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Quality evaluation of masks marketed in Brazil during the COVID-19 pandemic for the presence of silver and silver nanoparticles

Avaliação da qualidade das máscaras comercializadas no Brasil em tempos de pandemia da COVID-19 quanto à presença de prata e de nanopartículas de prata

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ABSTRACT

Introduction: The COVID-19 pandemic caused by the new coronavirus is a disease without specific treatment and has become one of the great challenges of the current century. One of the alternatives to minimize the direct transmission rate of the virus is the use of face masks. Currently, there is a diversity of masks on the market, both in the textile composition and in the presence or absence of the silver (Ag) element, in the form of ions or silver nanoparticles (AgNP), which have biocidal activity. **Objective:** To evaluate the presence of total Ag and AgNP in masks produced to protect the population from COVID-19 being sold in Brazil during the pandemic. **Method:** This is a cross-sectional descriptive observational study with convenience sampling. The samples were analyzed by ICP-MS in the standard mode and in the single particle mode. **Results:** The concentrations of total Ag in the studied samples varied from 14 to 72 $\mu g g^{-1}$. It was observed that 50% of the evaluated samples that claim to have AgNP had a size distribution between 17 and 57 nm. When subjected to the washing cycles, there was a reduction in the concentration of Ag, which raises the question as to its real biocidal effectiveness over time. **Conclusions:** The data generated can provide the current scenario of the concentration of silver in the masks and thus assess the potential benefit or risk of use for human and environmental health. In addition, this knowledge can provide technical-scientific support for the sanitary inspection of quality control and implementation of regulatory standards in this field of activity.

KEYWORDS: COVID-19; Pandemic; Silver; Nanoparticles; ICP-MS

RESUMO

Introdução: A pandemia COVID-19 causada pelo novo coronavírus é uma doença sem tratamento específico que se tornou um dos grandes desafios do século atual. Uma das alternativas para minimizar a taxa de transmissão direta do vírus é o uso das máscaras faciais. Atualmente, há no mercado uma diversidade de máscaras, tanto na composição têxtil, quanto na presença ou não do elemento prata (Ag), sob a forma de íon ou nanopartículas de prata (AgNP), que possui atividade biocida. **Objetivo:** Avaliar a presença da Ag total e de AgNP em máscaras produzidas para proteger a população da COVID-19 que estão sendo comercializadas no Brasil durante a pandemia. **Método:** O desenho utilizado para o estudo foi observacional descritivo do tipo transversal com amostragem por conveniência. As amostras foram analisadas por ICP-MS no modo padrão e no modo *single particle*. **Resultados:** As concentrações de prata total nas amostras estudadas apresentaram uma variação de 14-72 $\mu g g^{-1}$. Foi observado que 50% das amostras avaliadas que declaram ter AgNP apresentaram uma distribuição de tamanho entre 17-57 nm. Ao serem submetidas aos ciclos de lavagem, verificou-se uma redução na concentração de Ag, a cada novo ciclo, o que levanta o questionamento quanto a sua real efetividade biocida ao longo do tempo. **Conclusões:** Os dados gerados fornecem o atual cenário da concentração de Ag nas máscaras e, assim, avaliar o potencial benefício ou risco do uso para a saúde humana e ambiental. Além disso, este conhecimento pode dar subsídios técnico-científicos para a fiscalização sanitária do controle de qualidade e a implementação de normas regulatórias nesse ramo de atuação.

PALAVRAS-CHAVE: COVID-19; Pandemia; Prata; Nanopartículas; ICP-MS

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INTRODUCTION

COVID-19 is an infectious disease caused by SARS-CoV-2. It does not yet have a specific treatment and has become one of the greatest challenges of the 21st century.[1] Since it is a disease caused by a virus of the respiratory tract, COVID-19 spreads mainly through droplets, respiratory secretions, and direct contact with infected patients, from human to human (direct transmission).[1] With these characteristics, COVID-19 is currently found in more than 100 countries and five continents[2] and is thus classified as a pandemic.

