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## Review article

# Development of nanoparticle-delivery systems for antiviral agents: A review

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## ABSTRACT

The COVID-19 pandemic has resulted in unprecedented increases in sickness, death, economic disruption, and social disturbances globally. However, the virus (SARS-CoV-2) that caused this pandemic is only one of many viruses threatening public health. Consequently, it is important to have effective means of preventing viral transmission and reducing its devastating effects on human and animal health. Although many antivirals are already available, their efficacy is often limited because of factors such as poor solubility, low permeability, poor bioavailability, un-targeted release, adverse side effects, and antiviral resistance. Many of these problems can be overcome using advanced antiviral delivery systems constructed using nanotechnology principles. These delivery systems consist of antivirals loaded into nanoparticles, which may be fabricated from either synthetic or natural materials. Nevertheless, there is increasing emphasis on the development of antiviral delivery systems from natural substances, such as lipids, phospholipids, surfactants, proteins, and polysaccharides, due to health and environmental issues. The composition, morphology, dimensions, and interfacial characteristics of nanoparticles can be manipulated to improve the handling, stability, and potency of antivirals. This article outlines the major classes of antivirals, summarizes the challenges currently limiting their efficacy, and highlights how nanoparticles can be used to overcome these challenges. Recent studies on the application of antiviral nanoparticle-based delivery systems are reviewed and future directions are described.

## 1. Introduction

Viral infections pose a serious public health and economic threat globally, as demonstrated by the recent COVID-19 pandemic. It has been estimated that viruses are responsible for around two million deaths per year [41,149]. Multitudes of different kinds of viruses exist, but only about five thousand of them have currently been identified and characterized. These viruses can enter the human body through a variety of different routes, including the nose, mouth, eyes, and skin [24]. Human immunodeficiency virus (HIV), norovirus, hepatitis viruses, human papillomavirus (HPV), herpes simplex virus (HSV), and coronaviruses are major pathogenic viruses associated with a large amount of human morbidity and mortality, and antiviral platforms against them will be discussed for the purposes of this review [58,72]. Moreover, the

devastating effects of viral outbreaks can affect other areas of human life such as economic and social disruptions. The SARS-CoV-2 pandemic has disrupted the economic and social fabric of nearly every nation in the world, causing serious global health problems, as well such as negative economic and social effects [132]. Vaccination is a common method to inhibit viral infections; however, at present there are many important viruses for which effective vaccines are currently unavailable [35].

Another popular method to reduce the burden of viral disease is the use of antiviral drugs, which may be chemically synthesized or natural. Recently, there has been great emphasis on the identification and characterization of natural antiviral agents because of their perceived health and environmental benefits. A broad spectrum of these natural substances have already been identified, including various phenolic compounds, essential oils, and peptides. For example, curcumin, a

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phenolic compound isolated from turmeric, has been shown to exhibit antiviral activity against a variety of viruses. Including parainfluenza virus type 3 (PIV-3), feline infectious peritonitis virus (FIPV), vesicular stomatitis virus (VSV), herpes simplex virus (HSV), flock house virus (FHV), and respiratory syncytial virus (RSV) [186]. Various synthetic drugs are also available that disrupt viral infection and/or replication and thereby reduce the clinical severity of an infection. For instance, enfuvirtide, maraviroc, indinavir, acyclovir, foscarnet, abacavir, lamivudine, tenofovir, adefovir, entecavir, telbivudine, tenofovir, camptothecin, ribavirin, and interferons (siRNA) are commonly used in the medical treatment of viral infections [90].

Despite their antiviral activity, there are numerous challenges limiting the efficacy of many existing antiviral agents, including their poor solubility, their poor stability during storage or application, their low bioavailability, their potential to cause adverse side effects or toxicity, and the development of drug-resistant viruses. Using the pure form of an antiviral agent can therefore present major challenges. For example, when antivirals are ingested *via* the oral route, the harsh conditions of the human gastrointestinal tract (GIT) can damage them and decrease the amount of active antivirals absorbed. Many antivirals also have low permeability through cell membranes, mucosal layers, the epidermis (skin), and the epithelial surfaces of the GIT, which also limits their efficacy. The rapid metabolism and excretion of antivirals after uptake are other problems that can limit their efficacy [38,43,62].

