Silver Nanoparticle as an Effective Antiviral Agent

Hiran Mayookh Lal, Arya Uthaman, and Sabu Thomas

Abstract The infections from viral pathogens pose a significant global health challenge. The emergence of viral strains resistant to conventional antiviruses and the adverse side effects due to their prolonged use slow down the application of many antiviral therapies. The silver nanoparticles are considered a potentially useful tool for preventing various pathogens. The silver nanoparticles have already proven its potential as an efficient antiviral agent offered by their unique physical and chemical properties. The silver nanoparticles provide an excellent opportunity for novel antiviral therapies as it can attack a broad range of viruses with a lower possibility for developing resistant antiviral strains compared to conventional antiviral drugs. This chapter discusses the application of silver nanoparticles as an efficient antiviral agent against human immunodeficiency virus, respiratory syncytial virus, hepatitis B virus, monkeypox virus. Furthermore, the effect of silver nanoparticles against coronaviruses and the development of silver nanoparticles on their application as an effective antiviral therapeutic agent against pathogenic viruses have been discussed in this chapter.

Keywords Silver nanoparticles · Virus infection · Antiviral therapy · Antivirus mechanism

1 Introduction

The viruses are sub microscopic infectious agents that replicate only inside the living cells of organisms. It can infect any type of living form that varies from plants and animals to microorganisms such as bacteria and archaea. The viruses constitute one of the prime causes of diseases and death worldwide. The vaccination programs are

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an effective way of preventing infections by viruses. The vaccinations could permanently disable numerous diseases that have killed many and others have been eradicated, such as smallpox in 1979. In the case of paralytic disease poliomyelitis, the development of vaccination programs greatly reduces the burden of disease. However, for some of today's critical viral pathogens, there is still no vaccine has been developed. The viruses contain genetic materials RNA or DNA, capsid proteins, and a lipid envelop. The viruses are classified as enveloped and non-enveloped viruses based on the presence or absence of a lipid envelope. The human pathogenic viruses such as Hepatitis C and B, Dengue, Yellow fever, Measles, Influenza, Ebola, Respiratory syncytial, and most recently of COVID-19 (1.12 million people have died so far from the COVID-19 outbreak as of October 21, 2020, 01:34 GMT) (WHO 2020) are enveloped viruses. Some of the viruses are capable to cause persistent infections that may lead to cancer or acquired immunodeficiency, for e.g. human immunodeficiency virus (HIV) and hepatitis (mainly HBV and HCV). Up-to-date a numerous effort has been expended in attempts for developing vaccination programs for these viruses. The new vaccines for viruses such as HIV, HCV, and some herpesviruses have been developed without appreciable success.

The development of vaccines against diseases can save millions of lives each year. If a human body is exposed to infection- causing viruses or bacteria later, the body is immediately ready to destroy them. The vaccines work by preparing the body's immune system (natural defense) to recognize and ruin the bacteria and viruses they target. The emerging and re-emerging of viruses are a threat to human health because of their ability to adapt their present host, to their transition to a new host, to undergo an evolution of various adaptive conditions, and to escape from different antiviral measures. The development of a new vaccine for viruses such as COVID-19, HIV, HCV, etc., seems likely to continue to be elusive. The field of development of a new vaccine is very promising even together with the risk of emerging and re-emerging of the viruses. Currently, technological advances have led to the discovery and characterization of molecules that are essential for the replication of viruses, so the development of new antiviral agents is possible to inhibit viral infections. Great progress has been achieved in the field of antiviral therapy, but still there exists a margin of ineffectiveness. The development of new antiviral agents is very much essential to continue the fight between the host responses and invading viruses.

The effects of viral infection are administrated by the interaction between the host cells and the virus. When a cell of an organism is exposed to a virus it first binds to the cells and then the virus or its genome gets penetrate into the cytoplasm. The genome is released from the capsid, and it is transcribed either in the nucleus or in the cytoplasm. The mRNA of the virus directs protein synthesis in a well-regulated manner. Finally, the virus goes through the genome replication and together with viral proteins get together and forms a new virus which is then released from the cell (Galdiero et al. 2011). The virus replication cycle is represented in Fig. 1, showing the HBV replication as an example (Zoulim 2004).

The development of drugs that target viral entrance or attachment and possibly inhibit viral infections have proven very difficult to invent. Due to the emerging and
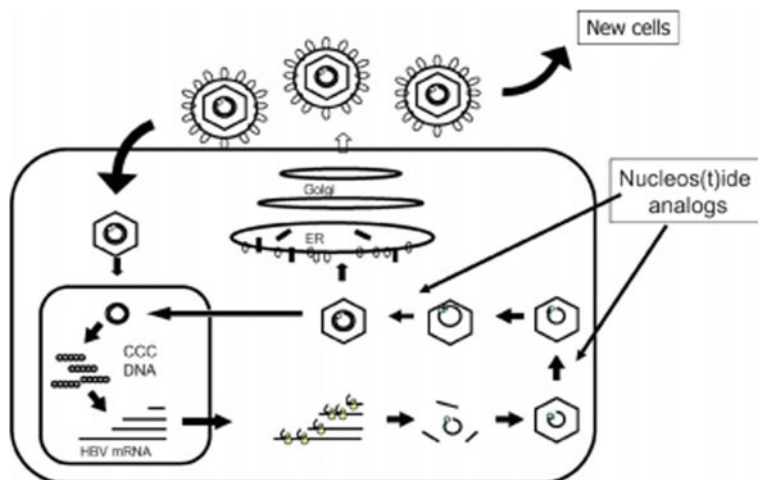
Fig. 1 Major steps involved in the HBV replication cycle. Reused with permission from Zoulim (2004)

re-emerging of diseases caused by various pathogenic viruses and the rising of antiviral resistance to conventional antivirus drugs, researchers and pharmaceutical companies are in search of the development of new effective antiviral drugs.

