

# Silver and Silver Nanoparticles for Treatment of COVID-19

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COVID-19 is an epizootic and life-threatening outbreak affecting millions of people globally. Coronavirus variants have emerged in different locations since their origin. Silver and its compounds, including silver nanoparticles (AgNPs), have been used in the medical field for a long period, especially in surgical treatments. The anti-microbial and anti-viral properties of silver are well documented. These properties depend on the size of the particles, concentration, precursor, method of preparation, and the presence of other benefiting compounds. Several experiments were conducted by researchers worldwide to prove the anti-bacterial and anti-viral properties of silver (Ag) and AgNPs, emphasizing that silver can be introduced to multiple organs in the human body and exhibit the expected antiviral characteristics.

Keywords: silver ions ; silver nanoparticles (AgNPs) ; SARS-CoV-2

## 1. Introduction

Since the outbreak, numerous variants of the SARS-CoV-2 virus have emerged in various parts of the world. Several researchers worldwide have monitored and assessed the evolution of COVID-19 since January 2020. Based on the risk posed to the public, coronaviruses are classified under the specifications of 'variants of interest' (VOI) and 'variants of concern' (VOC). VOCs exhibit increased transmissibility, virulence, and threat to public health. Based on these effects, many variants are classified as VOC. The primary variants under this category are alpha (B.1.1.7) and beta (B.1.35.1) variants, which were named on the 18 December 2020 [1][2]. The alpha variant was found in United Kingdom samples collected in September 2020, whereas the beta variant was discovered in South African samples procured in May 2020 [1][2]. The alpha variant was transmitted to more than 122 countries, including North American and European countries [4]. Due to continuous evolution, the alpha variant had undergone 13 mutations, making it difficult to track and detect using the RT-PCR method [2]. Furthermore, the beta variant has spread across 85 countries worldwide and is found at present in three different forms: B.1.35.1.1 (Botswana), B.1.35.1.2 (Mayotte), and B.1.35.1.3 (Bangladesh and Singapore) [2]. Over time, the beta variant had also undergone over 10 mutations, resulting in improved binding capability, immune resistance, and transmissibility of the virus [5]. The gamma (P.1) variant was the next major deadly variant (labeled on 11 January 2021) found in samples collected from Brazil in November 2020 [6][7]. This variant was also extensively found in the United States of America [8], Italy [9], and 46 other countries, including Canada [10]. This strain was found to have several mutations (approximately 11) and is said to possess a potential immune escape mutation (E484K) [7], causing severe complications, especially in people of age group 20 to 39 [11]. The variant that caused high distress in India was the delta variant (B.1.61.7.2; named on the 4 April 2021) [2]. The same delta variant was found responsible for Brazil's increased percentage of stillbirths and placental dysfunction [12]. The variant omicron (B.1.1.529) is thus far the latest type of coronavirus found on the 24 November 2021 as Variants under Monitoring (VUM), which was later classified as a VOC on the 26 November 2021. This category was found in samples procured from various parts of the world and was found to have a higher transmissibility rate [2]. Another variant, epsilon (B.1.429), which was identified in the United States and another 31 countries in June 2020, was not considered under VOC owing to its lower virulent abilities [2][13].

Variants of interest (VOI) are the category of coronavirus species anticipating to undergo genetic alterations over time that enhance their transmissibility, severity, as well as diagnostic or therapeutic escaping capacity, etc. Hence, they can emerge as threats to global public health [14]. The lambda (C.37) and mu (B.1.621) variants are the main examples of this category. The lambda variant was named on 14 June 2021 and mu on 30 August 2021 after the primary cases were reported from Peru in December 2020 and Colombia in January 2021, respectively [2][15]. Variants under monitoring (VOM) are the class of least concerned coronaviruses, which have not caused much trouble currently. Some of the variants in this category are C.1.2, B.1.617.1 (kappa), B.1.526 (iota), and B.1.525 (eta) [2]. However, these variants can undergo mutation and create havoc in the future and hence are monitored and assessed regularly [16].