Because of the lack of vaccines or specific treatments, the most effective measures to prevent contamination are based on preventing the spread of the virus from person to person through social distancing or isolation. In addition, the competent bodies in the area of health recommend the use of masks to help reduce the spread of the disease on a large scale for greater collective protection.[3] Masks act as physical barriers that reduce the contamination of the virus through droplets from sneezes or coughs and prevent hand-to-mouth contamination.[4]

As a result, the textile industry is investing in fabrics with antimicrobial activity. Some of these fabrics contain silver (Ag), which is a natural element with unique physical-chemical, optical, and biological characteristics. In addition, Ag is well known for its strong toxicity to a wide range of microorganisms.[5] Today, Ag is added to various consumer products, including clothes, refrigerators, and washing machines—to deodorize or sanitize—, plastic food packaging, and also drinking water for disinfectant purposes, without any damage to health described yet.[6]

However, the way Ag is present in these products is relevant. The ionic form of Ag has bactericidal activity by inhibiting a series of biological processes of bacteria, especially gram-negative. It has been used for a long time without having shown carcinogenic or mutagenic activity in living beings.[7] However, this ion has low stability and tends to react with anions like Cl^-, HS^- and SO_4^- in water, thus forming precipitates that decrease its biocidal activity.[8,9]

The emergence of nanotechnology, a multidisciplinary and innovative science, enabled the production of nanoscale substances[10,11,12] with enhanced effects. This includes, for example, the use of silver nanoparticles (AgNP), which have proven antimicrobial activity. Thus, AgNP can be used in masks as a surface barrier to reduce the number of pathogens and the chances of contamination. It can also be used to fight the SARS-CoV-2 virus.[8,9]

The objective of this study was to evaluate the presence of total Ag and AgNP in masks produced to protect the population from SARS-CoV-2 marketed in Brazil during the COVID-19 pandemic. The data generated will provide the competent bodies with further information about the concentration of silver in these masks. In addition, the validation of the technique may be used by other laboratories to control these products, as long as the laboratories also carry out intra-laboratory validation.

METHOD

This study was conducted in the Sector of Inorganic Elements of the Chemistry Department of the National Institute for Quality Control in Health (INCQS), a unit of the Oswaldo Cruz Foundation (Fiocruz), Brazil.

The experiments were performed on a mass spectrometer with inductively coupled plasma operated in the standard mode and in the single particle mode (spICP-MS), model NexION 300D (Perkin Elmer, USA). The inductively coupled plasma mass spectrometer (ICP-MS) was equipped with concentric nebulizer (Meinhard), glass cyclonic nebulization chamber, cone, nickel skimmer, and hyper-skimmer. Argon gas with a minimum purity of 99.996% was supplied by White Martins (São Paulo, Brazil). The instrumental and data acquisition parameters are listed in Table 1.

Standards

Monodisperse suspensions of spherical silver nanoparticles were prepared from standard solutions of 40 nm and 50 nm (Sigma-Aldrich - Saint Louis, Missouri, USA), containing 7.2×10^{10} and 7.4×10^9 particles/mL, respectively.

The nanoparticle suspensions were sonicated for 1 min at a frequency of 25 kHz, after successive dilutions, and prepared in deionized water (Milli-Q Advantage, Molsheim, France). After dilution and before every analysis, the suspensions were sonicated for 1 min at a frequency of 25 kHz and homogenized in vortex for 1 min.

Dissolved Ag solutions for making the calibration curve (1, 5, 10, 15, 20 μgL^{-1}) were prepared by successive dilutions in deionized water from the 1 mgL^{-1} stock standard solution (Sigma-Aldrich

Chart 1. Instrumental parameters for mass spectrometry analysis with inductively coupled plasma (ICP-MS).

