Migration of silver nanoparticles from plastic materials, with antimicrobial action, destined for food contact

Fabio Silvestre Bazilio[1] · Lisia Maria Gobbo dos Santos[1] · Cristiane Barata Silva[1] ·
Santos Alves Vicentini Neto[1] · Carlos Alberto Senna[2] · Bráulio Soares Archanjo[2] ·
Silvana do Couto Jacob[1] · Shirley de Mello Pereira Abrantes[1]

Abstract Five materials with antimicrobial function, by adding silver, were investigated to evaluate total silver concentration in the polymers and migration of silver nanoparticles from the materials in contact with food. The migration test was carried out by contacting plastic material with food simulant. Migration concentrations and average silver particle sizes were determined by mass spectrometry with inductively coupled plasma, performed in single particle mode (spICP-MS). Additionally, silver particles size and shape were characterized by scanning electron microscopy (SEM) with chemical identification by energy-dispersive X-ray spectroscopy (EDS). Most of samples showed detectable total silver concentrations and all samples showed migration of silver nanoparticles, with concentrations found between 0.00433 and 1.35 ng kg^{-1}. Indeed, the migration study indicated the presence of silver nanoparticles in all food simulants, with sizes bellow 95 nm. The average particle size determined for acetic acid was greater than that observed in the other simulants. In the images obtained by SEM/EDS also confirmed the presence of spherical silver nanoparticles, between 17 and 80 nm. The findings reported herein will aid the health area concerning of human health risk assessments, aiming at regulating this type of material from a food safety point of view.

✉ Lisia Maria Gobbo dos Santos
lisia.gobbo@incqs.fiocruz.br

1 Chemistry Department, National Institute of Quality Control in Health-INCQS/FIOCRUZ, Av Brasil 4365, Rio de Janeiro, RJ CEP: 21040-900, Brazil

2 Directorate of Scientific Metrology and Materials Division, National Institute of Metrology, Quality and Technology INMETRO, Duque de Caxias, Brazil

Introduction

Food packaging technology is constantly evolving in response to the growing challenges of modern society. In this context, one of the main challenges for the packaging sector concerns food shelf-life extension (Realini and Marcos 2014), which requires the incorporation of appropriate technologies to food packaging production (Vanderroost et al. 2014). The use of active food packaging is a good innovation example that goes beyond traditional packaging functions, where the product and packaging interact to extend a product shelf life or to improve its safety or sensory properties (Realini and Marcos 2014). Most studies in this field are directed towards active packaging, developed with antimicrobial agents (Barbosa-Pereira et al. 2014). This type of packaging is manufactured from nanocomposites formed by incorporating metal nanoparticles into polymer films. The most common nanocomposites used as antimicrobial films for food packaging are based on silver, well known for its strong toxicity towards a wide range of microorganisms, also exhibiting high temperature stability and low volatility (Peters et al. 2014; Simbine et al. 2019).

Although silver zeolites have been used in the development of antibacterial polymers for some time, AgNP-based nanocomposites offer greater stability and slower silver ion release rates to stored foods compared to the elemental form, which is important for sustained antimicrobial activity (Duncan 2011). Furthermore, due to their reduced particle size, the total surface area in solution is maximized, leading to greater effects (Panyala et al. 2008). However, these intrinsic AgNP properties also make them potentially harmful to

humans (Van der Zande et al. 2012), as silver nanomaterial effects seem to be linked to the release of silver ions from these materials, while some studies also suggest the in vivo formation of silver nanoparticles resulting from silver ion absorption (Peters et al. 2014).

The main risk of consumer exposure to food packaging nanoparticles is likely due to nanoparticle migration into the packaged food and beverages (Echegoyen and Nerín 2013; Kuorwel et al. 2015). Yet, the migration and diffusion mechanism potentials concerning food packaging nanoparticles is a nanotechnology field issue that has not yet received the same attention as nano-aerosols, nanofluids and drugs (Hannon et al. 2015). Therefore, a significant knowledge gap regarding nanoparticle migration processes is observed, and no data on whether whole nanoparticle migration occurs, or only in the form of dissolved ions are available. Therefore, studies prior to the quantification process are required to understand if nanoparticles are released directly or formed from ions during post-migration treatment. Many studies indicate only a total migration amount through the analysis of the simulant solution.

Consequently, complete test material characterization involving not only the particles originated from the migration process, but also the host polymers in their pristine state, are essential (Istiqola and Syafiuddin 2020; Morais et al. 2020). In view of this, further in-depth studies are paramount with regard to nanoparticle migration, with an emphasis not only on their quantification. But also their characterization, since food safety issues associated to nanotechnology applications in the food packaging sector are directly associated to the physicochemical nature of the nanoparticles that migrate to food items in contact with packaging (Aldossari et al. 2015).

Food contamination due to monomer or additive migration is a Health Surveillance issue (Kato and Conte-Junior 2021). In Brazil, the Resolution of the Collegiate Board of Directors (RDC) No. 91 of the National Health Surveillance Agency established in May 11, 2001 determines that components used in materials intended to come into contact with food items must be included in positive lists and their use must be authorized for the manufacture of these kinds of materials (Brasil 2001). The positive list of permitted plastic material additives intended for the preparation of food packaging and equipment is established by ANVISA RDC No. 326 was set on December 3, 2019, but does not include AgNP (Brasil 2019). Concerning the European Union's (EU) Regulation No. 10/2011 of January 14, 2011, substances in nanoforms can only be used if they have been expressly authorized and mentioned in this regulation's Annex I, which deals with the list of authorized substances in the EU. AgNP, however, are also not included in the list (EU 2011).

Routine food packaging tests, called migration tests, aim to assess the amount of substances likely to migrate from the packaging to the food item. These tests simulate, as far as possible, the conditions to which the food package and food item will be submitted to, depending on type of food, contact time and temperature (Germano and Germano 2001). ANVISA's RDC No. 51 of November 26, 2010 (Brasil 2010) establishes conditions for migration test carried out in food simulants, chosen based on the characteristics of investigated each food or food category. For whole, condensed, skimmed or partially skimmed milk, for example, the migration test must be carried out in a 50% (v/v) food simulant ethanol solution prepared in distilled or deionized water.