B.1.1.529 (omicron) is a variant that must be considered carefully. These viruses were first reported in South Africa on 24 November 2021, the first of which was identified in a sample collected from a person on the 9 November 2021 [17]. These variants pose a significantly high transmissibility rate evident in reports collected from South Africa and many other countries [18]. This variant underwent 30 mutations in the genomic proteins, enhancing its permeability, transmissibility, and resistance to antibodies [17]. Of these 30 mutations, 23 were distinctive and were not found in other variants of coronaviruses. Evolutionary studies were conducted to identify the ancestral variant of these viruses, and it was found that the gamma variant (P.1) was closely related in the phylogenetic analysis [19]. Mutations such as E484A and Y505H are the primary causes of the absence of communication between S-RBD and antibodies. At the same time, Asn501Tyr improves ACE2 receptor binding, which is responsible for the high transmission of viruses. The low affinity of S-RBD suggests that the current vaccination processes cannot guarantee protection against these variants [20][21][22]. The WHO has created several guidelines to prevent the transmission of these strains, but their effectiveness in tackling the viruses is minimal. Due to researchers' tireless efforts worldwide, several vaccines were designed for reducing the mortality rate worldwide. Some of the most widely used vaccines include mRNA-1273 (Moderna), BNT162b2 mRNA (Pfizer) [23], and Janssen Ad26.CoV2. S (Johnson & Johnson) [24] developed in the USA, AZD1222 (Oxford-AstraZeneca) [25] developed in the UK, BBIBP-CorV (Sinopharm) and CoronaVac (Sinovac Biotech) [26] developed in China, Sputnik [27] developed in Russia, and Covaxin and Covishield developed in India [28].

## **2. Treatment of COVID-19 Using Silver and Silver Nanoparticles (AgNPs)**

Considering the tremendous success of silver ions and silver nanoparticles in the inhibition of varieties of microbes and in the treatment of infectious diseases (vide supra), their utilization in the successful inhibition of the SARS-CoV-2 virus was highly anticipated. It is well understood that viruses bind to cell receptors of the host and undergo rapid multiplication and ultimately mutilate the cell. The dead cells act as carriers of the virus in the host body and spread the virus to neighboring cells. Hence, strong inhibiting strategies would be necessary for preventing and treating viral infections. Reactive oxygen species (ROS) play a key role in the regulation of viral replication and functioning of organelles, potentially providing new insights into the prevention and treatment of coronavirus infections. Silver-based nanoparticles and composite materials are known to facilitate the production of ROS in cells and hence are extensively considered for COVID-19 treatments [29]. Although their action on SARS-CoV-2 remains unclear, due to the high order inhibition efficacy exhibited by the silver nanoparticles on other viruses, four classes of silver composite materials are identified, viz., Glutathione-capped silver sulfide nanoclusters (GSH-Ag<sub>2</sub>S NCs), PVP-coated silver nanomaterials (PVP-AgNMs), silver nanoparticle-anchored graphene oxide nanoparticles (GO-AgNPs) and PDDA-coated PVP-functionalized graphene oxide-silver nanocomposites (PDDA-PVP-GO-AgNCs) [30]. These materials promote and facilitate the formation of free radicals inhibiting the physiological processes of virus-cell [30]. It is observed that the binding of silver ion to the GP-120 protein subunit can prominently inhibit the normal functioning of the coronavirus. Binding of the silver ion results in the rendering of the S-protein subunits that are responsible for the binding of the beta variant of the SARS-CoV-2 virus spike to the host cell ACE 2 receptor, preventing the binding and fusion of the virus and host cell. Graphene oxide silver nano-composite ink was proven to have excellent inhibitory properties against influenza A virus and OC43 beta coronavirus, both of which are RNA viruses and come under the same lineage as SARS-CoV-2, indicating the potential use of these materials in the treatment of COVID-19 [31]. Inhalation of the AgNPs (average size of 2–10 nm) was recommended for first-line treatment for COVID-19 [32]. Suitably designed AgNPs with a higher negative zeta potential can bind to the viruses that have predominantly positively charged spike proteins. Considering the well-documented antibacterial efficacy of the AgNPs, the suggested inhalation treatment was also expected to control the biofilm formation in the upper respiratory system [32]. The size of the AgNPs is believed to have an important role in the binding process. Smaller particles, due to the larger surface area, were found to interact more effectively with the viral protein. The ideal size of the AgNPs for the optimum binding is around 2–15 nm [30]. Morita et al. showed that considering the stability of viral proteins, the most suitable size of AgNPs for this application is 10 nm. Larger nanoparticles were found to be less stable [33]. Further, it was shown that the stability of the nanoparticles can be enhanced by means of coating the nanoparticles with suitable polymers. Coated AgNPs exhibited better stability, lower agglomeration rate, and higher activities [30].