The introduction of nanotechnology widely for numerous biomedical applications has also extended their contributions to the development of antiviral drugs that act by preventing viral infections during the viral attachment and entry. Among nanoparticles, metal nanoparticles are a promising candidate for various biomedical applications due to their unique physicochemical properties. The dimension of nanoparticles is less than 100 nm and possess unique physical and chemical properties that are derived from the presence of a higher quantity of surface atoms and also the surface area to volume ratio. Among the metal nanoparticles, the silver nanoparticles (Ag NPs) are well known antimicrobial materials that are effective against numerous types of bacteria and fungi. The antimicrobial and antifungal activity of Ag NPs is mainly due to the obstruction of respiratory enzymes, electron transport component, and tampering the DNA function by released Ag+ ions. One of the main advantages of silver is the microorganisms are unlikely to evolve resistance against silver as compared to conventional antibiotics, as silver can fight against a broad range of targets in microbes. The application of Ag NPs in the biomedical field has been found in various forms such as wound dressing, silver-impregnated textile fabrics, coating for medical devices, etc. Ag NPs have received significant attention as antimicrobial agents and was proven their effectiveness against Gram-positive and Gram-negative bacteria. The biological interaction between the microbes and the host cells is multivalent that includes multiple copies of ligands and receptors that bind together in a coordinated manner. This multivalent bond enhances the efficiency and strength of such interactions that allow the microbes to invade cells under attack. Interfering these identified

interactions between microbes and cells, and thus blocking the viral entry into the cell membranes is the promising strategy being perused for developing a new antiviral drug and preventive microbicides.

2 Ag NPs as an Emerging Antiviral Agent

Silver nanoparticles have been proven to be an excellent antimicrobial agent however, the antiviral properties of Ag NPs remain still undeveloped. Nowadays, viruses represent one of the leading causes of diseases and death worldwide. We have witnessed several examples occurring each year, and presently on October 2020, the world is suffering very seriously from the Coronavirus disease (COVID-19) pandemic which has already taken the life of 1.13 million human beings as reported by WHO (2020). The other known examples are SARS coronavirus, monkeypox virus, West Nile virus, Nipah virus, Chikungunya virus, Hantavirus, Influenza virus, etc. As we discussed earlier in this chapter, the emerging and re-emerging of viruses are a major threat for discovering a new antiviral drug. The changes to the ecosystem that disorder the balance between microbes and the host species cells, and the changes in human behaviour and increase in urbanization are some of the factors responsible for the outbreak of a pandemic. Therefore, there is a greater need for a new methodology for treatment with developed antiviral agents that can also overcome the problem of antiviral resistance.

Due to the potential antiviral properties of Ag NPs, they are emerging as one of the choices of action for the management of viral diseases. The significant application of Ag NPs in the treatment of viral infections that require maintenance of circulating drug concentration or long-lasting therapeutic regimens. Ag NPs are active against a broad range of viruses and, the possibility for the development of bacterial resistance is lower compared to traditional antiviral drugs (Rai et al. 2014). Ag NPs received considerable attraction as an antiviral drug due to their intrinsic properties since they have shown antiviral behavior against several viruses regardless of the specific family. There are only a limited amount of studies emerged to demonstrate that silver nanoparticles can apply as effective antiviral agents. Several studies have arisen since the last few years showing the effectiveness of Ag NPs as an excellent antiviral agent against the viruses such as HIV, respiratory syncytial virus, influenza virus, hepatitis B virus (HBV), hepatitis C virus (HCV), and monkeypox virus. Some of the studies showing the inhibitory effect of Ag NPs against each virus are mentioned in Table 1.

Considering the application of metal nanoparticles as an antiviral agent, the properties of each nanoparticle vary depending on their size, capping agents, level of dispersion, and shape. The available research data regarding the antiviral usage of nanoparticles are heterogeneous and difficult to categorize. From the various nanoparticles, Ag NPs are commonly used as an antiviral agent for a different type of viruses. So far, from the antiviral properties of silver three key aspects can be extrapolated.

Table 1 A review on antiviral properties of silver nanoparticles

Virus	Family	Ag NPs type/composition/size	References
HIV 1		PVP—Coated AgNPs (1–10 nm)	Elechiguerra et al. (2005) Sun et al. (2005) Lara et al. (2010a)
HIV 1	Retroviridae	Mangrove-mediated green synthesised AgNPs (12–28 nm)	Kumar et al. (2017)
HIV 1		Curcumin stabilized Ag NPs (45 nm)	Sharma et al. (2017)
HIV		$NH_2fMWCNT$ with Ag NPs (10 nm) (anti-HIV drug RIL)	Aftab et al. (2019)
HIV 1		Ag NPs (0.92–2.48 nm)	Tsai et al. (2019)
RSV		PVP coated Ag NPs (69 ± 3 nm)	Lova et al. (2008)
RSV	Paramyxoviridae	Curcumin modified AgNPs	Yang et al. (2016)
RSV		PVP coated Ag NPs (8–12 nm)	Morris et al. (2019)
Influenza virus H1N1		Ag NPs solution (5–20 nm)	Xiang et al. (2011)
Influenza virus H3N2		Ag NPs solution (9.5 nm)	Xiang et al. (2013)
Influenza virus H1N1	Orthomyxoviridae	Amantadine modified AgNPs (3–2 nm)	Li et al. (2016a)
Influenza virus H1N1		Oseltamivir decorated AgNPs (3–2 nm)	Li et al. (2016b)
Monkey pox	Poxviridae	AgNPs and Polysaccharide coated AgNPs (10–80 nm)	Rogers et al. (2008)
HBV	Hepadnaviridae	Ag NPs (10–50 nm)	Lu et al. (2007)
HCV		Green synthesised Ag NPs from total extract and petroleum ether fraction of *Amphimedon* (8.22–14.30 nm)	Shady et al. (2020)

Expanded abbreviations from this Table, Human immunodeficiency virus (HIV), Respiratory sincitial virus (RSV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), poly vinylpyrolidone (PVP)

(1) The Ag NPs used for the viral inhibition also depends on their size. From Table 1, it is clear that the average size of Ag NPs are generally less than 25 nm. The lower size of nanoparticles resulted in more effective antiviral infectivity inhibition.