Materials and methods

Standards

To prepare the dissolved silver solutions, a multi-elemental standard containing 10,000 μg L^{-1} of silver (Perkin Elmer, Massachusetts, USA) was used. The nanoparticle solutions were prepared from 30, 40 and 80 nm spherical silver nanoparticle standards (Sigma Aldrich, Missouri, USA), containing 1.6×10^{11}, 7.2×10^{10} and 7.4×10^{9} particles mL^{-1}, respectively.

Instrumentation

The measurement and quantification of silver nanoparticles were performed on a NexION 300D inductively coupled plasma mass spectrometer operated in single particle mode (spICP-MS) (Perkin Elmer, Massachusetts, USA). The mass spectrometer (ICP-MS) was equipped with a concentric nebulizer (Meinhard), glass cyclonic nebulizer chamber, cone, skimmer and nickel hyper-skimmer. Argon gas with a minimum purity of 99.996% was supplied by White Martins (São Paulo, Brazil). ICP-MS instrumental and data acquisition parameters are listed in Table 1. The validation of the methodology used is described in Bazilio et al. (2021).

To quantify the silver present in plastic materials, the samples were digested in a Speedwave microwave (Berghof, Eningen unter Achalm, Germany). For characterization of the nanoparticles present in the sample, a Helios Nanolab 650 electron microscope (FEI Company, Oregon, USA) was used, equipped with an energy dispersive X-ray spectrometer.

Sample selection

Five commercial plastic food packaging materials presenting antimicrobial characteristics due to the addition of silver nanoparticles were selected, in the form of bottles, flexible films, zip locks and laminated films, comprising different polymers, namely low-density polyethylene (LDPE),

Table 1 Default spICP-MS instrumental and data acquisition parameters

Instrumental parameters		
RF power	1400 W	
Argon gas flow rate		
Plasma	18 L min^{-1}	
Auxiliary	1.2 L min^{-1}	
Nebulizer	1 L min^{-1}	
Data acquisition parameters		
Measurement unit	Standard	Single particle detection
Point per spectral peak	1	1
Sweeps	20	1
Dwell time	50 ms	50 μs
Readings per replicate	1	2000.000
Integration time	1 s	100 s

spICP-MS mass spectrometry with inductively coupled plasma, performed in single particle mode

high-density polyethylene (HDPE), and polyvinyl chloride (PVC). The samples were uniquely identified with the code "AM-XX", with XX being a sequential number from 01 to 05 (AM-01 to AM-5) (Table 2). Samples AM-01, AM-04 and AM-05 were purchased directly from their sellers. Samples AM-02 and AM-03 were obtained through partnerships established with their manufacturers.

Sample nanoparticle characterization

The AgNP present in the selected plastic materials were characterized as follows: a total of 10 g of each material were first calcined in a muffle at 600 °C for 4 h. The ashes were then homogenized and a small amount of each sample was deposited on a carbon strip. The excess was eliminated under a nitrogen flow. Metal supports (stubs) containing the carbon strips were analyzed by SEM and AgNP identification was performed by Energy Dispersive X-Ray Spectroscopy (EDS) (Huang et al. 2011; Liu et al. 2016).

Table 2 Tested samples

Sample	Polymer	Food in contact	Presentation
AM-01	PEBD	Not specified	Reclosable bags
AM-02	PEAD	Milk	Plastic bottles
AM-03	PEBD	Milk	Flexible plastic bags
AM-04	PVC	Not specified	Transparent PVC film
AM-05	PVC	Not specified	Transparent PVC film

AM-01 to AM-05 Sample identification codes, *LDPE* low-density polyethylene, *HDPE* high-density polyethylene, *PVC* polyvinyl chloride

Migration tests

For the specific silver nanoparticle migration test, 1 dm^2 of each sample was cut with the aid of a calibrated mold and placed in contact with 50 mL of the simulant solvent in a 250 mL capped Erlenmeyer and stored in an oven.

The analytical migration method follows ANVISA RDC No. 51 of November 26, 2010 (Brasil 2010), and the test conditions were defined based on established provisions for materials that will encounter fatty, acidic aqueous and non-acidic aqueous foods. In this regard, ANVISA's Resolution establishes a 95% (v/v) ethanol solution in deionized water as one of the possible simulants for fatty foods. For acidic aqueous foods, a 3% (v/v) acetic acid solution in deionized water is determined, while deionized water is used for non-acidic aqueous foods. Moreover, a 50% (v/v) ethanol solution in deionized water was used for milk package testing, established for simulating whole, skimmed and partially skimmed milk and dairy beverages (Brasil 2010).

Concerning the milk package samples, the migration test was carried out at 20 °C for 10 days, as recommended by Brazilian and European legislations for contact times greater than 24 h at temperatures over 5 °C and less than or equal to 20 °C employing the selected simulants. Regarding the other samples, the test was carried out at 40 °C for 10 days, as recommended for contact times longer than 24 h at temperatures ranging from 20 to 40 °C. All conditions were established based on the worst package food exposure case. Table 3 presents the test conditions employed herein.

All migration test solutions were evaporated to dryness at the end of the migration test, and then resuspended in 10 mL of deionized water, followed by a triplicate analysis (three readings) employing spICP-MS.

Silver concentration determinations employing spICP-MS

Working solutions ranging from 1.5 to 20 μg L^{-1} were prepared for the construction of the Ag(i) curve and analyzed by spICP-MS. The signal intensities (counts) of each standard were associated to their dissolved silver concentrations, in μg L^{-1}.

Table 3 Migration test conditions according to RDC No. 51 (ANVISA, Brazil)

Sample	Food simulant	Migration conditions
Milk packaging	Ethanol 50% (v/v)	20 °C, 10 days
Other materials	Ethanol 95% (v/v)	40 °C, 10 days
	Acetic acid 3% (m/v)	
	Deionized water	

ANVISA Brazilian National Health Surveillance Agency

Mass flow curve definition

The Ag(i) concentrations of the working solutions were correlated to the silver mass of each reading ("dwell time"). The ratio was further adjusted by the *TE*, comprising the ratio between the amount of analyte that enters the plasma and the amount effectively aspirated (Bazilio et al. 2021).

Average migration test solution silver particle size determinations

To determine the average size of the silver particles present in the migration test solutions, the intensity of the signal determined for each particle was correlated with its mass by means of the mass flow curve, allowing for size calculations (Bazilio et al. 2021; Naasz et al. 2018).