Many of these challenges can be overcome by using well-designed delivery systems to encapsulate antiviral agents, protect them during storage, and then deliver them to a desired target, thereby leading to an increase in antiviral activity. Nano-enabled delivery systems, which consist of bioactive agents trapped inside nanoparticles, are particularly suitable for this purpose because their compositions, structures, and functional attributes can easily be manipulated [101,115,116]. The nanoparticles in these delivery systems can be fabricated from either chemically synthesized or natural components. Recently, there has been great emphasis on the construction of nanoparticles from biocompatible ingredients, such as biopolymers, lipids, phospholipids and biosurfactants, because of their versatile functional properties, high biocompatibility, and good biodegradability. Nanoparticle-delivery systems are characterized by small particle sizes and high specific surface areas, which can be beneficial for certain applications due to their rapid digestion, penetration, and/or absorption [26,102]. Moreover, the composition, structure, and interfacial properties of nanoparticles can be designed to improve the dispersibility and stability of the encapsulated bioactive agents.

Given the public health and societal burden viruses currently pose, as well as the possibility of increased zoonotic viral exposure in the future, new approaches like nanotechnology-based delivery systems should be considered to improve the efficacy of existing and newly-discovered antivirals. Therefore, the major objective of this paper is to discuss improving the activities of antivirals by using nanoparticle-delivery systems. For the purposes of the review, some of the most important synthetic and natural antiviral agents available will be discussed, as well as challenges that currently limit their efficacy. Major types of nanoparticle-delivery systems available for encapsulating antiviral agents will be presented with an emphasis on those constructed from natural components, such as biopolymers, lipids, phospholipids, and/or surfactants. Finally, recent studies on the application of nano-enabled antiviral delivery systems are highlighted.

## 2. Major viral infections

Different types of viruses are transmitted and replicate in different ways and cause different diseases of varying clinical severity [11,59]. In many cases, antivirals are chosen to generally target nonstructural proteins (proteins encoded in the viral genome that help it replicate once inside the host cell) involved in viral replication; though a number of antivirals also act on structural viral proteins. For the purposes of this

review, we will only present relevant information on viruses for which we discuss antivirals and not the entire replication cycles of the relevant viruses. However, we refer interested readers to multiple excellent works on these viruses, including: human immunodeficiency virus (HIV) [69,123,170], hepatitis viruses [1,42,55,105], herpes simplex viruses (HSV) [22,57], human papillomavirus (HPV) [29,105], norovirus ([95,105,137,168,187]), influenza virus [17,21,105], and the human coronaviruses [105] (include SARS-CoV-2) [68,154,172].

## 3. Antiviral substances and their mechanisms of action

A broad spectrum of synthetic and natural antiviral substances is available to inhibit viral transmission or treat viral infections. In this section, we highlight some of the most important ones currently in use, as well as the antiviral mechanisms involved.

### 3.1. Selected synthetic antivirals and their general mechanisms

Different synthetic antivirals can prevent viral infections by inhibition of one or more steps of viral attachment and/or replication [111] (Table 1). Entry of the virus particle into the host cell is often the first step of infection, which can be prevented by blocking virus receptors on the surface of the host cell membrane or the attachment apparatus on the virus itself [185]. For example, enfuvirtide prevents the entry of HIV into host cells. Enfuvirtide (also known as T-20) is a synthetic 36-amino-acid peptide that blocks HIV-1 attachment to the CD4+ host cell membrane by binding to the envelope glycoprotein 41 of HIV-1 in a manner that inhibits the fusion of the virus with the membrane of the CD4+ host cell [100]. Maraviroc is another virus entry inhibitor that has been used for the treatment of HIV-1. Maraviroc prevents HIV-1 binding to the CCR5 receptor of the CD4+ cell surface by attaching to CCR5 [73].

Protease inhibitors are another type of antiviral agent that have been widely used for treating hepatitis C and HIV [166,169]. These antivirals act upon the proteases that are essential for the replication of viruses. Viral proteases catalyze the cleavage of peptide bonds in cellular proteins or viral polyprotein precursors. Protease inhibitors selectively attach to viral proteases and block proteolytic cleavage of protein precursors, leading to the inhibition of viral replication. For example, indinavir is a popular antiviral to treat HIV and hepatitis B or C [33,48].

