposites; (ii) “active packaging”, where the nanoparticles interact directly with the food or the environment to allow a better protection of the food, such as silver nanoparticles as potent antimicrobial agents (Duncan, 2011). Metal nanoparticles with their potent antimicrobial properties are therefore used as “active packaging”. Emerging metal nanoparticles with biocidal properties are Cu, Zn, Au, Ti, and Ag (Toker et al., 2013). Among them silver nanoparticles (AgNPs) demonstrated to have the most effective bactericidal properties against a wide range of pathogenic microorganisms, including bacteria, yeasts, fungi and viruses (Rai et al., 2009; Martinez-Abad et al., 2012). AgNPs showed better antimicrobial properties compared to metallic silver thanks to their extremely large surface area which can provide a better contact with the microorganism (Toker et al., 2013). Furthermore, they exhibit low volatility and stability at high temperatures (Youssef and Abdel-Aziz, 2013). AgNPs can be hosted in different matrices such as polymers and stabilizing agents

(citrates and long chain alcohols) (Toker et al., 2013), through different strategies: they can be coated, absorbed, or directly incorporated in the synthesis processes (Martinez-Abad et al., 2012).

Although the use of AgNPs as antimicrobial agents in food packaging is a mature technology, concerns on the risks associated with the potential ingestion of the Ag ions migrated into food and drinks still exist. This leads to a prudent attitude of food safety authorities (Cushen et al., 2012).

The European Food Safety Authority (EFSA) panel on Food Additives and Nutrient Sources added to Food stated its inability to assess the safety of silver hydrosol (EFSA, 2011; Shenir, 2014) and, for extension, products for food packaging and food supplements that contain AgNPs are not allowed in the EU unless authorized (Bumbudsanpharoke and Ko, 2015). EFSA did provide upper limits of Ag migration from packaging. Recommendations are not to exceed 0.05 mg/L in water and 0.05 mg/kg in food. This implies that evaluations of silver migration profiles are necessary to assure antimicrobial effectiveness while complying with the current legislation. EFSA published in 2011 a document (EFSA, 2011) which indicates that *in vitro* genotoxicity, absorption, distribution, metabolism and excretion tests are required by manufacturers.

Similarly, in the United States the USFDA published in 2014 a document which provides guidance to manufacturers of food ingredients and food contact substances. The USFDA recommends that manufacturers should study and prepare a toxicological profile for each container with nanomaterials (USFDA, 2014). In March 2014 the United States Environmental Protection Agency (EPA) prohibited the sale of plastic food containers with nanosilver produced by an American company because their products have not been tested according to USFDA regulations (Martin, 2014). At the moment Canada does not have any regulation on nanomaterials and in many other countries only incomplete food safety regulations are introduced (Berekaa, 2015).

In this mini review, we overview the latest studies focused on the evaluation of the efficacy of AgNPs-containing hybrid materials to assure fresh food safety. The use of AgNPs in food packaging was investigated in order to obtain information about their benefits to food packaging and also about their possible negative and toxicological effects.

2. AgNPs based nanomaterials for food packaging

AgNPs based antimicrobial packaging is a promising form of active food packaging which plays an important role in extending shelf-life of foods and reducing the risk of pathogens.

A subdivision has been made according on the type of matrix used to host the AgNPs. Two subsections are made depending on the polymeric matrix employed, i.e. whether it is a (i) non-degradable polymeric one or a (ii) biodegradable edible coating film made by either a polymer or a stabilizing

Table 1 Nanocomposite packaging based on AgNPs/degradable polymeric matrices.

Polymer matrix	Tested food	Tested microorganisms	Reference
LDPE + Ag, ZnO NPs	Orange juice	Yeast, molds, total aerobic bacteria	Emamifar et al. (2010)
LDPE + AgNPs	Barberry	Total aerobic bacteria	Motlagh et al. (2012)
LDPE + AgNPs, TiO ₂ , kaolin	Strawberry	–	Yang et al. (2010)
PVC + AgNPs	Minced beef	Total mesophilic bacteria, <i>E. coli</i> , <i>S. aureus</i>	Mahdi et al. (2012)
EVOH + AgNPs	Chicken, pork, cheese, lettuce, apples, peels, eggshells	<i>Salmonella</i> spp., <i>L. monocytogenes</i>	Martinez-Abad et al. (2012)
Polyurethane + AgNPs	–	<i>E. coli</i> , <i>S. aureus</i>	Toker et al. (2013)
Polystyrene + AgNPs	–	<i>S. aureus</i> , <i>Bacillus subtilis</i> , <i>Enterococcus faecalis</i> , <i>Pseudomonas aeruginosa</i> , <i>E. coli</i> , <i>salmonella typhimurium</i> , <i>Candida albicans</i> , <i>A. niger</i>	Youssef and Abdel-Aziz (2013)
Polyethylene + Ag, TiO ₂ NPs	Fresh apples, white slice bread, fresh carrots, soft cheese, atmosphere packaging milk powder, fresh orange juice	<i>Penicillium</i> , <i>Lactobacillus</i>	Metak and Ajaal (2013)
Polyethylene + Ag, TiO ₂ NPs	Fresh apples, white slice bread, fresh carrots, soft cheese, atmosphere packaging milk powder, fresh orange juice	<i>S. aureus</i> , <i>Coliforms</i> , <i>E. coli</i> , <i>Listeria</i>	Metak (2015)