The average sizes of the silver particles in the solutions were calculated by averaging the mean size values of the particles, in nm, determined in the three readings performed for the migration test solutions.

Silver nanoparticle concentration determinations

The AgNP concentration, expressed as number of particles per mL, was calculated from the number of determined particles, and AgNP migration concentrations were reported as number of particles per kg of food (Eq. 1), considering a 6 dm² sample ratio per kg of food and the sample size-migration volume ratio, referring to the migration test (Brasil 2019; EU 2011).

$$C_N = \overline{N} \times \frac{R}{T_A} \times V_R \qquad (1)$$

where,

C_N = Concentration in number of particles per kg of food (partículas kg^{-1}),

$\overline{N}$ = Mean particle concentrations (*N*), in particles mL^{-1}, calculated for the three readings of the migration solution,

V_R = Resuspension volume in the migration test, in mL,

T_A = Size of the sample section, used in the migration test, in dm²,

R = Surface/volume ratio, equal to 6 dm² kg^{-1} (Brasil 2019; EU 2011).

Additionally, particle concentrations were expressed as mass of particles, in ng, per kg of food (Eq. 2).

$$C_M = C_N \times \left(\frac{d^3 \times \pi \times \rho}{6} \right) \qquad (2)$$

where,

C_M = Concentration in mass of particles per kg of food (ng kg^{-1}),

C_N = Concentration in number of particles per kg of food (partículas kg^{-1}),

d = average size of silver particles in the solutions from the migration test, in cm,

π = 3,14,159,265,359,

ρ = particle density, in ng cm^{-3}.

Determination of dissolved silver concentration

The concentration of dissolved silver, *D* in ng L^{-1}, was calculated by means of the continuous signal intensity (*counts*), correlated to the concentration of dissolved silver in solution, by interpolation in the dissolved silver curve (silver concentration, μg L^{-1} × signal, *counts*). The concentration of dissolved silver C_{dissol}, in the solutions from the migration test, was reported in μg of dissolved silver per kg of food (Eq. 3).

$$C_{dissol} = \overline{D} \times \frac{R}{T_A} \times V_R \qquad (3)$$

where,

C_{dissol} = Concentration of silver dissolved in the migration solution, in ng per kg of food (ng kg^{-1}).

$\overline{D}$ = Mean dissolved silver concentrations, in ng L^{-1}, calculated for the three readings of the migration solution.

V_R = Resuspension volume in the migration test, in L.

T_A = Size of the sample section, used in the migration test, in dm.²

R = Surface/volume ratio, equal to 6 dm² kg^{-1} (EU 2011; Brasil 2019).

Determination of detection limits

The detection limits of size LOD_T, in nm, and concentration of dissolved silver LOD_d, in ng kg^{-1}, were determined by the mean and standard deviation of the most frequent size found for fifteen readings of a white solution of deionized water, free of silver nanoparticles (Bazilio et al. 2021). Limits were calculated for a 95% confidence level (α = 0.05).

The detection limit of particle concentration, in particles mL^{-1}, was associated with the ability of the method to count 3 particles, as described by Laborda et al. (2013) and Bazilio et al. (2021), considering the transport efficiency (%), the sampling flow (mL ms^{-1}) and the analysis time (ms). Thus the LOD_p, in particles kg^{-1}, was determined by Eq. 4.

$$LOD_p = \frac{3}{F \times t_a} \times \frac{100}{TE} \times \frac{R}{T_A} \times V_R \qquad (4)$$

where,

TE = transport efficiency in %,

F = sampling flow in mL ms^{-1},

t_a = analysis time in ms,

V_R = resuspension volume in the migration test, in mL,

T_A = Size of the sample section, used in the migration test, in dm^2,

R = Surface/volume ratio, equal to 6 dm^2 kg^{-1} (EU 2011; Brasil 2019).

Additionally, the LOD_p was calculated, in ng kg^{-1} of food, referring to the particle size determined for the LOD_T.

Total polymeric sample silver concentration determinations

To determine total silver Ag(t) polymer concentrations, the samples were cut into small pieces and about 100 mg of each sample were weighed, in duplicate, and placed in Teflon-type plastic tubes. Then, 5 ml of H$_2$O$_2$ 30% (Merck, Darmstad, Germany) and 5 ml of HNO$_3$ 65% (Merck, Darmstad, Germany) were added and the solutions were then microwaved. After cooling, the samples were transferred to 15 mL volumetric flasks, type Falcon, and made-up with ultrapure water.

The concentration of Ag(t) $C_{ma}repe_i$, in each i repetition of the sample, was calculated using Eq. 5. The final concentration of Ag(t) (C_{ma}) for each sample was calculated by the mean of the concentrations ($C_{ma}repe_i$) calculated for the i repetitions of the sample.

$$C_{ma}repe_i(\mu g g^{-1}) = \left(\frac{\overline{D} \times V_f}{V_a} \times \frac{V_d}{1000}\right)/m_a \qquad (5)$$

where,

$C_{ma}repe_i$= Total silver concentration, in μg g^{-1},

$\overline{D}$ = Mean dissolved silver concentrations, in μg L^{-1}, calculated for the three sample readings,

V_f = Final dilution volume, in mL,

V_a = Volume of the dilution aliquot, in mL,

V_d = Digestion volume, in mL,

m_a = Sample mass, in g.

To evaluate Ag(t) polymer recoveries, 105.06 mg of each sample were transferred to a digestion tube and 50 μL of a standard containing 20 μg mL^{-1} of 50 nm AgNPs were added. For concentrations in the order of 10 mg kg^{-1} (ppm), recoveries are considered adequate when ranging between 80 and 110%, as established by the AOAC (AOAC International 2016) and indicated by the Brazilian National Institute of Metrology Standardization and Industrial Quality (INMETRO 2020).

Results and discussion

Plastic material AgNP characterization and identification

After calcination, the PVC sample (AM-04) produced a yellowish, fine and light powder partially soluble in 99% ethanol. This solution was placed on an aluminum support containing a carbon tape and analyzed by SEM. Spherical nanoparticles between 17 and 80 nm were observed (Fig. 1d). Images were acquired employing a backscattered electron detector (CBS). Figure 1 presents the SEM images for sample AM-04. The presence of silver nanoparticles was confirmed by EDS. The low AgNP mass in relation to the amount of matrix sample made their identification difficult. Thus, to maximize the relationship between particle mass and matrix mass, we obtained an EDS spectrogram of the nanoparticle with 80 nm diameter in Fig. 1d. It is shown in the inset of Fig. 1c.