Several scientists have conducted experiments to validate the effect of silver compounds against COVID-19. Bui et al. [34] theoretically evaluated the inhibitory action of the mono silver-carbene and bis silver-carbene compounds on human protein ACE2 and SARS-CoV-2 protease PDB6LU7 with the help of molecular docking simulations. The simulations helped to understand the bond dissociation energy (BDE) of the hierarchical order of NHC-Ag > NHSi-Ag > NHGe-Ag for the mono silver compounds and NHSi-Ag-bis > NHGe-Ag-bis > NHC-Ag-bis for the bis-silver compounds. These simulations explain that NHC-Ag and NHC-Ag-bis compounds could inhibit SARS-CoV-2 receptors, with NHC-Ag-bis compounds showing superior inhibitory effects. These silver compounds' inhibitory actions were comparable to the known antiviral drugs reported by Ribavirin and Remdesivir [34]. Hydroxychloroquine was used for the early treatment of COVID

during the outbreak in 2019 [35]. Ghaffari et al., with the help of molecular dynamic simulations [35], showed that silver and gold nanoparticles could be used as drug delivery systems for medicines such as hydroxychloroquine or chloroquine without causing significant damage or side effects [36]. Hybrid components formed by combining silver and gold particles could also provide better options for this purpose [30]. During the pandemic, the streets of Milan in Italy were sterilized with a mixture containing silver ions ( $\text{Ag}^+$ ) and titanium dioxide [37]. This led to a significant decrease in the spread of coronaviruses during the initial outbreak of COVID-19 in these regions. Titanium dioxide nanoparticles are also good anti-viral agents used to inhibit the viral activity of influenza viruses (H3N2) [38].

Jeremiah et al. [33] experimented with viral pretreatment assay (VPrA), cell pretreatment assay (CprA), and cell post-treatment assay (CpoA) with AgNPs in VeroE6/TMPRSS2 to understand the mechanism of action of silver on intracellular and extracellular viruses. VPrA results indicate the death and reduction of the viral load of extracellular viruses. From the CpoA results, the inhibitory properties of AgNPs were established. VeroE6/TMPRSS2 cells infected with SARS-CoV-2 virus, when washed with 2 ppm of PVP-AgNP10 silver nanocomposite solution, showed an excellent suppression rate. The CprA studies showed that the VeroE6/TMPRSS2 cells pretreated with 2 ppm PVP-AgNP10, when infected with SARS-CoV-2, showed partial inhibition only after 2 days, indicating the high order viral resistance provided by the silver nanocomposite material [29]. Sanea et al. [39] demonstrated the antiviral properties of AgNPs prepared using natural extracts against SARS-CoV-2. The silver nanoparticles synthesized from the strawberry extract exhibited better antiviral properties than the ginger extract silver nanoparticles. With the help of molecular docking and dynamic simulation studies, the authors monitored the interaction of about 30 possible natural products from both the extracts against seven SARS-CoV-2 protein targets (AAK1, Cathepsin L—human proteins; Mpro, ADP ribose phosphatase, NSP14, NSP16, PLpro—viral proteins). Among all the components of the extracts investigated, neohesperidin, a flavanone glycoside present in strawberry extract, exhibited a higher potential to bind the NSP16 protein of the SARS-CoV-2 virus and to a human AAK1 protein, indicating its key role in the antiviral properties exhibited by the nanoparticles synthesized by strawberry extracts alongside the virucidal effect of silver. Rodrigues et al. [34] with the help of DFT studies explained that the potential virucidal property of the silver originates from the spontaneous chemical reaction between the amino acids of the SARS-CoV-2 virus and  $\text{Ag}^+$  ions. Alveolar inflammation and lung fibrosis are the primary symptoms of COVID-19 patients initiated by a cytokine storm. AgNPs are effective protectors against inflammation and fibrosis because of their ability to change the transcriptional activity of cytokines [40]. Silver nanoparticles effectively reduce the number of inflammatory cytokines such as interleukin (IL)-1 $\beta$ , IL-6, and IL-17, transforming growth factor-beta (TGF- $\beta$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ), thereby reducing inflammation and fibrosis via the NF $\kappa$ B and MAPKinase pathways. They further reduce collagen deposition by controlling the profibrotic gene expression of Col 1a1 and Col 1a2 [35].

Inhalation of colloidal silver particles improves any form of respiratory disease [41][42]. However, inhalation could occur via either oral or nasal pathways. Zachar [43] observed a significant difference between them because the deposition of particles in the bronchial tree when consumed through nasal breathing was only 10%, which is minimal compared to the oral counterpart, which was about 30%. These findings led the authors to conclude that inhalation by oral breathing is the best method to introduce AgNPs into the system. Although silver can be introduced to the body in both colloidal or ionic forms, it is observed that the antiviral properties of the colloidal silver (AgNPs) are 10-fold greater than that of silver ionic particles, making the colloidal form more suitable for antiviral applications [44]. Nevertheless, the size of nanoparticles is a critical parameter for the appropriate therapeutic applications. It is known that smaller particles have higher specific weight fraction, and much higher particle density, which is a good characteristic for fighting different viral pathogens at a minimum inhibitory concentration (MIC) level [44]. Moreover, smaller particles can easily penetrate the cell wall and ultimately destroy the viral cells. In the case of HIV cells (cell size ~120 nm), the MIC estimate of silver nanoparticles was found to be 10  $\mu\text{g/mL}$  [45]. Considering that the SARS-CoV-2 viral cell size is in the same range (~90–100 nm) [46], it is speculated that a similar MIC level of AgNPs would be suitable to treat COVID-19 [29].