agent. A summary of the selected papers for the two subsections are reported in [Tables 1 and 2](#).

It is important to highlight that in both cases the addition of AgNPs into polymeric matrices is able to largely influence the film permeability with a subsequent influence on product quality, as shown in the examples reported below.

2.1. AgNPs/non-degradable polymeric matrix based nanocomposite packaging

Among the non-degradable polymers polyethylene (PE), polyvinyl chloride (PVC) and ethylene vinyl alcohol (EVOH) are the most largely used to host AgNPs for food packaging.

PE is the most common plastic made by addition of ethylene in a polymerization process. Between the two basic forms of polyethylene, high density polyethylene (HDPE) and low density polyethylene (LDPE), the latter is more flexible, transparent and resistant to moisture and therefore only this form is used to make covering films suitable for fresh food storage. PVC is a transparent, stiff and ductile thermoplastic material obtained by polymerization of vinyl chloride polymer. It is an excellent barrier to acids, bases and oil and it's largely used for food containers and packaging films (Mahdi et al., 2012). EVOH is a copolymer of ethylene and vinyl alcohol. It is a thin film with excellent resistance to oil, fat and oxygen. However, EVOH is moisture sensitive and therefore cannot be used in direct contact with liquid food (Martinez-Abad et al., 2012).

Some examples of these polymers employed after incorporation with AgNPs for packaging of different foodstuffs are reported herein.

Low-density polyethylene (LDPE) polymer matrix containing Ag and ZnO nanoparticles was studied to preserve and extend the shelf life of orange juice (Emamifar et al., 2010). This active-nanocomposite showed to be very effective as

antimicrobial nanomaterial in combination with heat treatment at the pasteurization temperature (55 °C–65 °C). The antimicrobial activity of LDPE with nanosilver was significantly more effective against fungi (yeast and molds) with respect to the other active polymer matrices containing ZnO nanoparticles. The antimicrobial activity of AgNPs allowed markedly decreasing of the pasteurization temperature of orange juice by 10 °C.

Similar effects were obtained testing Ag-LDPE packages for the preservation of appearance and sensory quality of stored barberries (Motlagh et al., 2012) and for the keeping of quality of strawberries during extended storage (Yang et al., 2010). Both works showed that nano-packaging obtained with LDPE and AgNPs was able to maintain the sensory, physicochemical and physiological qualities of barberry and strawberry fruits at a higher level compared with normal packaging realized with polyethylene bags.

Food “ready-to-use” requires a refrigerator step to ensure the quality and keep the nutritional compounds. The shelf life of meat and its derivatives depends largely on the quality of the raw material. Mahdi et al. (2012) evaluated the antimicrobial effect of AgNPs-PVC nanopackaging in minced beef, during storage at refrigerator temperature (+4 °C). After 7 days of study the indications are of an inhibitory effect of AgNPs packaging on microbial growth. In particular, these inhibitory effects are better against *Escherichia coli* growth with respect to *Staphylococcus aureus* growth. Really, bacteria growth was slowed down significantly, allowing an increased shelf-life of minced meat that usually spoiled after 2 days of storage in common food packaging.

The effects of Ag and TiO₂ nanoparticles in PE polymer packaging were investigated by Metak et al. (Metak and Ajaal, 2013; Metak, 2015) on solid, liquid, high fat containing and high acidic food samples, comparing them to conventional

Table 2 Nanocomposite packaging based on AgNPs/biodegradable edible polymeric matrices.