For PVC (AM-05), HDPE (AM-01), HDPE (AM-02) and LDPE (AM-03) samples, no AgNP were observed in the obtained images, possibly due to the high amount of matrix present in the sample ashes. Figure 2 and 3 show SEM images for the HDPE (AM-02) and LDPE (AM-03) samples respectively. No images were obtained for the HDPE (AM-01) sample.

Total polymeric silver concentration determinations

The Ag(t) concentrations was determined by spICP-MS and reported as μg g^{-1} of polymer and as m/m percentages of silver in each polymer. The results for each polymer sample are presented in Table 4.

The milk packaging HDPE (AM-02) and LDPE (AM-03) samples contained similar total silver concentrations, with mean values of 5.3 and 5.2 μg g^{-1}, respectively. The LDPE (AM-01), PVC (AM-04) and PVC (AM-05) samples, in turn, contained higher total silver concentrations than the milk packaging samples, of 9.2, 9.9 and 7.6 μg g^{-1}, respectively.

ANVISA's RDC No. 326, established on December 3, 2019, sets a maximum silver and zinc zeolite composition limit of 3% m/m of the plastic material, to be used only as antimicrobial agents (Brasil 2019). The findings reported herein indicate the presence of silver in all analyzed polymer samples, although below the maximum permissible limit. It was not possible, however, to determine whether the determined silver was added in the form of silver and zinc zeolites, requiring further material composition assessments. Furthermore, the determination of total silver only does not indicate whether this metal is present in nano form. According to ANVISA RDC No. 326, substances in nanoform can only be used if they have been expressly authorized, The use of AgNP as potential
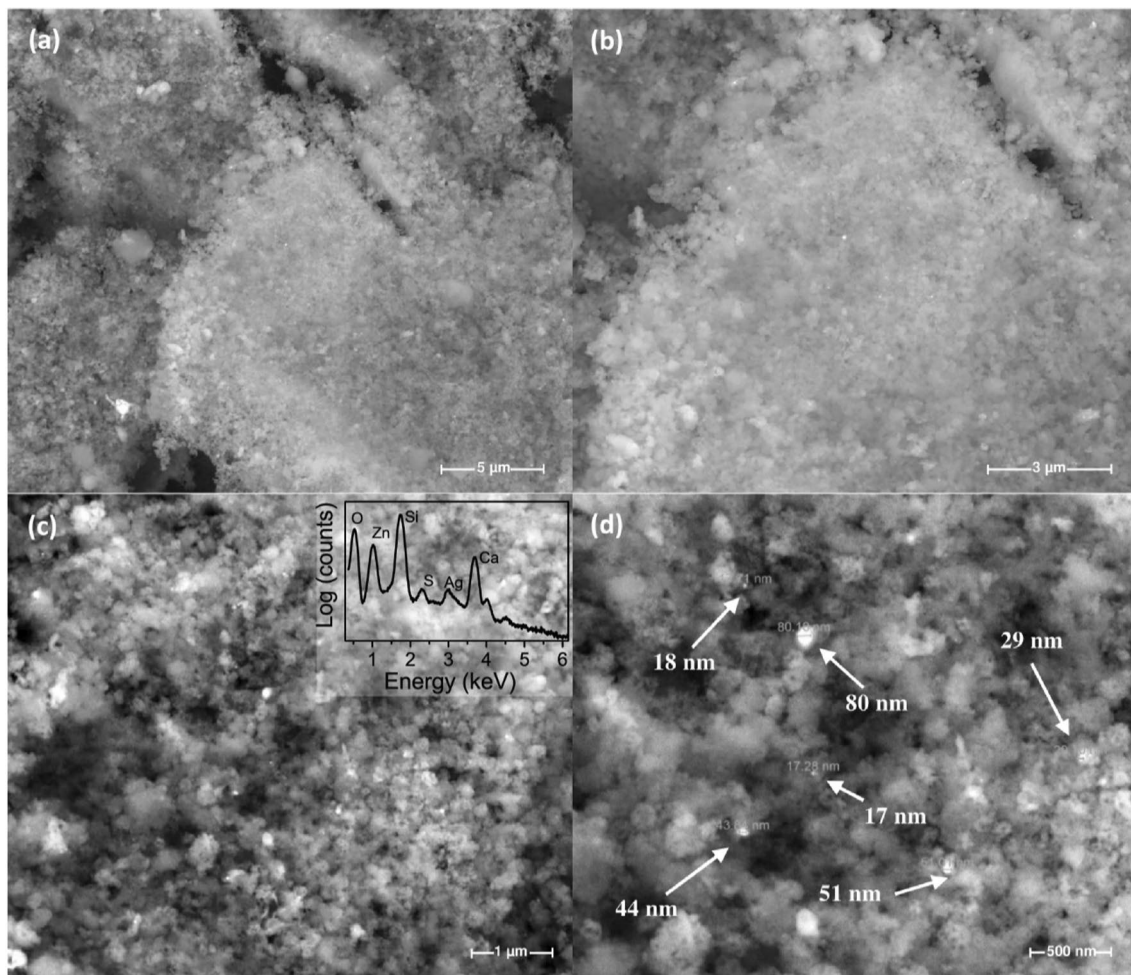
Fig. 1 SEM images of the calcined PVC sample (AM-04) acquired using a Circular Backscatter Detector (CBS) at 10 kV and 0.20 nA. From **a** to **d** different magnifications were used. Image **d** shows the silver particles found, with sizes between 17 and 80 nm. The inset in (**c**) shows the EDS acquired for the 80 nm nanoparticle (**d**)

plastic material additives intended for food item contact is not, however, present in ANVISA's positive list.

Total silver determinations by spICP-MS following acid digestion employing a microwave led to a 107% recovery for the fortified polymer sample, within AOAC recommendations, 80–110% (AOAC International 2016).

Limits of detection determinations

Under the applied experimental conditions of this study, the limits of detection for size LOD_T and dissolved silver concentration LOD_d, were 21 nm and 44 ng kg^{-1} of food, respectively. The LOD_p particle concentration limit was 96 particles mL^{-1}, equivalent to 5.7×10^3 particles kg^{-1} of food for an average particle size of 21 nm, sampling flow rate of 0.2 ml min^{-1} and TE of 9.387%.