### **3. Application of Silver Nanoparticles for Controlling COVID-19**

AgNPs can be used in various forms to tackle COVID-19. AgNPs can be used as nano-based protective equipment, virus-controlling sanitizers, or nano vaccines to improve immunity and antigen carriers, etc. [47]. AgNPs can be used to manufacture clothes for protection against COVID-19 infection. Textile fibers containing AgNPs exhibit improved antibacterial and anti-viral properties [48]. Edible chitosan/pectin-supported silver nanoparticle films were found to enhance the antimicrobial properties and durability of the textiles significantly [49]. Composite materials containing lignin extracted from sugarcane bagasse and silver nanoparticles are successfully used in commercial antibacterial textiles. Cotton fibers combined with guanozole, zinc, and silver nanocomposites are also extensively utilized in manufacturing antimicrobial cloths [48].

Radiochemical deposition of silver nanoparticles on cotton fiber was effective against Influenza A and Feline caliciviruses [48]. Kumar et al. [50] manufactured and analyzed a cloth with silver being photo-deposited over it and found that these clothes showed 97% efficiency in restraining the growth of the SARS-CoV-2 virus. The proposed mechanism of antiviral action demonstrates that the binding of silver ions with the glycoprotein possessed by the virus is the main reason for the inactivation of the virus, which ultimately leads to the death of the virus [50].

Even after the proper usage of personal protection equipment (PPE) by health workers, the spread of the SARS-CoV-2 virus among the categories has heavily risen [51]. This happened because of the prolonged existence of the virus in the air, environment, and PPE kits. To prevent this, the development of antibacterial and anti-viral surfaces is highly essential. It was found that metal-grafted graphene oxide (GO)-based fabrics could effectively reduce PPE contamination [52]. Of these, silver- or copper-based GO is found to be the best in performance. Embedding the silver/copper—GO composites in polymeric materials and utilizing them in the manufacturing of masks can also be considered. A higher level of viral inhibition was observed in the case of masks manufactured by fabric materials containing metals such as copper, silver, and gold [52]. Balagna et al. [53] studied the effects of coating masks with AgNPs. The coating was carried over a glass substrate, and it was found that there was a decreased possibility of infection for the coated mask as compared with the uncoated mask. The coating was sufficient to bring the titer value of SARS-CoV-2 to almost zero and improved the mask's filtering effects significantly, which also helps to reduce unnecessary waste disposal [53].

Apart from clothing and masks, several other products used for COVID-19 treatments contain silver or AgNPs. SHEPROS, a Malaysian company, developed a suspension named 'Nanosilver sanitizer' [54] using AgNPs of a size ~25 nm, which is assured to destroy a wide variety of microorganisms and viruses. The oral and nasal cleaning agent ARGOVIT mouthwash containing AgNPs was found to prevent the spread of SARS-CoV-2 virus among health workers [55]. A similar product, Silvo Clean Spray, containing colloidal AgNPs, was developed by Weinovate solutions, a startup company in collaboration with the Indian Department of Science and Technology and the Department of Biotechnology [56].

## 4. Future Scope

Vaccination is the only effective method for eradicating the uncontrollable spread of any virus. Nanomedicines could prove to be the best means of improving vaccine efficacy. Electrostatic binding of the S protein with gold nanoparticles result in s-AuNP nano vaccines showing high responses. Due to the enhanced anti-viral potency, there could be future chances of using silver-based nano vaccines instead of gold nano vaccines. Currently, even though detection of SARS-CoV-2 is performed through reverse transcription-polymerase chain reaction (RT-PCR) methods. Several nanoparticle-based detection methods have also been developed, while most of them include gold nanoparticles as their main constituent. However, silver nanoparticles can be a potential and economically viable replacement. To improve the efficiency of viral control, a careful and detailed study of the impact of various undetected protein enzymes on spreading and their subsequent inhibition must be performed. Based on these studies, the development of silver-based components can be developed to tackle future variants of coronavirus.

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