Polymer matrix	Tested food	Tested microorganisms	References
Cellulose + AgNPs	Beef meat	Total aerobic bacteria, lactic acid bacteria, <i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i>	Fernandez et al. (2010a)
Cellulose + AgNPs	Fresh-cut melon	Total mesophilic aerobic bacteria, psychrotrophic bacteria, yeasts, molds	Fernandez et al. (2010b)
HPMC + AgNPs	–	<i>E. coli</i> , <i>S. aureus</i>	de Moura et al. (2012)
Pullulan + AgNPs	Turkey deli meat	<i>L. monocytogenes</i> , <i>S. aureus</i>	Khalaf et al. (2013)
Pullulan + ZnO NPs	–	–	–
Pullulan + oregano oil	–	–	–
Pullulan + rosemary oil	–	–	–
Pullulan + AgNPs, EOs	Meat and poultry products	<i>S. aureus</i> , <i>L. monocytogenes</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>E. coli</i> , <i>S. Typhimurium</i>	Morsy et al. (2014)
Pullulan + ZnO NPs, EOs	–	–	–
Agar hydrogel + Ag-montmorillonite NPs	Fior di Latte cheese	<i>Pseudomonas</i> spp.	Incoronato et al. (2011)
Sodium alginate + AgNPs	Pears, carrots	<i>E. coli</i> , <i>S. aureus</i>	Fayaz et al. (2009)
Calcium alginate + Ag-montmorillonite NPs	Fresh-cut carrots	–	Costa et al. (2012)
Sodium alginate + Ag-montmorillonite NPs	Fior di Latte cheese	–	Gammariello et al. (2011)
Sodium alginate + CaCl ₂ + AgNPs	Fior di Latte cheese	<i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i> , <i>E. Coli</i>	Mastromatteo et al. (2015)
AgNPs/starch	–	<i>S. aureus</i> , <i>E. coli</i> , <i>Candida albicans</i>	Abreu et al. (2015)
AgNPs/starch/sodium alginate/lemongrass oil	–	<i>E. coli</i>	Maizura et al. (2007)

HPMC: hydroxypropyl methylcellulose.
EOs: essential oils.

containers. Energy-dispersive-X-ray spectroscopy (EDX) studies indicated that the Ag and TiO₂ NPs are distinctly layered and embedded within the bulk polymer instead of existing as a coating on the polymer surface. AgNPs containers showed significant antifungal activity by inhibition of the microorganism growth after several days of storage. A significantly lower rate of deterioration was observed up to day 10 in all samples examined, due to the antibacterial activity of Ag and TiO₂ NPs, compared to normal packaging.

To assess the antimicrobial activity of the nanocomposites, food samples should be differentiated according to their protein contents as inactivation of silver is favored by the presence of proteins (Martinez-Abad et al., 2012). In this work silver ions were incorporated into an EVOH polymer matrix and the antibacterial efficacy was tested in contact with different kinds of food. In particular, two sets of experiments were carried out, the first with food samples with low protein content such as fresh cut fruit, fresh fruit juice and vegetables, and the second involving food with high protein content such as meat and cheese. The results of the experiments showed that in food samples with low protein content inactivation of the silver ions might occur to a lesser extent and high bactericidal effect is achieved only for 10% Ag-EVOH films. However, even with low protein samples, a 0.1% silver content in the EVOH film is not enough to ensure not to surpass the restriction limits recommended by the EFSA.

Other polymers have also been used to prepare new nanocomposites for food packaging. Toker et al. (2013) described the synthesis and characterization of a new AgNPs containing polyurethane hybrid coating which showed antibacterial activity against *E. Coli* and *S. aureus*. Youssef et al. (Youssef and Abdel-Aziz, 2013) soaked AgNPs into a

polystyrene (PS) matrix to form a Ag/PS nanocomposite. The nanocomposite was characterized using SEM, TEM and FTIR and demonstrated to significantly inhibit the growth of important pathogenic gram-positive bacteria such as *Bacillus subtilis* and *Enterococcus faecalis*, gram-negative bacteria such as *E. Coli* and *Salmonella typhimurium* and yeast (*Candida albicans*).

2.2. AgNPs/biodegradable edible matrix based nanocomposite packaging

Biodegradable polymeric films represent an alternative option in food packaging because they may be obtained at low costs from renewable sources with no contribution to the environmental pollution. For these reasons, biodegradable polymers, which already encounter extensive use in several fields (Mazzuca et al., 2014), have been attracting much interest as alternatives to non-degradable polymers currently used in food packaging. Among them the most commonly used are polysaccharides such as cellulose, pullulan, agarose, starch and chitosan (Valencia-Chamorro et al., 2011; Dhall, 2013; Elsabee and Abdou, 2013). Cellulose binds electropositive transition metal atoms by electrostatic interactions. Consequently silver ions are adsorbed during immersion in silver nitrate and the porous structure of cellulose favors the synthesis and stabilizations of AgNPs. Absorbent pads are realized in this polymer material which is widely used in fresh food modern packaging strategies (Fernandez et al., 2010a,b). Abundant in nature and at low cost of production, cellulose is biocompatible and may be synthesized in edible format too.