Polymerase inhibitors are another large group of antiviral agents that play a key role in preventing viral infections [75]. Viral genome replication and transcription play a key role in viral replication, which are controlled by viral polymerases. These polymerases are proteins that can often accomplish multiple functions and have a main role in viral genome synthesis. Converting viral genomes into mRNA, which has the capability of translation into viral proteins, is an essential step for the replication of many viruses. The nature of the viral genome and the cellular location have a major effect on genome replication. For example, DNA viruses need a DNA-dependent DNA polymerase, and

**Table 1**  
Common synthetic antiviral substances.

Synthetic antivirals	Mechanism	Virus	References
Pleconaril, Enfuvirtide	Block attachment and uncoating	Picornavirus	[140], [100]
Saquinavir, Ritonavir, Indinavir	Protease inhibitors	HIV and hepatitis B or C	[146], [80], [89]
Acyclovir, Foscarnet, Abacavir, Lamivudine, Adefovir, Telbivudine, Tenofovir, tenofovir, Ribavirin, Zidovudine	Polymerase inhibitors	HSV, HIV, Hepatitis B	[67], [79], [76], [46], [91], [150], [120], [122], [85]
Interferons	Cell defense protein activated	Hepatitis B or C	[88]

RNA viruses need an RNA-dependent RNA polymerase to accomplish genome replication [121]. Therefore, targeting viral polymerases can drastically disrupt viral replication and reduce the time and/or severity of viral infection.

Acyclovir (ACV) is the first nucleoside analog that selectively prevents replication of herpes simplex virus (HSV) and varicella-zoster virus (VZV). Nucleoside analogs block cellular division or viral replication by impairment of DNA/RNA synthesis or by inhibition of cellular or viral enzymes involved in nucleoside/tide metabolism. ACV can be taken orally, injected, or applied as a cream [153]. Foscarnet is another polymerase inhibitor that has been used for the treatment of herpesviruses, by direct inhibition of viral DNA polymerase through blocking the release of pyrophosphate from the terminal nucleoside triphosphate when added onto the growing DNA chain [7]. It can be taken through intravenous injection or infusion. Abacavir is an oral antiviral that treats HIV by inhibiting viral replication due to nucleoside reverse transcriptase inhibition [76]. Lamivudine is an oral antiviral used to treat HIV and hepatitis B, which inhibits viruses by phosphorylating active metabolites that compete for incorporation into viral DNA [79]. Adefovir is an antiviral used to treat hepatitis B, which prevents viral replication by blocking a reverse transcriptase [46]. Telbivudine is another oral antiviral used to treat hepatitis B that inhibits a reverse transcriptase, which has been reported to be more effective than lamivudine and adefovir [120]. Tenofovir is another antiviral used for the treatment of HIV and hepatitis B, which inhibits viral replication by preventing DNA replication, reverse transcription, and transcription [150]. Ribavirin is an effective antiviral against hepatitis C, human orthopneumovirus, and viral hemorrhagic fevers, due to its ability to inhibit nucleosides. It can be administered orally or through inhalation [67].

Interferons (IFNs) (a family of cytokines) are released by host cells when they are attacked by viruses. IFNs are a significant part of the innate immune response and defense against many viral infections. Currently, IFNs have been investigated for therapeutic potential against various viruses, especially the hepatitis C virus. IFNs are classified into three major types (I, II and III) based on the receptor complex they signal through. Type I IFNs attach to a particular receptor complex on the cell surface known as the IFN- $\alpha/\beta$  receptor (IFNAR). When they are released, they attach to specific receptors on the target cells, which causes protein expression to inhibit the virus producing and replicating its RNA and DNA. Type I IFNs are critical for providing a strong general immune response against multiple viral infections. Type II IFNs are activated by Interleukin-12 (IL-12) and are released by cytotoxic T cells and T helper cells. They attach to the IFN $\gamma$  receptor (IFNGR) complex and induce a wide immune response to pathogens. Type III IFNs include three IFN $\lambda$  gene products that signal through receptors containing IFNLR1 and IL-10R2 [152].