(2) Ag NPs exhibits excellent antiviral properties against a large number of viruses infecting both eukaryotic and procotic organisms. Thus, Ag NPs could be considered a true broad-spectrum antiviral agent.

(3) The early infection might be the common time frame where Ag NPs exert their antiviral activity inhibiting the rest of the virus replication cycle.

3 Mechanism of Ag NPs Antiviral Activity

Several studies have been reported the excellence of Ag NPs as an antiviral agent inhibiting several bacterial infections. However, the exact stage of viral infection at which the silver exerts their antiviral property and the precise mechanism behind the antiviral activity of Ag NPs have yet to be determined. Comprising much literature; (Rai et al. 2014) demonstrated the possible mechanism behind the antiviral activity of Ag NPs and represented in Fig. 2.

Different studies based on the antiviral activity of Ag NPs without a capping agent revealed that it could inhibit several viruses. Lu et al. (2007) showed that the smaller particle size Ag NPs (10 and 50 nm) could effectively inhibit HBV due to

Fig. 2 Antiviral mechanism of silver on different stages of viral replication. 1—Bonding/interaction with viral surface, 2—Interference with viral attachments, 3—resisting the penetration of virus into host cell, 4—interaction with viral genome, 5—inhibition of genome replication, 6—inhibition of protein synthesis, 7—inhibition of viral assembly and release of virions. Reproduced from Ref. (Rai et al. 2014) with permission
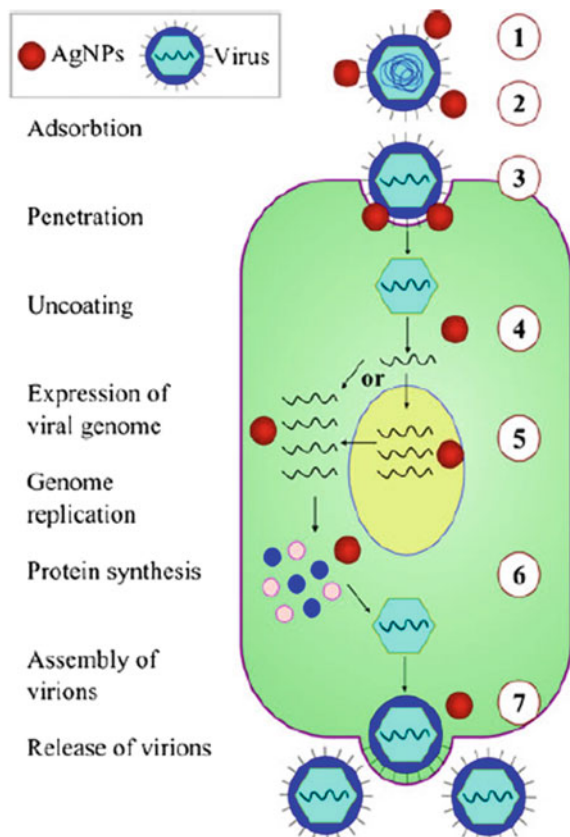
the specific interaction between smaller size nanoparticles and DNA of HBV virus. Similarly, the Ag NPs with 10 and 50 nm showed a strong binding affinity with HBV virions and thus preventing the virions from entering the host cells than Ag NPs with 50 nm size. Larger nanoparticle size (800 nm) induced several cytotoxic effects in cell culture-based assays, but the smaller size Ag NPs showed less cytotoxic effects. The in vitro anti-HBV activities of the different sized Ag NPs are determined using the HepAD38 cell line that secretes HBV particles and exhibits a high level of HBV DNA to the surrounding medium. The authors explained feasible mechanisms for the anti-HBV effect of Ag NPs is due to their reduced size. The smaller size facilitates their higher possibility of interaction with other smaller molecules. The Ag NPs with 10 nm size might imide the RNA of the virus by directly binding or interacting with the HBV DNA, which serves as a template of RNA synthesis. Xiang et al. (2011) studied the inhibitory effect of Ag NPs (10 nm) against the influenza virus. The authors found that the virus presumed to be dependent on the soluble silver ions (Ag+ ions), which impede strongly the growth of pathogens via the suspension of electron transport components, respiratory enzymes, and interface with DNA function. Speshock et al. (2010) studied the inhibition effect of Ag NPs against Tacaribe virus (TCRV). The 10 nm size Ag NPs exhibited excellent anti-TCRV activity compared to 25 nm-sized Ag NPs. The authors demonstrated that the Ag NPs seem to interact with TCRV before the viral infection due to exposure of virus into host cells. The Ag NPs interfere with cellular receptors binding or the internalization of Ag NPs together with the virus and develop an inhibitory effect on viral replication interfering with virus RNA-dependent RNA polymerase.

Numerous researches have emerged in the last few years on the antiviral effect of modified Ag NPs and green synthesized Ag NPs. Lara et al. (2010a) demonstrated the PVP modified Ag NP exert anti-HIV activity at the early stage of viral replication. Ag NPs inhibited all strains of HIV 1 isolates include T-tropic, M-tropic, dual-tropic and resistant isolates. The anti-HIV mechanism of Ag NPS is based on the inhibition of the initial stage of the HIV-1 cycle that can be attributed to the inhibition of virus fusion or binding with the host cells. The Ag NPS inhibits the interaction between HIV-1 gp120 glycoprotein and the target cell membrane receptor. The Ag NPs might interact with disulfide bonds located in the carboxyl half of HIV-1, which has been implicated in binding to CD4 host cell receptors. Lara et al. (2010b) observed the PVP-coated Ag NPs could function as effective microbicides with virucidal properties, they are capable of inhibiting the transmission of HIV-1, and offers long-lasting protection of cervical tissue from infection for 48 h, authors observed no trace of cytotoxicity exists in the explants. From these studies, we can conclude that the precise mechanism of interaction between Ag NPs and HIV-1 still has to explore. However, various data points to direct interaction with HIV-1 glycoproteins and Ag NPs could interfere with the fusion of viral penetration into the host cell. Ag NPs can also inhibit the post-entry stages of infection by preventing other functional HIV-1 proteins or by decreasing reverse transcription or proviral transcription rates by directly interacting with the DNA or RNA molecules (Rai et al. 2014).
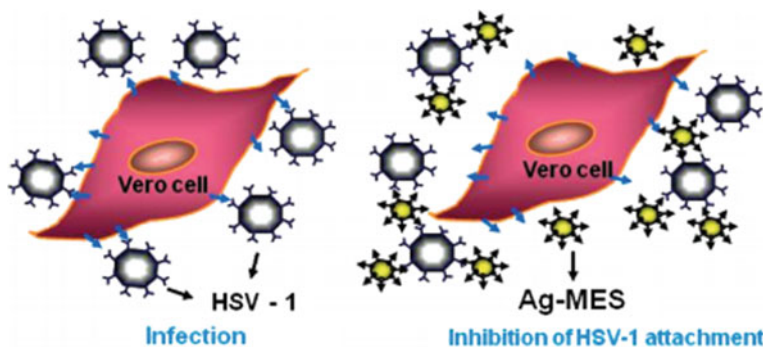
Fig. 3 Proposed mechanism of inhibition of HSV-1 by Ag NPs decorated with MES. Reproduced from Ref. (Baram-Pinto et al. 2009) with permission