Instrumental parameters		
Radio Frequency (RF) Power	1,400 W 1400	
Argon gas flow rate		
Plasma	18 L min^{-1}	
Auxiliary	1.2 L min^{-1}	
Nebulizer	1.0 L min^{-1}	
Sample flow	0.2 mL min^{-1}	
Data Acquisition parameters		
Meansuring mode	Standard	Single particle detection
Sweeps	20	1
Dwell time	50 ms	50 µs
Reading per Replicates	1	200000
Integration Time	1 s	100 s

Source: Prepared by the authors, 2020.

- Saint Louis, Missouri, USA). A rhodium (Rh) solution of 1000 mgL^{-1} (Merck, Germany) was used as the internal standard.

Samples

The study has an observational, descriptive, cross-sectional design, with the description and analysis of masks containing Ag in the form of ions or AgNP, currently marketed in Brazil and either homemade or industrially manufactured. The study was based on convenience sampling at random, and the samples were acquired in the domestic market in different businesses located in the state of Rio de Janeiro and online.

From May to July 2020, samples of protective masks of different prices and textile compositions were selected. Ten samples of masks were analyzed and identified by letters. Six of these masks claimed to have Ag (A-F) and anti-COVID-19 action in their composition according to ISO 18184:2019,[13] and four (G-J) did not claim to have such properties in their promotional material in the Brazilian market. The latter were used as control in this study.

Sample preparation

Each sample (mask) was divided into quadrants of 3 cm^2 (equivalent 0.1 g) so that the analyzed pieces had the same area. These pieces were selected so that the results represented the concentration of Ag present in the entire sample area. For that, the analyzed quadrants were based on the extension of the two diagonals of the fabric. The objective was to comprise the ends and the central area of the mask.

Total silver

The masks were subjected to acid digestion by Speed Wave microwave (Berghof, Germany) for 45 min.[14,15] In this procedure, approximately 0.3 g (about three 3-cm^2 quadrants) of the fabric sample were weighed in hexaplicate and packed in Teflon-type plastic tubes, to which we added 3 mL of deionized water (Millipore, Brazil) and 5 mL of Suprapur 65% (w/v) nitric acid (Merck, Germany). After the time of digestion and cooling of the tubes, the solutions resulting from the digestion process of the samples were transferred to volumetric flasks of 15 mL. The samples received internal Rh standard in the final concentration of 10 μgL^{-1} and the final volume was topped with ultrapure water.

Silver nanoparticles

About 0.3 g (about three 3-cm^2 quadrants) of the sample were weighed in triplicate and cut into fragments ≤ 5 mm to increase the sample's contact surface with water. They were then packed in Falcon tubes, to which 15 mL of water were added, and then the samples were subjected to ultrasound for 2 h, centrifugation at a speed of 4,500 rcf for 30 min and filtration in a 0.22 µm porosity membrane.

The filtration step was used to remove unwanted organic matter. In this stage, particle losses may occur due to size or possible interaction with the filter material, but they were not considered in this study. The samples were prepared in triplicate.

Loss of Ag in the washing process

Samples of masks that claimed to have Ag in their composition were subjected to a washing and drying process following the guidelines of Brazil's National Health Surveillance Agency (Anvisa)[16] and the manufacturers. Thirty wash cycles were performed to assess whether there was any loss of the total concentration of Ag in the fabric. Every five washing and drying cycles, a quantity of 0.1 g of each mask (equivalent to 3 cm^2) was collected and the total Ag was determined. The analyses were performed in duplicate.