Pullulan is an edible polysaccharide polymer consisting of maltotriose units. Pullulan films are colorless, tasteless, resis-

tant to oil and low permeable to oxygen, but high moisture sensible. In food storage, the water sensibility is a disadvantage for the microbial growth but is advantageous during cooking process (Khalaf et al., 2013; Morsy et al., 2014).

Agarose is a polysaccharide obtained from red algae. It is a linear polymer made up of the repeating unit of agarobiose, a disaccharide formed by D-galactose and 3,6-anhydro-L-galactopyranose. Agar-agar is a jelly-like substance derived from agarose. This gel is water-insoluble, no-toxic, biodegradable and non-immunogenic. For its characteristics agar-agar is used to make coatings for “ready to eat” food and has been proposed for liquid food gel packaging (Incoronato et al., 2011).

Cellulose-based absorbent pads are used as a vehicle for AgNPs formed *in situ* by physical and chemical reduction methods (Fernandez et al., 2010a,b). Microbiological analyses have demonstrated a microbial growth reduction during storage of beef meat (Fernandez et al., 2010a). They were carried out following different conditions: total aerobic microflora, yeast and molds were enumerated with plate count agar test performed at 30 °C–25 °C respectively; pathogens as *Salmonella*, *E. coli* (*E. coli*) O157:H7, *S. aureus* (*S. aureus*), and *Listeria monocytogenes* (*L. monocytogenes*) were tested by viable count cell test after incubation at 37 °C in specific culture media. Total aerobic bacteria were significantly reduced during the storage period analyzed whereas lactic acid bacteria were less sensitive. *Pseudomonas* spp. and *Enterobacteriaceae* counts resulted to be below those of the control samples (< 1 log CFU/g) (Fernandez et al., 2010a). Similar results were obtained with fresh-cut melon samples. The silver loaded absorbent pads reduced the microbial loads also in cut melon (Fernandez et al., 2010b).

A different type of cellulose was used by de Moura et al. (2012) who incorporated AgNPs into a hydroxypropyl methylcellulose (HPMC) matrix for applications in food packaging. The matrix showed good antibacterial properties against *E. coli* and *S. aureus*.

The addition of essential oils of spices (rosemary and oregano) already known as antibacterial agents as well as AgNPs may further improve the safety and the quality of foods. Khalaf and coworkers (Khalaf et al., 2013) studied the antimicrobial activity of edible pullulan films incorporated with either nanoparticles (AgNPs, ZnO NPs) and essential oils (EOs) such as oregano oil or rosemary oil, in turkey deli meat conservation. The results demonstrated that AgNPs and oregano oil edible films resulted to be more active than ZnO NPs and rosemary oil. The antimicrobial activity was studied against the Gram-positive bacteria *L. monocytogenes* and *S. aureus* during 7 weeks of storage at different temperatures (4°, 25°, 37°, 55 °C). The optimum temperature condition for storage in pullulan edible films with AgNPs and EOs was 4° and 25 °C. Food samples packaged with pullulan film with AgNPs and oregano oil demonstrated a significant decrease in the population of both bacteria.

In a similar work Morsy et al. (2014) demonstrated the activity of AgNPs incorporated into pullulan films in addition to essential oils to control pathogens such as *S. aureus* and *L. monocytogenes* on meat and poultry products.

Agar hydrogel was also used as hosting matrix for NPs (Incoronato et al., 2011). Silver montmorillonite (Ag-MMT) nanoparticles, obtained replacing Na⁺ ions in the natural sodium montmorillonite with Ag⁺ ions, were loaded into an

agar hydrogel to prolong the shelf life of Fior di Latte cheese. The results confirmed the inhibitory effect exerted by the Ag-MMT matrix on the growth of *Pseudomonas* spp.

Among the edible materials used to incorporate AgNPs for food packaging creating a biopolymer matrix also stabilizing agents such as salts of the anionic polysaccharide alginic acid (sodium alginate) have been successfully used (Fayaz et al., 2009). Film-gels made with sodium alginate show good tensile strength, flexibility, resistance to tearing and they are oil-proof. The low temperature used in the alginate gel coating synthesis minimizes the inactivation of the antimicrobial agent and preserves the foodstuff characteristics. An odium alginate film incorporating silver nanoparticles was synthesized and investigated demonstrating a reduction in fruit and vegetable decay rate after several storage days (Fayaz et al., 2009). Antibacterial activity was demonstrated against microorganisms of both Gram-positive and Gram-negative types. In another work, sodium alginate was used with calcium ions as active coating loaded with silver montmorillonite nanoparticles to realize a significant shelf life prolongation of fresh-cut carrots (Costa et al., 2012). In other works, sodium alginate was used to form a bio-composite coating to preserve quality of Fior di latte cheese (Gammariello et al., 2011; Mastromatteo et al., 2015).