The sulfonate capped Ag NPs (4 ± 1 nm) have been introduced as an anti-HSV-1 infection (Baram-Pinto et al. 2009). The authors used mercaptoethane sulphonate (MES) as a capping agent. The entry and attachment of HSV-1 into cells involve the bonding between the viral glycoprotein and cell surface heparan sulfate (HS). Ag NPs are expected to block the interaction between the virus and cells and thus prevents the virus from entering the cell and thus inhibit the cell-to-cell spread of the virus. The authors found that the Ag NPs capped with MES served as a multivalent inhibitor that mimics the HS of the host cell as shown in Fig. 3.

There are different studies reported demonstrating the Ag NPs alone and with their different compositions as an excellent antiviral agent. However, the precise mechanism behind the antiviral activity has yet to be determined. In this section, we have explained the antiviral activity of Ag NPs compiled from the available literature. The overall picture from the literature showed a tendency of smaller size Ag NPS is more incisive in inhibiting the infectivity of each virus regardless of the viral specious. The smaller particle exerts minor cytotoxicity. With our limited knowledge, we have tried our best to refer to various research studies in this chapter although, there have been many studies that we may not be able to include in this chapter regarding the mechanism of Ag NP's antiviral activity.

4 Ag NPs and Their Antiviral Activity

4.1 Retroviridae

A retrovirus family of virus inserts a copy of its RNA genome into the DNA of the host cell's cytoplasm that it invades. Once this virus entered the host cell's cytoplasm, it uses its reverse transcriptase to develop DNA from its RNA genome. The acquired immunodeficiency syndrome (AIDS) is the disease caused by HIV. The cure for HIV does not exist. The treatment of HIV involves taking medicine that

slows down the progression of the virus inside the human body. Highly active antiretroviral therapy (HAART) is a treatment regimen that includes taking a cocktail of drugs that suppresses the HIV infection of an HIV infected patient. This treatment has significantly improved the life expectancy and quality of millions of HIV infected individuals.

The replication of HIV-1 is a complicated multistep process that is only dependent on both the virus and host cell factors. The entry of the virus into target cells is achieved via fusion of the viral lipid envelope and the cellular plasma membrane. The viral components glycoproteins composed of two subunits such as gp120 (binds to the cellular receptors), and gp41 (which is subunits bearing the transmembrane segments, and executes the fusion. After the fusion following gp120 binding to the CD4 (cellular receptor), and the following interaction with CXCR4 or CCR5 (co-receptors), a conformational change in gp41 leads to membrane fusion and delivery of capsid to the cytoplasm. Soon after the entry, RNA is reverse transcribed to a complimentary DNA that converted to a double stranded DNA, and integrated into the host cellular genome. The integrated viral DNA is transcribed to develop full-length progeny viral RNA and a number of spliced mRNA transcripts. This results in the synthesis of viral proteins together with progeny viral RNA are transported to the site of virus particle assembly at plasma membrane, which is the place where the virus gains access to extracellular milieu upon building events.

Elechiguerra et al. (2005) first demonstrated the size-dependent interaction of Ag NPs with HIV-1, with the Ag NPs size ranges of 1–10 nm attached to the virus. The authors suggested that the sulphur bearing residues of the virus glycoproteins are attractive sites for nanoparticle interaction with HIV-1 through preferential binding to gp120 glycoprotein knobs. In their investigation, they demonstrated the interaction of Ag NPs with capping agents opens the possibility for their physiochemical properties. For this reason, they used three different surface treatments of Ag NPs. (1) Foamy carbon, (2) poly (N-vinyl–2-pyrrolidone) (PVP), and (3) bovine serum albumin (BSA). The authors found the BSA and PVP coated AG NPS exhibits a slightly lower inhibition effect to HIV-1 may due to the Ag NP's surface are directly bond and encapsulated to those capping agents. The Ag NPs released from the carbon matrix observed a higher inhibitory effect because of the indispensably free surface area. Lara et al. (2010a) showed the Ag NPs coated with PVP were an effective antiviral agent against HIV-1. A luciferase-based assay showed an effective virucidal effect against cell-free virus including laboratory strains, clinical isolates T and M tropical strains, and resistant strains, and cell-associated virus. The authors observed the antiviral effect of Ag NPs is due to the nanoparticle itself, rather than just to the Ag ions present solution. The antimicrobial effect of Ag salts through Ag ions inhibited HIV-1 12 times lower than the one of Ag NPs. The mechanism of inhibition of HIV-1 is based on inhibiting the interaction between HIV-1 glycoprotein gp120– and host cell receptor CD4. Lara et al. (2010b) showed the PVP—coated Ag NPS as an excellent antiviral drug against HIV-1 using an in vitro human cervical tissue based organ that simulates in vivo conditions. Mohammed Fayaz et al. (2012) developed Ag NPs coated polyurethane condoms

(PUC). They demonstrated that the Ag NPs coated PUC could effectively inhibit and inactivate HIV-1 and HIV-2 on contact. It can also develop a defence line against other sexually transmitted infections.