Methodology validation

The analytical method was validated in-house according to the parameters described in the guidance document on Validation of Analytical Methods of the National Institute of Metrology, Quality and Technology (Inmetro) (DOQ-CGCRE-008)[17] and ABNT NBR ISO/IEC 17025:2017.[18] The established working range was 1–20 μgL^{-1}. The detection limit for total Ag (LOD_T) in standard mode was obtained by reading ten independent replicates of the blank solution and calculated with a 95% confidence interval.[17,19]

The identification and quantification of AgNP by spICP-MS depends on two factors: (i) nanoparticle size, which must be large enough to generate a number of ions that is detectable by the spectrometer, and (ii) the numerical concentration of nanoparticles, which must be high enough to enable a minimum number of events to be counted. As a result, two detection limits can be calculated: size limit of detection (LOD_d, diameter for solid nanoparticles) and limit of detection of the concentration of the number of nanoparticles (LOD_{NP}). LOD_d and LOD_{NP} in spICP-MS mode were obtained experimentally from the mean reading of ten independent solutions of the blank with a dwell time of 50 µs, sample flow of 0.2 $mLmin^{-1}$, and transport efficiency (TE) of 8.87%.[17,19,20]

To evaluate the matrix effect, an analytical curve prepared in solvent (water) was compared with a curve prepared in the presence of the sample.[21] The recovery parameter was evaluated in standard and single particle modes using three times the amount of 0.3 g from the same mask. Each piece was fortified with a final silver solution of 30 μgL^{-1} and then subjected to microwave digestion. For AgNP, the fabrics were fortified with a solution containing particles of average size of 40 nm with 50000 ± 1000 particles/mL. They were left to rest for 24 h to absorb the solution. After the fabric was completely dry, sample preparation was performed as previously described. The repeatability, expressed as relative standard deviation ratio (SDR), was evaluated using readings from five repetitions of the samples done in the standard mode and in the single particle mode, under the same analytical conditions, the shortest possible time, and the same analyst.[17,21]

Statistical analysis

A descriptive statistical analysis was performed on Microsoft Excel 2010 and included arithmetic means and standard deviation. In addition, Student's t-test and the statistical test of analysis of variance (ANOVA) were applied to compare the means. The linearity of the analytical curve was assessed with the aid of a spreadsheet called "*planilha para avaliação de premissas*", prepared by Bazilio et al.[22]

RESULTS AND DISCUSSION

The results obtained in the validation of the methodology for determining Ag and AgNP in the standard mode and in the single particle mode are shown in Table 1.

The matrix effect, recovery, and repeatability were evaluated according to Inmetro.[17] To evaluate recovery and accuracy, three mask samples were selected, of which two claimed to have silver in their composition and one did not claim to have silver in its composition. The results about recovery and accuracy are shown in Table 2.

The analytical curves were considered homogeneous, with a 95% confidence level, and compared using Student's t test. This test enabled us to conclude that the curves are equivalent, that is, the matrix does not have significant interference in the quantification of Ag in the studied samples. The results of the evaluation of recovery and repeatability are adequate with recoveries obtained within the acceptable range 60% -115%, and the percentage of the relative standard deviation (% RSD) did not exceed 21%, as recommended by the Inmetro guidelines.[17]

According to a bibliographic survey, the accuracy study must be assessed using certified reference material (CRM), since factors like matrix, size, density, stoichiometry, and type of nanoparticle influence the recovery.[13] We were not able to use CRM because none was available for purchase in either the domestic or the international market. However, the results we achieved demonstrate that the method has good accuracy.

Identification and quantification of total Ag and AgNP in samples

Each sample was prepared as previously described and, for each replicate, five independent readings were performed. The results were used to calculate the mean and standard deviation, as shown in Table 3.

When comparing the results of the analyzed samples, we could quantify Ag in all samples, even in those that did not claim to have Ag in their composition. The observed variation was in the concentration. This is because in the samples that did not claim to have Ag, the concentration was less than 2 $\mu g g^{-1}$, whereas in the samples that claimed to have Ag in their composition, the value found was at least seven times higher, except for sample F, which claimed antimicrobial action but whose concentration of Ag was close to that of samples that did not claim to have this action. Nevertheless, this action may be caused by other substances not evaluated in this study.