Migration concentration determinations

AgNP migration from the sampled plastic materials was assessed for five different polymers samples, namely two LDPE, one HDPE and two PVC samples. To this end, the average size and number of the silver particles that migrated to the food simulant and dissolved silver concentrations were determined. The results obtained are shown in Table 5. Figure 4 and 5 shows concentrations of migration of silver particles and dissolved silver migration, respectively, determined for samples AM-01 to AM-05, by spICP-MS.

Samples AM-02 and AM-03, destined for milk packaging, contained statistically similar dissolved silver migration concentrations in the simulant ethanol 50% solution, calculated as 51 ± 33 and 46 ± 32 ng kg^{-1} food, respectively. The silver migration rate in relation to the silver content present in the polymeric sample, however, was approximately
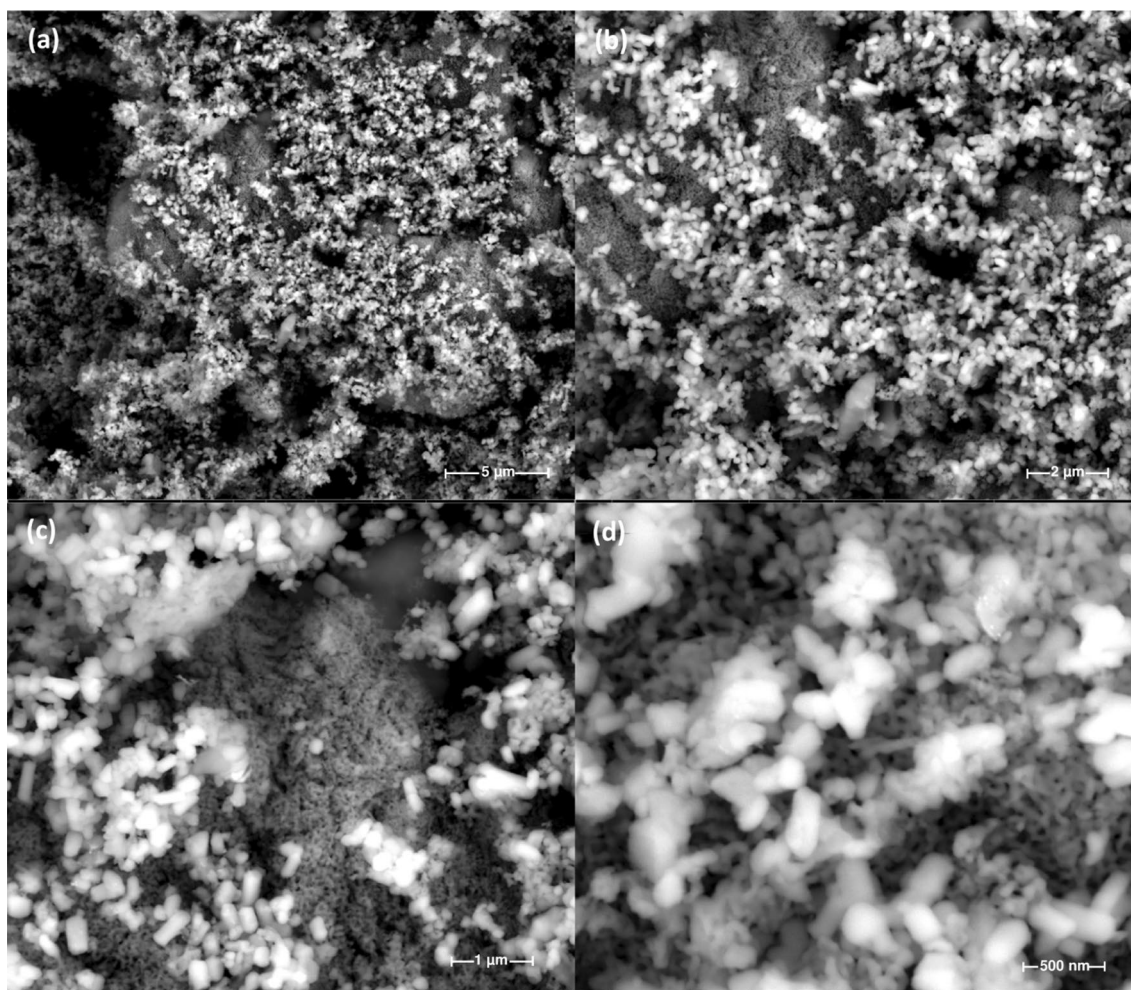
Fig. 2 SEM images of the calcined HDPE sample (AM-02) acquired using a Circular Backscatter Detector (CBS) at 10 kV and 0.20 nA. From **a** to **d** different magnifications were used

nine-fold higher in the AM-03 sample, comprising 0.24% for the LDPE sample, and 0.027% for the AM-02 (HDPE). The higher total silver migration rate in sample AM-03 may be due to greater silver migration difficulty compared to sample AM-02, associated to the nature of the polymer. Silver particle migration, in mass of particles per kg of food, in sample AM-03 (LDPE) was about 2.2-fold higher than sample AM-02 (HDPE), of 0.131 ± 0.014 and 0.0589 ± 0.0054 ng kg^{-1}, respectively. The mean particle sizes in samples AM-02 and AM-03 were 33.9 ± 1.7 and 27.6 ± 2.0 nm, respectively.

Samples AM-04 and AM-05 (PVC), destined for contact with unspecified foods, exhibited dissolved silver migrations below the method LOD (< 44 ng kg^{-1}), for the 95% ethanol simulant, while sample AM-01 (LDPE) contained 46 ± 32 ng kg^{-1}, slightly higher than the LOD. Silver particle migration concentrations, in mass of particles per kg of food, were 0.0766 ± 0.0089, 0.134 ± 0.013 and 0.113 ± 0.011 ng kg^{-1}, for AM-01, AM-04 and AM-05,

respectively, with mean particle sizes ranging from 25.8 ± 2.3 to 31.6 ± 1.8 nm. The silver particle migration rates to the simulant for AM-04 and AM-05 (PVC) were 0.0020 and 0.0022%, respectively, increasing to 0.00027% for sample AM-01 (LDPE). The migration rates for the 95% ethanol simulant were determined in relation to the simulant particle concentrations, and not total silver, as the AM-04 and AM-05 PVC samples presented dissolved silver concentrations below the LOD.