Kumar et al. (2017) prepared green synthesized Ag NPs by aqueous leaf extract of mangrove *Rizophora lamarckii* with a size range from (12–28 nm). The Ag NPs showed high HIV-1 (RTase) reverse transcript inhibitory activity. The authors claimed the mangrove-synthesized Ag NPs is a promising candidate against HIV and other viruses.

Whiteley et al. (2016) investigated the interaction between HIV aspartic protease (PR) and AgNPs (2.12 nm) using molecular dynamic simulations. The interacting docking of Ag NPs and HIVPR is represented in Fig. 4. The authors found the Ag NPs coated with citrate molecules implied strongly anionic particles interacted with HIVPR through van der Waals forced of interaction. The naked Ag NPs with zero charges interacted with HIVPR via π π* hydrophobic-hydrophobic interactions with aromatic moieties of tryptophan, phenylalanine, or tyrosine residues via the side chain of amino acids including sulphur bearing residues of cysteine and methionine. The in silico investigations revealed the interaction of Ag NPs with Trp6, Trp42, Phe,53, and Cys95, as represented in Fig. 4.
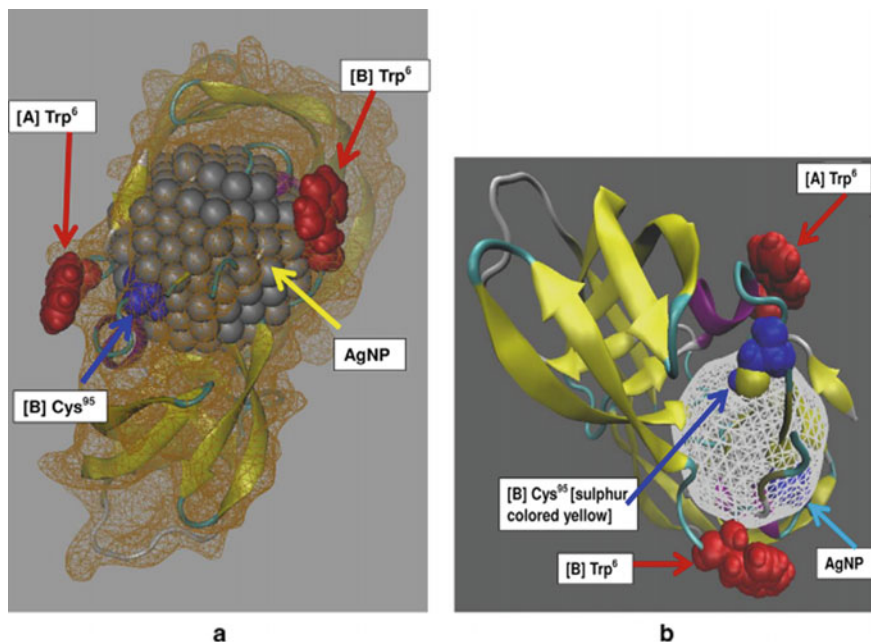

Fig. 4 HIVPR docked with Ag NPs: **a** HIVPR represented as an electron density map. **b** Ag NPs represented as an electron density map. Reproduced from Ref. (Whiteley et al. 2016) with permission

Tsai et al. (2019) demonstrated the interaction between Ag NPs (0.92–2.48 nm), HIV-1 protease (PR), and target peptides (synthesized). These interactions have been studied using three protocols: (1) Ag NPs + HIV-1 PR (24 h incubation) + peptides; (2) Ag NPS + peptides (24 h) + HIV-1 PR; (3) Ag NPs + peptides + HIV-1 PR. (Ag NPs, HIV-1 PR, and synthesized peptides were mixed inside HEPES buffer solution). The incubation of Ag NPs and HIV-1 PR allows the nanoparticles to attach on the surface of HIV-1 PR, and thus active sites of virus forming a HIVPR-Ag NPs complex as shown in Fig. 5. Further, the incubation of peptides with the Ag NPs allows Ag NPs to interact or bond with peptides to form a peptide*nanoparticle complex. This complex can protect the peptides from being cleaved by HIV-1 and let the peptide to remain intact. The HIV-1 PR and peptides interact faster than the Ag NPs interact with peptide. If the AG NPs, Peptides, and HIV-1 PR mixed, HIV-1 PR will cleave some of the initial peptides, and those peptides that remain intact will interact with Ag NPs. Thus, the authors demonstrated the late presence of Ag NPs would have no inhibition effect on HIV-1 PR activity.

4.2 Paramyxoviridae

This family of viruses consists of large enveloped RNA viruses infecting mammals and birds or in some cases reptiles and fish. Many of the Paramyxoviridae such as a respiratory syncytial virus (RSV), measles virus, Nipah virus, measles virus, Hendra virus, and several parainfluenza viruses are pathogenic for humans.
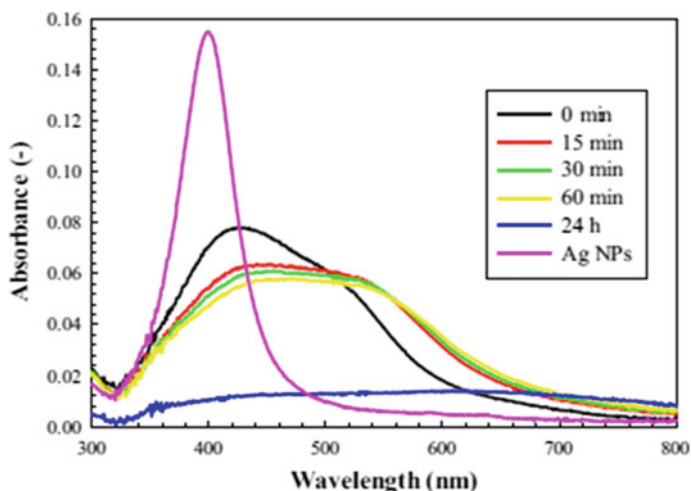
Fig. 5 UV-vis spectra of Ag NPs + HIV-1 PR. The peak at 393 nm for Ag NPs disappears with increase in incubation period. The shift of absorbance to broad band between 400 and 550 nm, and no clear peaks on 24 h contact shows the interaction of Ag NPs with the HIV-1 PR, and complete interaction at 24 h contact. Reproduced the image from Ref. (Tsai et al. 2019), with permission