### 3.2. Natural antivirals

Antiviral drugs based on natural compounds have attracted considerable attention from researchers because of their low toxicity, low cost, low side effects, and reduced likelihood of promoting antiviral resistance [8]. Several bioactive compounds have been found to have activity against viruses, including flavonoids, terpenoids, limonoids, organosulfur compounds, sulfides, polyphenolics, lignans, saponins, coumarins, chlorophyllins, alkaloids, furyl compounds, thiophenes, polyines, proteins, and peptides [130]. The antiviral activity of these compounds has been attributed to various mechanisms of action, including antioxidant activities, inhibition of DNA and RNA synthesis, and prevention of viral particle entry [8,130]. Curcumin is one of the widely studied phenolic compounds for treating viral infections [186]. Curcumin prevents viral long terminal repeat (LTR) activity, which has a main role in the transcription of HIV-1. Moreover, it inhibits inosine monophosphate dehydrogenase (IMPDH) activity, an enzyme involved in the *de novo* synthesis of guanine nucleotides. It was demonstrated that curcumin has antiviral activity against a broad group of viruses including HIV, HSV,

HBV, HCV, and influenza [165,186].

Essential oils are non-polar substances found in many plants, particularly herbs and spices [147]. These compounds are commonly used as flavoring agents in foods, as well as for their antimicrobial properties. Essential oils are mainly composed of volatile compounds, including monoterpenes (e.g., limonene,  $\alpha$ -pinene, P-cymene, and sabinene), phenylpropanoids (e.g., cinnamaldehyde, vanillin, eugenol, and safrole), and monoterpenoids (e.g., thymol, citronellal, carvone, carvacrol, and borneol) [133]. Many essential oils have been shown to possess antiviral activity against a variety of enveloped and nonenveloped viruses such as HSV, influenza, adenovirus type 3, Junin virus, poliovirus, norovirus, and coxsackie virus B1 (Table 2).

### 3.3. Antiviral delivery routes and potential biological barriers

Antiviral agents can be utilized in a variety of ways. They may be used to clean surfaces or hands so as to prevent viral transmission (antiviral wipes or hand sanitizers), or they may be used as treatments to fight viral infections or their symptoms (drugs). In the latter case, they may be administered through oral, injection, skin, or ocular routes depending on the nature of the formulation and the intended target.

#### 3.3.1. Oral delivery

When antivirals are taken orally, they have to pass through the gastrointestinal tract to reach the small intestine where they are usually absorbed and transported to the systemic circulation [181]. There are numerous factors that may impact the bioavailability ( $B_A$ ) of ingested antivirals, *i.e.*, the fraction that actually reaches the systemic circulation [119]:

$$B_A = B^* \times A^* \times S^* \quad (1)$$

Here,  $B^*$  is the *bioaccessibility* of the antiviral, *i.e.*, the fraction present in the intestinal fluids that is in a form that can be absorbed.  $S^*$  is the *stability* of the antiviral, *i.e.*, the fraction that is stable to chemical or enzymatic transformation.  $A^*$  is the *absorption* of the antiviral, *i.e.*, the fraction in the intestine that is taken up by the body.

Some antiviral agents are highly hydrophobic molecules with a low water-solubility (such as essential oils and some phytochemicals). As a result, they may not fully dissolve in the gastrointestinal fluids so they are not fully bioaccessible. The bioaccessibility of hydrophobic

**Table 2**  
Antiviral activity of various essential oils.

Essential oil compounds	Virus	Reference
Linalool, myrcene, carvacrol, bicyclogermacrene, <i>p</i> -cymene, <i>E</i> -caryophyllene, Germacrene D, estragole, eugenol	HSV-1	[164] [174] [23]
Gurjunene, eudesmol, and muurolene	Avian influenza A virus (H5N1)	[78]
Carvacrol, <i>p</i> -cymene, $\gamma$ -terpinene, and $\beta$ -caryophyllene	Murine norovirus	[56]
Thymol, carvacrol, <i>p</i> -cymene, $\beta$ -caryophyllene, $\gamma$ -terpinene, $\alpha$ -zingiberene, camphene, $\beta$ -sesquiphellandrene, <i>ar</i> -curcumene, $\beta$ -phellandrene, and $\beta$ -bisabolene	HSV-2	[96]
Thymol, <i>p</i> -cymene, $\gamma$ -terpinene, and ( <i>E</i> )-Cinnamaldehyde	Influenza A virus subtype H1N1	[159] [74]
Camphor, limonene, <i>p</i> -mentha-1, 8-diene, $\alpha$ -pinene, $\gamma$ -terpinene, germacrene D, $\beta$ -caryophyllene, calamine, leptospermone, flavesone, viridiflorene, isoleptospermone, geranial, menthol, menthone, and isomenthone	HSV-1 and HSV-2	[23] [148] [155] [156]
Patchoulol, $\delta$ -guaïeno; gurjunene- $\alpha$ , $\alpha$ -guaïene, aromadendrene, and $\beta$ -patchoulene	Influenza A (H2N2) virus	[179] [178]