Also starch was used as edible film. A nanostructured starch based film containing clay and AgNPs has been recently proposed (Abreu et al., 2015). In their native forms, starches are organized into granules and show poor mechanical properties and high water affinity. The presence of AgNPs, which can be easily complexed by the large number of hydroxyl groups of the biopolymer, showed to improve mechanical and gas barrier properties with excellent antimicrobial results.

In another paper, starch and sodium alginate are mixed together to form an edible film with improved mechanical properties (Maizura et al., 2007). Alginate has a potential to form biopolymer films because of its unique colloidal properties but exhibits poor water resistance because of its hydrophilic nature. The mixture of the two biopolymers allowed to improve the mechanical properties of the film. In this paper lemongrass oil was added to the film which resulted to be effective in inhibiting the growth of *E. coli*. The antimicrobial effect resulted to be enhanced in the films containing also glycerol compared to films without glycerol.

3. AgNPs release studies

It is extremely important to assess hazards and risks of potential migration of packaging constituents into food, for consumer safety.

An exhaustive study was performed by Echevoyen and Nerín (2013) on the migration of silver from different types of nanocomposites (LDPE and polypropylene) into food simulants. In particular, they made an analysis of the form of silver migrating, whether ions or particles. Their results showed not only that silver migrated into food simulants, but also that the migration was food and heating dependent, the acidic food and the classical oven presenting the highest migration level. The authors suggested two different migration mechanisms, related both to the release of the detached silver nanoparticles from the composites, and dissolution of silver ions upon oxidation. However, in their study they found that the Ag migration

is well below the maximum migration limits stated by the European Union legislation.

Cushen et al. (2014) studied the effect of time and temperature on the migration of silver and copper from polyethylene (PE) nanocomposites to boneless chicken breasts. Migration of silver occurred in a range from 0.003 to 0.005 mg/dm², and migration of copper in a range from 0.024 to 0.049 mg/dm². The authors showed that critical parameter driving the migration was the percentage of nanofiller in the nanocomposites more than particle size or storage temperature and time. In another work, Cushen et al. (2013) employed inductively coupled plasma mass spectrometry (ICPMS) to evaluate the silver migration from PVC nanocomposite to chicken meat. Once again silver migration appears to be below current migration limits established by the European Union legislation.

Among the articles scanned in this review Metak performed migration assessments of AgNPs and TiO₂ NPs from the packages in a wide range of food matrices. The analyses have been carried out using plasma mass spectrometry (ICP-MS). The results showed that there was a migration of AgNPs and TiO₂ NPs from the packaging to the food materials, in a long time span, i.e. after 7 and 10 days. Furthermore, the highest migration level was achieved from orange juice samples, followed by apple sample. Bread samples had the lowest level. Regardless of the food type, the migrating amounts of both Ag and TiO₂ NPs were less than its concentration limit of 10 mg/mL (2002/72/EC).

4. Conclusions

Nanosilver-including containers are innovative concepts in active food packaging to prolong the shelf-life of food and the quality maintenance. In modern packaging strategies the adsorbent pads are extensively used to adsorb fluids exuded from fresh food. To avoid the health risks associated with food storage it is very important to exert a control of the loads of spoilage-related microflora of adsorbent pads, over all for meat and fish storage containers. This is effectively achieved by food packing in AgNPs embedded matrices. Two main distinctions can be made, based on the type of matrix, whether it is edible or non-edible. In both cases, the shelf life of fresh foods such as, meat fruit and dairy products are significantly increased by biocide effects against *E. Coli*, *Salmonella*, *L. monocytogenes* and *S. aureus*, *Pseudomonas* spp. *Pseudomonas* spp. Concerns about possible AgNPs toxicity caused EU and USA food safety authorities to enforce regulations in the their use in actual food packaging, the limits being set on the Ag + migration to the packed food.

Evaluations made on the Ag+ migration from AgNPs to packed food in several usage conditions indicates that acidic food, microwave heating and the use of nanofillers may increase the migration, the actual Ag+ concentration, staying below the limits set by safety authority.

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