Of the six samples that claimed to have antiviral action, none declared the concentration of silver in the fabric and only two declared their effectiveness against SARS-CoV-2 as described by ISO 18184:2019.[13]

The concentrations of Ag in the studied samples had a great variation between them: 14–72 $\mu g g^{-1}$. This indicates that different technologies of incorporation of Ag in the textile fiber were used and may have led to this variation. Assuming that Ag is the virucidal component of the mask, the efficiency of this action may be different among the products under study.

Table 1. Results of validation tests for silver (Ag) and silver nanoparticles (AgNP) obtained by mass spectrometry with inductively coupled plasma (ICP-MS).

Element	mode	LOD	LOQ	Working range
Ag	Standard	0.002 $\mu g g^{-1}$	0.05 $\mu g g^{-1}$	1,5,10,15,20 $\mu g L^{-1}$
	Single particle	LOD_d -16 nm LOD_{NP} 3.5 x 10^5 particle/g	- -	1.47 x 10^{-10}- 2.95 x 10^{-11} µg/event

Source: Prepared by the authors, 2020.
Ag: silver; LOD: limit of detection; LOQ: limit of quantification; LOD_d: size detection limit; LOD_{NP}: limit of detection of the concentration of the number of nanoparticles.

Table 2. Results of recovery and repeatability for total silver and silver nanoparticles by mass spectrometry with inductively coupled plasma (ICP-MS).

Sample	Total Ag added $\mu g L^{-1}$	Total Ag measured $\mu g L^{-1}$	AgNP added particles/mL	AgNP measured particles/mL	% REC	% RSD
A	30 -	31.6 -	- 50,000	- 39,737	105 83	1.0 2.5
B	30 -	22.4 -	- 50,000	- 30,827	75 64	10.0 18.0
G	30 -	26.3 -	- 50,000	- 50,271	92 105	7.0 5.0

Source: Prepared by the authors, 2020.
Ag: silver; AgNP: silver nanoparticle; REC: recovery; RSD: relative standard deviation.

Table 3. Results obtained for total silver and for silver nanoparticles in the masks analyzed in this study.

Samples	Ag total µgg^{-1}	Size of the AgNP (nm)	Concentration	
			Particles/g	Particles/cm²
A	14.3 ± 7.2	24 ± 3	$2.3 \times 10^6 \pm 5.7 \times 10^4$	$2.43 \times 10^4 \pm 6.1 \times 10^2$
B	25.9 ± 4.1	17 ± 4	< LOD	-
C	40.2 ± 9.2	57 ± 3	$8.3 \times 10^5 \pm 4.1 \times 10^4$	$9.9 \times 10^3 \pm 4.9 \times 10^2$
D	72.0 ± 4.1	36 ± 8	< LOD	-
E	8.6 ± 0.1	19 ± 3	$1.5 \times 10^6 \pm 7.5 \times 10^4$	$1.7 \times 10^4 \pm 8.5 \times 10^2$
F	0.3 ± 0.1	-	-	-
G	0.5 ± 0.1	-	-	-
H	1.9 ± 0.4	-	-	-
I	0.6 ± 0.1	-	-	-
J	0.4 ± 0.1	-	-	-

Source: Prepared by the authors, 2020.
Ag: silver; AgNP: silver nanoparticle; LOD: limit of detection.

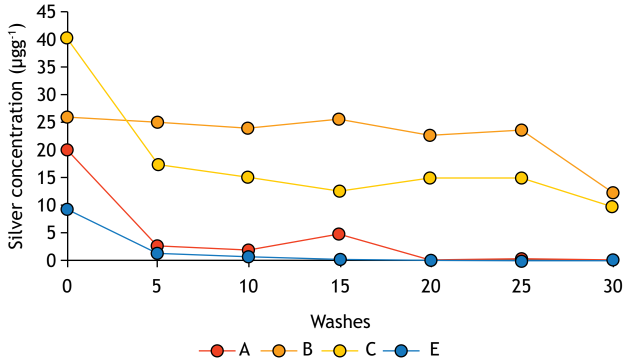
However, nothing can be stated, since how much Ag should be applied to obtain antiviral efficacy has not been established yet. Another factor we observed was the lack of homogeneity in the distribution of Ag in the fabric used to make each sample. The results have shown an RSD of 15%-51%. This indicates some heterogeneity, which can directly affect the action of Ag against SARS-CoV-2.