Samples AM-01 (LDPE), AM-04 and AM-05 (PVC) presented migration concentrations for the simulant deionized water of 53 ± 33, 83 ± 15 and 66 ± 13 ng kg^{-1}. The PVC AM-04 and AM-05 presented simulant silver migration rates about 6.3- and 6.8- fold higher, respectively, compared to sample AM-01 (LDPE). The silver particle migration concentrations, in mass of particles per kg of food, were 1.32 ± 0.12, 0.0339 ± 0.0030 and 0.0744 ± 0.0065 ng kg^{-1} for AM-01, AM-04 and AM-05, respectively, with mean particle sizes ranging from 37.7 ± 1.7 to 60.2 ± 2.5 nm.
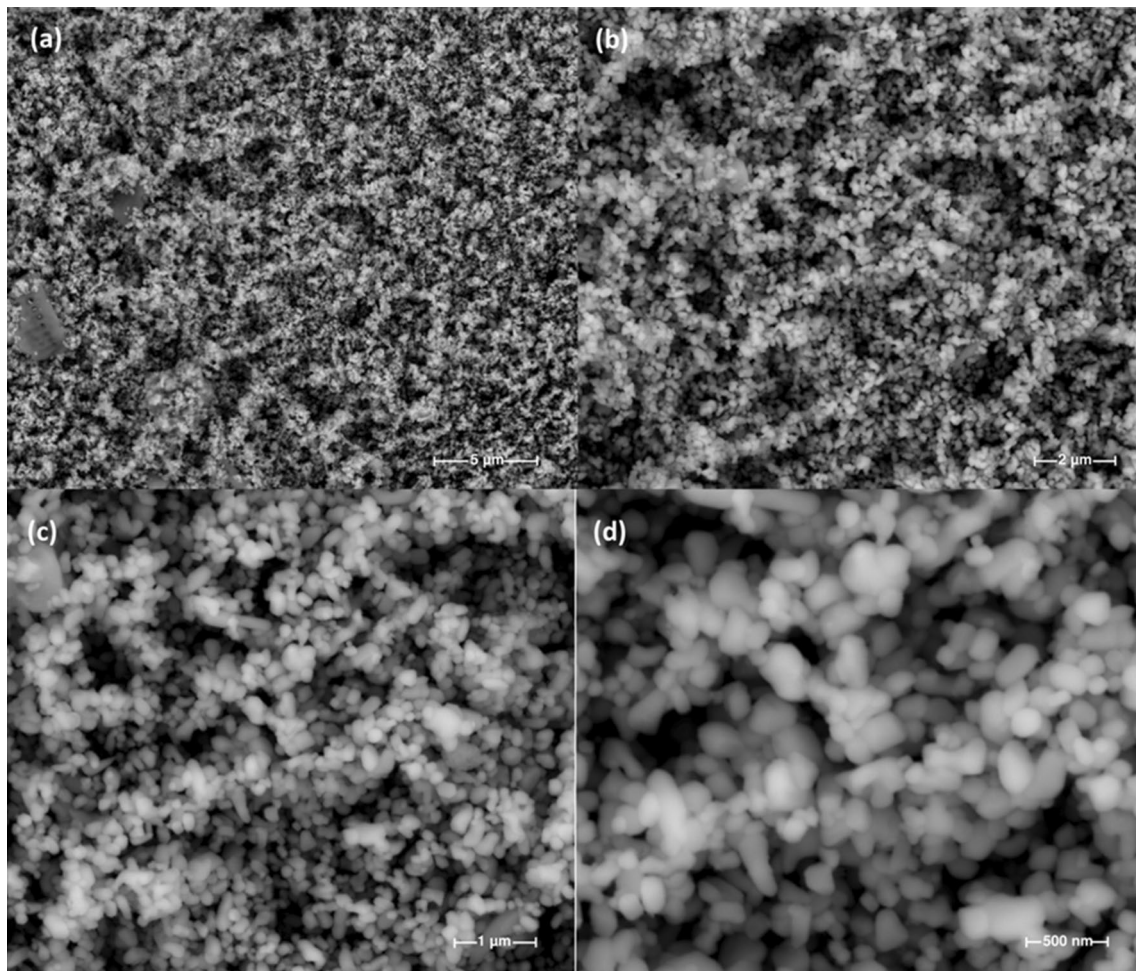
Fig. 3 SEM images of the calcined LDPE sample (AM-03) acquired using a Circular Backscatter Detector (CBS) at 10 kV and 0.20 nA. From **a** to **d** different magnifications were used

Table 4 Total silver concentrations in polymer samples determined by spICP-MS

Sample	Polymer	Ag(t) concentration in polymer	
		($\mu g\ g^{-1}$)	(% m/m)
AM-01	PEBD	9.2	9.2×10^{-4}
AM-02	PEAD	5.3	5.3×10^{-4}
AM-03	PEBD	5.2	5.2×10^{-4}
AM-04	PVC	9.9	9.9×10^{-4}
AM-05	PVC	7.6	7.6×10^{-4}

Mean particle sizes between 31.6 ± 1.8 and $94, 5 \pm 4.1$ nm, with a mean of 61.4 nm, were observed for sample AM-04 for the food simulants acetic acid, deionized water and 95% ethanol, while the microscopy test indicated particle sizes between 17 and 80 nm. Although these are consistent results, it is important to note that the nanoparticles observed in the

migration test may have suffered ionization due to the presence of the food simulants. Furthermore, the SEM assay revealed that the observed nanoparticles were located in small sample area, which may not statistically represent the average size that would be observed in the sample.