RSV causes infections of the lungs and respiratory tract. This disease can cause severe infection in humans especially premature babies, infants, and adults with lung and heart diseases, or humans with a weak immune system. RSV genome consists of single RNA molecules of negative-sense RNA that encodes among others. The RSV viral envelope is exposed to two surface glycoproteins, which are (G) protein (serves as receptor binding proteins), and (F) proteins (responsible for fusion between the viral envelop and cell membrane. Following the infection of the host cell, the F protein is expressed on the surface of the cells and fuse to the adjacent cells, giving rise to syncytia development.

Sun et al. (2005) utilized Ag NPs conjugated to different proteins to analyse the inhibition of RSV infection in Hep-2 cell culture. The authors used three types of capping agents for Ag NPs such as BSA, PVP, and a recombinant F protein from RSV (RF 412). The interaction between the RSV and Ag NPs was characterized by utilizing transmission electron microscopy. The authors found that the PVP-coated Ag NPs were able to interact with the viral surface with a specific association or a regular spatial arrangement that possibly provides interactions with G proteins that are uniquely distributed on the envelope of the RSV virion. Ag NPS conjugated with BSA also interacted with RSV but without a spatial arrangement, while RF 412—conjugated Ag NPs appeared to be floating freely without any proof of regular interaction. The hypothesized interpretation for having good interaction of PVP coated Ag NPs with G proteins is because of the uniformity and smaller size of Ag NPs (4–8 nm) compared to those of RF 412 and BSA coated Ag NPs (3–38 nm). Since metal nanoparticles have to be regarded cytotoxic, especially intended for treating respiratory diseases such as RSV. Using the Trypan Blue Exclusion Assay the authors revealed the Ag NPs decorated with capping agents such as PVP, BSA, and RF 412 showed only less than 20% cytotoxicity up to a concentration of 100 µg/ml.

4.3   Hepadnaviridae

This family of the virus has a small genome of partially single-stranded, partially double-stranded circular DNA. The genome of this virus consists of two variable-length strands, a longer negative-sense strand, and a shorter positive-sense strand. These strands are arranged in such a way that the two ends of the long strand meet, and they are not covalently bonded. The shorter strand overlaps this divide and is connected to either side of the split of the longer strand through a direct repeat (DR) segment, which connects both strands. In the viral replication, the viral partially double-stranded and partially single-stranded DNA is converted into the host cell nucleus to covalently closed circular DNA formed by the viral polymerase.

There are eighteen viral species in the Hepadnaviridae family, Hepatitis-B virus is the commonly known species from this family. HBV is a partially double-stranded DNA virus that is provided with a lipid envelope. HBV consists of a string tropism for hepatocytes, and once it has penetrated the cell then the viral

particles can enter into the nucleus where the HBV viral genome is completed to form a covalently closed circular DNA which, is a template for the viral mRNA transcription and the development of pre-genomic RNA (pg RNA). This pg RNA develop the template for reverse transcription by the viral encoded reverse transcriptase and develop new viral genomes. The antivirals drugs developed against HBV virus that includes nucleotide and nucleoside analogue inhibitors represent the approved pharmaceuticals exhibits excellent antiviral activity against HBV virus. However, it is well known that the effectiveness of these antiviral drugs is less due to the fast development of drug-resistant HBV strains. Lu et al. (2007) studied the inhibitory effect of Ag NPs against HBV replication. The Ag NPs used in their study are with mean diameters 10, 50, and 800 nm. The Ag NPs were prepared from AgNO3 in the HEPES buffer. The authors found that the Ag NPs with a diameter of 800 nm is highly toxic compared to the smaller size Ag NPs. Ag NPs with smaller sizes like 10 and 50 nm showed higher antiviral activity against HBV activities. The 10 nm Ag NPs can inhibit HBV of 38% for 5 μM and 80% for 50 μM, monodispersed Ag NPs solution. The authors concluded that the Ag NPs could inhibit the production of HBV RNA through the specific interaction between double-stranded DNA of HBV and the nanoparticles.

Shady et al. (2020) studied the antiviral inhibitory effect against HCV NS3 helicase and protease using green synthesized Ag NPs. The total extract and petroleum ether fraction of marine sponge (Amphimedon) were used for green synthesis of Ag NPs. The Ag NPs synthesized from Amphimedon total extract have particle size 8.22–14.30 nm, and those prepared from petroleum ether revealed particle size 8.22–9.97 nm. The authors found a diverse phytochemical class of natural products identified utilizing LCMS based metabolic investigations, followed by the reorganization of 14 known compounds through bioassay-guided isolation. The docking studies of those compounds suggested their mechanism of action, which were further proved by in vitro assays. The authors found that, among the Amphimedon sponge nakinadine B and 3,4-dihydro-6-hydroxymanzamine A, phytochemicals were found as an efficient anti-HCV drug candidate.