In addition, 50% of the samples had Ag in the form of nanoparticles, consistent with the information found on the package. The other samples contained Ag in the form of ions and, according to some authors, ionic Ag has low stability,[8,9] which raises the question as to its real biocidal effectiveness over time. However, Ag ions are still used because of their low cost to manufacturers when compared with cost of nanoparticles.

Evaluation of Ag concentration after washing cycles

The variation in the concentration of Ag in the samples in relation to each five wash cycles is shown in Figure 2. The percentage of loss was higher after the first wash cycle, except for sample B, whose loss was lower after the first cycle and which only started to lose Ag after the fifth wash cycle (25 washes). Samples A and E reached a concentration below 1 µgg^{-1} before 30 washes, which is contrary to the manufacturer's claims about the durability of the antiviral action. Although there was a reduction in the concentration of Ag in sample C after the first five washes, we observed that it remained at about 15 µgg^{-1} throughout the wash cycles. This could possibly be justified by the composition of the textile fiber and the technology used in the manufacturing process. Sample B has shown greater stability throughout the wash cycles, which can be attributed to the modern technology used in its manufacturing process. Sample D was not analyzed because unfortunately we did not have enough of it. Sample F was not analyzed either, since its initial silver concentration was close to that of samples that did not have silver in their composition.

In addition to the loss of biocidal action throughout the washing process, a worrying issue that cannot be overlooked is the environmental contamination of the water used to wash these masks. The residues of the washing process have a substantial concentration of Ag, either in ionic or nanoparticulate form. In this study alone, the washing of only four samples for six cycles discarded an estimated amount of 72.7 µgg^{-1} of Ag in the environment. If we assume that every individual has, on average, four masks of short useful life (about 30 washes), the amount of environmental contamination is very high, especially if we consider the size of the Brazilian population and its consumption of protective masks for COVID-19.



Source: Prepared by the authors, 2020.

Figure 1. Graphical representation of silver concentrations after wash cycles of samples A - E determined by mass spectrometry with inductively coupled plasma (ICP-MS).

CONCLUSIONS

The development and implementation of new methodologies to verify the quality of new products that arise as a result of public health emergencies have become major scientific challenges for inspection laboratories.

The ICP-MS technique used in routine laboratories for different products of health interest enables the quantification of Ag with precision and accuracy, whether in the form of an ion, using the standard mode, or AgNP using the single particle mode.

This study addressed the development and validation of a fabric analysis methodology, as well as the determination of the best way to prepare these samples containing AgNP. The results revealed very diverse amounts of Ag in the masks, which raises questions about their efficacy against COVID-19, since there is no minimum recommended value of Ag concentration to achieve 99.9% efficacy against the virus.

Research in the field of health surveillance plays an important role in responding to health emergencies, particularly with studies on new products that can be used to fight diseases and prevent future risks.

Therefore, there is a need to establish the minimum requirements for the composition and quality of these products by competent regulatory and inspection bodies, in view of the current demand for products containing AgNP in the domestic market. Legislative and inspection shortcomings eventually expose the population to higher health risks and can cause another emerging public health problem.

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Authors' Contributions

Barata-Silva C, Santos LMG – Conception, planning (study design), data acquisition, analysis and interpretation, and writing of the manuscript. Vicentini Neto SA, Magalhães CD – Data acquisition, analysis, and interpretation, and writing of the manuscript. Jacob SC, Moreira JC – Conception, planning (study design), and writing of the work. All authors approved the final draft of the manuscript.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.