antivirals can be increased by ingesting them with digestible lipids, such as triglyceride oils [119]. Triglycerides are hydrolyzed by gastric and pancreatic lipase in the stomach and small intestine, leading to the formation of free fatty acids (FFAs) and monoglycerides (MGs). The FFAs and MGs interact with bile salts and phospholipids in the intestinal fluids to form mixed micelles, which are small colloidal particles (micelles and vesicles) capable of solubilizing hydrophobic substances. The mixed micelles can then transport the hydrophobic substances to the epithelium cells where they can be absorbed. Consequently, it may be important to deliver hydrophobic antimicrobials intended for oral ingestion within digestible lipid particles, so as to increase their bioavailability.

Some antivirals may chemically degrade during storage or be damaged by the harsh conditions inside the human GIT, which decreases the concentration of active antivirals reaching the systemic circulation [64]. For instance, peptide-based antimicrobials may be hydrolyzed by proteases in the stomach and small intestine. This problem can be overcome by taking a higher oral dose of the antiviral agent, but this approach can lead to undesirable side effects [102,104]. For this reason, storage and gastrointestinal stability should be considered when designing effective antivirals intended for oral administration, which again may be enhanced by encapsulating the antimicrobials in nanoparticles.

Finally, some antiviral drugs have poor absorption in the gastrointestinal tract because the mucus layer or epithelium cells act as a biological barrier to their uptake [64]. For instance, antivirals with high molecular weights typically have low permeability through the intestinal epithelial layer, which affects the permeation and transcellular absorption of antimicrobials by passive diffusion [2]. The intestinal epithelium is covered by a thick mucus layer and consists of various different kinds of cells, including Paneth cells, enterocytes, goblet cells, M cells, and endocrine cells, which can decrease the uptake of antivirals [70].

### 3.3.2. Intravenous delivery

The intravenous administration of antiviral drugs into the veins by direct injection is the most effective route for emergencies, because many of the chemical, physical, and biological barriers to absorption are avoided [77]. The main limitations of this approach are the discomfort, cost, and inconvenience associated with injections, especially if they have to be carried out repeatedly. In addition, antiviral drugs may undergo opsonization in the systemic circulation and subsequent uptake by the mononuclear phagocyte system (MPS), which reduces their efficacy. This problem can be overcome by encapsulating the antiviral drugs in specially-designed particles that “hide” them from the MPS, thereby prolonging their circulation [18,53,129]. In general, the composition, size, and surface chemistry of the nanoparticles used in antiviral delivery systems must be carefully controlled to ensure that they exhibit their strong antiviral activity after injection, without causing adverse side effects. For instance, it has been reported that under normal flow conditions within blood vessels, the tendency for particles to stick to the endothelial cell surfaces increases as their size increases, which may be important for the uptake of antivirals into the body [20]. Moreover, the same authors reported that cationic particles tend to get rapidly opsonized and removed from the systemic circulation, whereas neutral particles tend to have prolonged circulation times. Consequently, it may be better to encapsulate antivirals in neutral nanoparticles so as to extend their lifetime in the body.

### 3.3.3. Transdermal delivery

The skin is the largest organ in the human body and is a popular site for antiviral administration because of its accessibility and ease of application. Some major antivirals are administered as topical formulations through the skin, including acyclovir [162]. In this case, the antiviral formulation should fuse with the skin lipids and then penetrate into the underlying tissues. The penetration of free antiviral drugs into

skin lipids is typically not high, leading to a reduction in their activity [135]. For this reason, there has been interest in encapsulating antiviral drugs into delivery systems to increase their bioavailability. However, various factors have to be considered for achieving a potent transdermal delivery system for antivirals, such as ensuring that the drug is absorbed into the systemic circulation and reaches a therapeutically active plasma level [143]. The epidermis layer of the skin is designed to protect the body from the loss of water and the penetration of harmful materials, and can therefore act as an effective barrier against drug absorption. The pure forms of many antiviral agents cannot rapidly penetrate through the skin, which leads to a low active plasma level and antiviral activity [173,177]. This problem can be overcome by designing antiviral delivery systems that contain carrier particles with dimensions and surface features that promote their penetration and absorbance.