4.4 Orthomyxoviridae

The influenza viruses constitute the genus Orthomyxovirus, which are classified into three types of species: A, B, and C. These viruses are responsible to cause influenza, an acute respiratory disease. Type A viruses cause periodic worldwide epidemics/pandemics; both Type A and B are reported to cause recurring regional and local epidemics. These viruses are highly contagious pathogens that cause much fear among humans for its potential to develop new viruses that can jump to humans from various animal species and causing pandemics. Xiang et al. (2011) investigated the inhibitory effect of Ag NPs on the H1N1 influenza A virus. The Ag NPs with an average particle size of 10 nm were prepared and analyzed for the hemagglutination test, the embryo inoculation assay, MTT assay (Mosmann-based

3-(4,5-dimethylthiozol-2yl)-2,5-diphenyltetrazolium bromide), where these tests were utilized for investigating the inhibitory effect of Ag NPs against the H1N1 virus. The authors used MDCK cells as the infection model. The cytotoxicity of MDCK cells after 24 and 48 h exposure to Ag NPs revealed that the higher concentration Ag NPs can cause cytotoxicity, where toxicity at a silver concentration of 200 µg/ml exhibited severe toxicity than 100 µg/ml. The lower concentration of silver for e.g. 50 µg/ml exhibited a lower toxicity effect. The authors found the Ag NPs concentration between 12 and 100 µg/ml showed potential anti-H1N1 influenza A virus activity at both 72 and 96 h exposures. The 98% of cell viability was observed at a concentration of Ag NPs of 6.25, 12.5, and 25 µg/ml at 24 and 48 h. From the test conducted the authors concluded that Ag NPs could effectively inhibit the H1N1 influenza A virus. Xiang et al. (2013) investigated the inhibition of A/ human/Hubei/32005 (H3N3) influenza virus by Ag NPs (9.05 nm) in vitro and in vivo. The authors demonstrated that the Ag NPs can effectively inhibit the growth of influenza virus in Mandin-Darby canine kidney cells via Hemagglutination assay. The H3N2 virus treated with Ag NPs interact with each other, resulting in the demolition of morphologic viral structures. The intranasal administration of Ag NPs in mice enhanced the survival of the mice after infected with the H3N2 virus. Also, the mice treated with Ag NPs have observed lower lung vital titer level and very small pathologic lesions inside lung tissue. The authors thus proved that the Ag Nps have a beneficial effect in inhibiting H3N2 influenza virus infection, and demonstrated that Ag NPs could be used as potential therapeutics for the prevention of outbreak of influenza virus. Li et al. (2016a) prepared Ag NPs decorated by Amantadine with antiviral properties. The authors developed the Ag NPS co-delivery of Amantadine to overcome the antiviral drug resistance. The results revealed that the Ag NPs decorated with Amantadine could inhibit H1N1 from infecting the host cell and prevent the DNA fragmentation, activity of capase-3, and chromatin condensation. Besides, this newly developed drug could prevent the accumulation of reactive oxygen species and reversed virus-induced apoptosis by the H1N1 influenza virus. Li et al. (2016b) introduced surface decoration of Ag NPs using oseltamivir (Ag@OTV) with antiviral properties to inhibit the activity of the H1N1 influenza virus. The newly developed Ag@OTV co-delivery system remarkably inhibited the accumulation of reactive oxygen species by the H1N1 influenza virus and activation of AKT and P53 phosphorylation. The authors found that this silver-based co-delivery system of oseltamivir could effectively inhibit the activity of the H1N1 influenza virus.

4.5 Coronaviridae

Coronaviride is a family of viruses enveloped, positive-strand RNA viruses with a crown-like morphology (corona is a Latin term for crown). This family of the virus includes subfamilies Letovirinae and Orthocoronavirinae. The coronaviruses belong to the subfamily of Orthocoronavirinae. The members of this family can cause

respiratory, hepatic, enteric, and neurological diseases in various animals including mammals, birds, and amphibians. Up-to-date there are seven human coronaviruses (CoVs) are reported to be capable of infecting humans, in which some of them are identified in the mid-1960s, and others were detected in the new millennium. The common coronavirus (CoV) that could affect humans are HCoV-229E, and HCoV-NL63 (alphaCoVs), HCoV-OC43, and HCoV-HKU1 (betaCoVs). These types of coronavirus can cause common colds and can cause upper respiratory infections by self-limiting immunocompetent individuals. The other CoVs that affect humans includes severe acute respiratory syndrome coronavirus (SARS-CoV) (from 2002 to 2003), the Middle East respiratory syndrome coronavirus (MERS CoV) (identified in Saudi Arbia 2012), and the coronavirus disease 2019 pandemic or known as COVID-19 is caused by SARS-CoV-2 (detected in Wuhan, China). It has been reported that 1.12 million people have died so far from the COVID-19 outbreak as of October 21, 2020, 01:34 GMT (WHO 2020). Currently, there is no specific antiviral treatments are recommended for COVID-19, and no vaccines are still available. Jeremiah et al. (2020) demonstrated the effect of Ag NPs against COVID-19. The diameter of Ag NPs $\approx$ 10 nm was found effective in inhibiting extracellular SARS-CoV-2 at a concentration between 1 and 10 ppm. However, the authors observed a cytotoxic effect at a concentration of 20 ppm. The Luciferase-based pseudovirus entry assay test revealed that the Ag NPs could prevent viral entry by disrupting viral entry. The Ag NPs can interact with the proteins on the surfaces of the extracellular viruses to prevent infection at the early stage of viral entry or viral attachment by damaging the surface proteins that affect the structural integrity of the virus. Du et al. (2020) developed Au/Ag nanorods (Au@Ag NRs) to inhibit porcine epidemic diarrhea virus (PEDV), a member of the coronaviradae family. Au@Ag NRs were synthesized by coating Au nanorods with silver. Viral titer analysis showed that the developed Au@Ag NRs could effectively inhibit the PEDV infection by a magnitude of 4 orders (at a non-toxic concentration of 0.04 μM) at 12 h post-infection. The mechanism of action of Au@Ag NRs against PEDV demonstrated that the inhibition of the virus at its entry stage and thus prevented the apoptosis induced by a viral infection. The Au@AgNRs decreased the potential of mitochondrial membrane and caspase 3 activity. The authors demonstrated that a large amount of virus proliferation causes the generation of reactive oxygen species in the cells and released Ag+ ions and the exposure to Au NRs from Ag@AuNRs after the stimulation of reactive oxygen species has a higher-level of antiviral activity that is capable for long term inhibition of PEDV replication cycle. The authors suggested that the Au@AgNRs could be further improved and effectively applied as a potential treatment strategy to inhibit the activity of all the other members of the coronavirus family such as SARS, MARS, and COVID-19. Chen et al. (2016) tested the antiviral activity of graphene oxide-AgNPs (GO-Ag) sheets against feline CoV and Infectious bursal disease virus (IBDV). The authors found the GO-Ag sheets could inhibit 25% of infection by feline CoV and 23% of infection by IBDV. The authors suggested that the application of GO-Ag could be considered for personal protection equipment (PPE) to reduce the transmission of viruses.