When in contact with 3% acetic acid, samples AM-01, AM-04 and AM-05 presented dissolved silver migrations of 215 ± 46, 473 ± 76 and 217 ± 35 ng kg^{-1}, with silver migration rates of 0.75, 7.0 and 4.1%, respectively. Food simulant silver particle concentrations, in mass of silver per kg of food were, respectively, determined as 0.00433 ± 0.00038, 1.35 ± 0.12 and 0.0650 ± 0.0057 ng kg^{-1}, for samples AM-01, AM-04 and AM-05, with mean particle sizes between 62.3 ± 2.6 and 94.5 ± 4.1 nm.
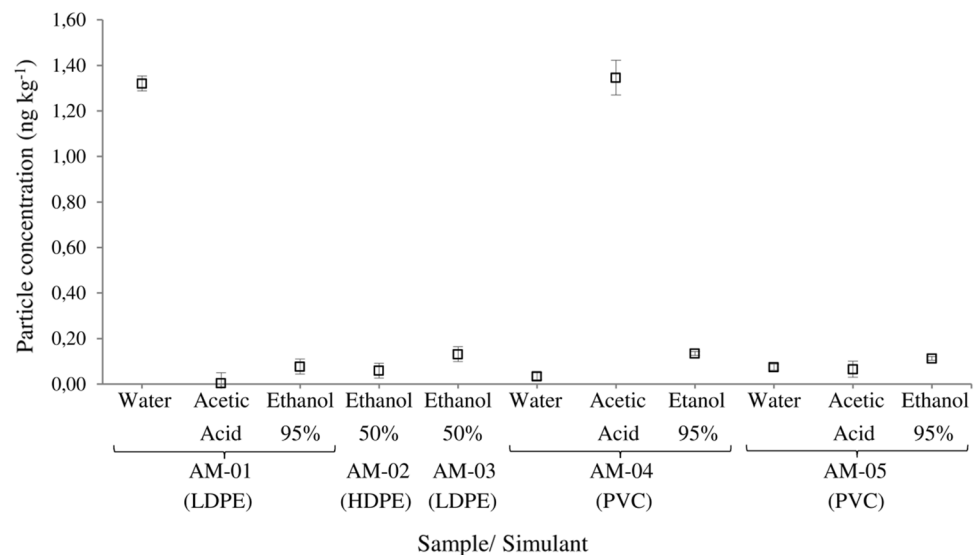
The migration rates determined for samples AM-01, AM-04 and AM-05 indicate greater total silver migration to the acetic acid simulant compared to the deionized water and 95% ethanol simulants, as expected and consistent with other literature reports. According to Mackevica et al. (2016),

Table 5 Concentrations of particles and dissolved silver determined, by spICP-MS, in food simulant solutions from the migration test

Sample	Simulant	Particle concentration		Mean size (nm)	Ag(i) concentration (ng kg⁻¹ food)	Ag(t) concentration[3] (ng kg⁻¹ food)	Ag(t) migration rate from packaging to simulant[4] (%)
		(particles kg⁻¹ food)	(ng kg⁻¹ food)				
AM-01 (LDPE)	Water	4.48×10^6	1.32 ± 0.12	37.7 ± 1.7	53 ± 33	54	0.19
	Acetic acid 3%	3.25×10^3	0.00433 ± 0.00038	62.3 ± 2.6	215 ± 46	215	0.75
	Ethanol 95%	8.15×10^5	0.0766 ± 0.0089	25.8 ± 2.3	46 ± 32	46	0.16 (0.00027[1])
AM-02 (HDPE—Milk)	Ethanol 50%	2.75×10^5	0.0589 ± 0.0054	33.9 ± 1.7	51 ± 33	51	0.027
AM-03 (LDPE—Milk)	Ethanol 50%	1.13×10^6	0.131 ± 0.014	27.6 ± 2.0	46 ± 32	46	0.24
AM-04 (PVC)	Water	3.14×10^4	0.0339 ± 0.0030	58.1 ± 2.4	83 ± 15	83	1.2
	Acetic acid 3%	2.90×10^5	1.35 ± 0.12	94.5 ± 4.1	473 ± 76	474	7
	Ethanol 95%	7.75×10^5	0.134 ± 0.013	31.6 ± 1.8	$< LOD_d$ (44 ng kg⁻¹)	0.134[2]	0.0020[2]
AM-05 (PVC)	Water	6.21×10^4	0.0744 ± 0.0065	60.2 ± 2.5	66 ± 13	67	1.3
	Acetic acid 3%	1.76×10^4	0.0650 ± 0.0057	87.6 ± 3.8	218 ± 35	218	4.1
	Ethanol 95%	7.98×10^5	0.113 ± 0.011	29.5 ± 1.9	$< LOD_d$ (44 ng kg⁻¹)	0.113[2]	0.0022[2]

LDPE low-density polyethylene, *HDPE* high-density polyethylene, *PVC* polyvinyl chloride, *LOD_d* limits of detection for dissolved silver concentration. (1)Migration rate considering only the migration of silver particles, (2) Only the concentration of particles was considered for the calculations of the total silver concentration and the migration rate, (3) Total silver concentration in the solution of migration, determined by the sum of the concentrations of particles and dissolved silver, (4) Ratio between the concentration of total silver determined in the polymer, and the concentration of total silver determined in the migration solution

Fig. 4 Concentrations of migration of silver particles determined for samples AM-01 to AM-05, by spICP-MS. Graphic presentation of particle concentrations (ng kg⁻¹), determined by spICP-MS (NexION 300D, Perkin Elmer), in solutions from the migration test in food simulant. spICP-MS: Inductively coupled plasma mass spectrometry, run in Single Detection Mode, AM-01 to AM-05: Sample identification codes; LDPE: Low density polyethylene; HDPE: High density polyethylene; PVC: Poly (vinyl chloride)

silver migration rates tend to increase in reduced pH, thus increasing migration to the 3% acetic acid solution compared to deionized water or 95% ethanol.