4.6   Poxviridae

Poxviridae family of a virus is classified among 22 genera, and are further cate-gorized into two subfamilies. Smallpox is a disease associated with this family of viruses. The Monkeypox virus (MPV) is an orthopoxvirus that is similar to the variola virus that could infect many species of non-human primates. However, MPV is also considered as human pathogens with their clinical representation similar to that of smallpox. Rogers et al. (2008) prepared silver-based nanoparticles to inhibit the infectivity induced by MPV. The authors evaluated the antiviral efficacy of various nanosized Ag NPS ranges from 10 to 80 nm with or without polysaccharide, and silver nitrate at a concentration of 12.5, 25, 50, and 100 µg/mL using a plaque reduction assay. Both the polysaccharide coated Ag NPs (25 nm) (Ag-PS-25) and non-coated Ag NPs (55 nm) (Ag-NP-55) exhibited a significant dose-dependent effect of the test compound concentration on the average number of PFU (plaque-forming unit). Besides, all concentrations of silver nitrate (excluding 100 µg/mL) and Ag-PS-10 exhibited a significant reduction in the number of PFU compared to untreated controls. The authors found no toxicity effect of silver by any test compounds except 100 µg/mL silver nitrate. The results demonstrated that the smaller-sized nanoparticle with a diameter of 10 nm was most effective at preventing MPV infection as showed by a statically significant decrease in MPV plaque formation. The possible mechanism behind the inhibition of MPV could be due to physical obstruction of interaction between the virus and the host cell. Also, there is a possibility for disruption of intercellular pathways that could diminish viral replication. The authors observed, some concentration of nanoparticle treat-ment led to an increase in MPV PFU that ranged from 1.04 to 1.8 fold above the control. The overall results demonstrated that Ag NPs of ≈10 nm could effectively inhibit MPV infection in vitro, and thus supporting the anti-viral therapeutic effi-ciency of Ag NPs.

4.7   Arenaviridae

This family of viruses composed of 18 various species of viruses and they are classified into two antigenic groups, the New World (Tacaribe complex) and the Old World group. The Tacaribe complex that mainly includes Tacaribe virus (TCRV), consists of the viral hemorrhagic fever inducing viruses such as Sabia, Guanaritio, Junin, and Mchupo. These virus groups have higher transmissibility from humans to humans through the respiratory route. TCRV is generally not a human pathogen, however, they exhibit a similar antigenic relationship with Guanarito and Junin viruses. Therefore, TCRV could serve as a model virus for Arenaviridae-derived diseases with adequate safety for laboratory analysis and without affecting humans. Speshock et al. (2010) analyzed the interaction of TCRV with Ag NPs. The authors demonstrated that the TCRV could interact with Ag NPs

before the host cellular exposure and thus inhibit the viral infectivity. Two types of Ag NPs were used for this study polysaccharide coated Ag NPs (PS-Ag) and uncoated Ag NPs. They treated TCRV with different concentrations of Ag NPs such as 50, 25, and 10 μg/mL, in which the 10 nm size Ag NPs exhibited a significant reduction in the progeny of the virus. The PS-Ag was found not effective compared to the uncoated Ag NPs, however, the polysaccharide coating indeed protects the cell's toxicity of Ag NPs. Ag NPs interact with TCRV prior to the attachment of virus with the host cells resulting in reduced infectivity with 10–25 nm Ag NPs, suggested that the Ag NPs may bind to the viral glycoproteins. The TCRV glycoproteins contain cysteine residues, and the Ag NPs can interact with the thiol groups found in cysteine residues. This interaction with TCRV and Ag NPs can inhibit the internalization of the viral particle by interfering with host cellular receptor interaction with the virus. In addition, the other possible mechanism of action suggested as the Ag NPs could interact with the viral glycoprotein and inhibit the virus uncoating in the endosome. The authors concluded that the Ag NPs are effective for inhibiting a prototype arenavirus at their non-toxic concentration, the Ag NPs can inhibit arenavirus replication when administrated early after initial exposure to the virus or prior to viral infection. The authors suggested the mode of action of inhibition of virus by Ag NPs occurs during the early phase of viral replication.

5   Conclusions

The emergence of viral resistant drugs and the severe side effects of the continuous usage of drugs represents enormous obstacles that are very difficult to overcome. The introduction of nanotechnology has evolved empower to explore their biological properties of already known antimicrobial compounds, such as metals, by manipulating their sizes. In this chapter, we have analyzed the application of metal nanoparticles peculiarly silver nanoparticles, which have proved its antiviral efficiency against a broad-spectrum of viruses. In most of the causes, the mechanism of interaction is between the silver nanoparticles and viral glycoproteins could be demonstrated. However, the exact mechanism or exact site of interaction is an intriguing problem to be solved. Besides the direct interaction of Ag NPs and viral surface glycoprotein, the Ag NPs are expected to get direct access to the cell and capable to exert their antiviral activity through the interaction with the viral genome.

The research advances with the advanced medical uses of Ag, including nanocrystalline silver, have been growing very fast and numerous medical products marketed, especially due to their excellent antiviral, antimicrobial, and anti-inflammatory properties. Recently the new results pointing to the exceptional usage of Ag NPs and silver-based products in particular to prevent the infections caused by bacterial, viruses, and fungi are reported on a regular basis. The development of new low-cost antimicrobial, antiviral and antifungal products through the nanochemistry aspects of Ag NPs, their controlled synthesis routes, and tailored

microencapsulation are purposeful topics to develop new lectures and laboratory research activities in renewed chemistry education curricula utilizing the recent research outcomes.

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