However, the silver particle concentrations observed for samples AM-01 and AM-05 in the 3% acetic acid simulant were lower than those observed for the deionized water and 95% ethanol simulants. Sample AM-04, on the other and, exhibited higher silver particle concentrations for the acetic

acid simulant than the other simulants, although 94% of the particles were over 73 nm. These findings indicate that part of the particles that migrate to the 3% acetic acid solution are oxidized to dissolved silver. The average particle size determined for the 3% acetic acid simulant was higher for the same sample in deionized water, which was, in turn, higher than for the 95% ethanol simulant, averaging 82 nm, 52 nm and 29 nm, respectively. These differences may be due to the

Fig. 5 Concentrations of dissolved silver migration determined for samples AM-01 to AM-05, by spICP-MS. Graphic presentation of dissolved silver concentrations (μg kg^{-1}), determined by spICP-MS (NexION 300D, Perkin Elmer), in solutions from the migration assay in food simulant. spICP-MS: Inductively coupled plasma mass spectrometry, run in Single Detection Mode, AM-01 to AM-05: Sample identification codes, LOD: Limit of detection; LDPE: Low density polyethylene; HDPE: High density polyethylene; PVC: Poly (vinyl chloride)
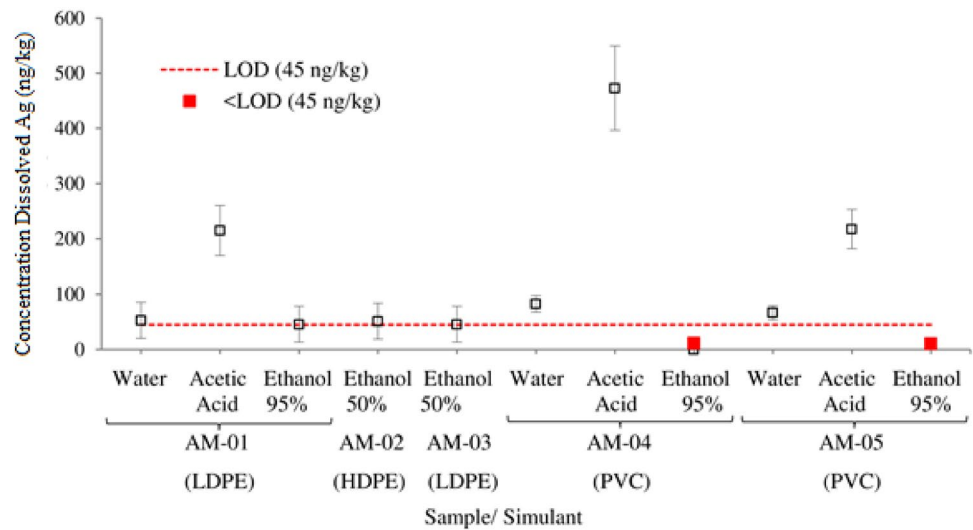
acidity of each simulant. These results indicate increasing mean particle size with increasing simulant acidity, as well as the prevalence of oxidation of smaller particles, consistent with previous literature reports.

Peretyazhko et al. (2014), for example, indicate that the average silver particle size in solution increases after dissolution, reporting that 6.2, 9.2, and 12.9 nm particle solutions increased to 10.4, 13.3, and 16.0 nm, respectively, in the presence of water. Furthermore, no statistically significant increase in mean particle size in the solution containing 70.5 nm particles was observed. Their results also indicate that smaller particles exhibit a higher solubilization rate.

Ntim et al. (2015) demonstrated reduced AgNP concentrations in the presence of 3% acetic acid, due to particle oxidation to dissolved silver. The same can be observed for ethanol 50% and ethanol 95% simulants, as indicated by Echegoyen and Nerín (2013), who report that AgNPs are unstable to oxidation and release ions through gradual oxidation reactions.

AgNP ion release occurs through oxidation, involving the combined effects of dissolved O_2 and H^+, and, under some conditions, may proceed to total dissolution (Liu and Hurt 2010). According to Ntim et al. (2015), the Ag^0/Ag^+ system is very sensitive to oxidation and reduction reactions and can be easily influenced by the presence of other reactive species.

All samples analyzed herein contained AgNP and dissolved silver migration rates below the specific silver migration limit (total) of 50 μg kg^{-1} established by legislation (Brasil 2019; EU 2011). However, silver nanoparticle migration ranging between 26 and 95 nm was observed in all analyzed plastic materials for all evaluated simulants. Thus, all evaluated samples were considered in violation of Brazilian and European legislation, as these establish that substances in nanoform can only be used if expressly authorized, and no

indication of the use of AgNP in plastic materials intended for food contact is noted (Brasil 2019; EU 2011).

According to Regulation No. 10/2011 of the European Union Commission, set on January 14, 2011, nanoparticles present significantly different chemical and physical properties than larger particles, which may result in different toxicological properties. Thus, nanoform substances must be evaluated on a case-by-case basis concerning potential risks until more information is obtained about this new technology. Given this scenario, authorizations based on the risk assessment of conventionally sized substances should not cover nanoparticles, as risks associated with silver nanoparticles are not addressed in the risk assessment established to date (EU 2011).

The toxicological data presented in the literature indicate that AgNP toxicity properties are related to their size, with smaller particles able to penetrate cells more easily. However, studies indicate that even particles on the order of 100 nm can cause cell damage. Thus, the particles observed herein, ranging from 26 to 95 nm, represent potential risks to human health.

Conclusion

The samples analyzed herein presented total silver concentrations ranging from non-detectable (< 44 ng kg^{-1}) to 473 ng kg^{-1} of the simulant. All samples exhibited silver nanoparticle migration, with concentrations ranging between 0.00433 and 1.35 ng kg^{-1}. The migration study revealed the presence of AgNP in all food simulants, from 26 to 95 nm in size. Although total silver concentrations in the migration solutions were below established limits, the absence of AgNP in the positive lists of additives in both Brazilian and European legislation indicate prohibited use to date.

The SEM/EDS results revealed the possible presence of AgNP in the analyzed samples. Thus, issues concerning AgNP migration from the evaluated plastic materials to food items are noted, indicating the need for greater control of the production of materials displaying antimicrobial characteristics through the addition of silver nanoparticles. Moreover, they also confirm the importance of implementing a validated method to direct health surveillance actions. Further studies on the safety of exposure to substances in nanoforms are still required, and current legislation does not allow their use to date.

Determining AgNP migration from packaging to food items is an important support tool for sanitary surveillance inspection, and the findings reported herein will aid the health area concerning of human health risk assessments, aiming at regulating this type of material from a food safety point of view. However, effective actions based on laboratory tests are paramount, as well as prevention actions through information dissemination.

Author contributions   Dr. FSB was responsible for conceiving the idea, carried out the work and wrote the MS, while DraLMGS and Dra. CBS supervised the work and corrected the manuscript; SAVN and CAS carried out the experiments; Dr BSA supervised the work and carried out the experiments; Dra. SCJ supervised the work and Dra SMA CBS supervised the work and review and editing.

Data availability   The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability   Not applicable.

Declarations

Conflicts of interest   The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval   Not applicable.

Consent to participate   Not applicable.

Consent for publication   Not applicable.

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