### 3.3.4. Ocular delivery

The human eye is a spheroidal organ of about 24 mm in diameter, which consists of anterior and posterior parts [163]. These parts prevent foreign agents entering the eye due to the presence of various specialized barriers. Not surprisingly, such barriers pose a significant hurdle to ocular delivery of antiviral compounds and other therapeutics to the eyes. The corneal, iris, lens, and aqueous humor are the major parts of the anterior eye, while the vitreous body, retina, choroid, and back of the sclera are the major parts of the posterior eye. The cornea consists of five layers: the epithelium, Bowman’s membrane, stroma, Descemet’s membrane, and endothelium. The epithelium forms one of the most important barriers against foreign agents because it consists of multiple layers of corneal epithelial cells interconnected by tight junctions. These junctions can restrict the penetration of some antiviral agents through the eye, especially hydrophilic antivirals [50]. Conversely, the stroma, which is partly assembled from hydrophilic collagen molecules, acts as a barrier against hydrophobic antivirals [144]. The blood-retina and blood-aqueous barriers also reduce drug penetration into the intraocular chamber [45,63]. Moreover, there are various other barriers that limit the topical administration of antivirals via the ocular route, including blinking, tear turnover, tear film, solution drainage, lacrimation, and clearance mechanisms on the corneal surface [66]. The presence of these different barriers reduces the amount of antivirals reaching the retina and vitreous body. Consequently, advanced antiviral delivery systems are needed to overcome these barriers and improve the efficiency of ocular administration.

### 3.3.5. Internal biological barriers

After entering the systemic circulation, there may be additional biological barriers that inhibit the ability of antivirals reaching their target tissues. In particular, the blood-brain barrier (BBB) and blood-testis barrier (BTB) decrease the penetration of antivirals into the brain and central nervous system, respectively [36,71]. The BBB is a highly selective semipermeable border of endothelial cells, which effectively separates the systemic circulation from the brain parenchyma and extracellular spaces [47]. The endothelial cells are tightly connected to one another, which inhibits transportation of compounds through the cell membranes. Hydrophilic antivirals cannot easily pass through these membranes because of the lipid domains they contain [37]. Increasing the lipid solubility of antivirals can enhance their penetration through cell membranes, but this can cause a greater tissue burden and higher efflux of the antivirals [25]. The BTB normally protects the developing germ cells from damage from chemical interactions and immunological effects, but it can also provide a sanctuary for the HIV virus during antiretroviral therapy [126].

It should also be noted that prolonged use of antivirals, especially in chronic diseases, leads to the development of antiviral resistance, which can reduce their efficiency [94,102]. Consequently, it is important to have a range of different antiviral agents that work by different mechanisms to tackle this problem.

### 3.3.6. Antiviral delivery systems: optimum requirements

As mentioned in the last section, there are several routes available for delivering antivirals, each with its own specific biological barriers that can decrease the effectiveness of antivirals. Nanoparticle-based delivery systems have considerable potential to overcome a number of these barriers and therefore increase the efficacy of antivirals ([13,97], Cojocaru, Botezat et al. 2020). In general, an effective antiviral delivery system should have a number of features:

- (i) It should be able to encapsulate an appropriate concentration of the antiviral agent to achieve the desired goal, *i.e.*, enough to prevent or treat viral infection without causing undesirable side-effects or toxicity;
- (ii) It should be in a physical form that is suitable for the chosen delivery route, *e.g.*, a low viscosity fluid (injection or oral), a viscous fluid or gel (transdermal or oral), or a powder, capsule or pill (oral);
- (iii) It should retain the antiviral agent and protect it from degradation during storage and transport through the body until it reaches the intended site of action;
- (iv) In the case of oral ingestion or topical application, it should deliver the antiviral agent in a form that is bioavailable, *i.e.*, a high fraction reaches the systemic circulation;
- (v) In some cases, it may be advantageous to have a targeted or controlled release of the antiviral agent.

In general, nanoparticles can be designed that can encapsulate antivirals so as to achieve many of the requirements just outlined.

## 4. Nanoparticle design: tuning functionality

Nanoparticle-based delivery systems have considerable versatility for the encapsulation, protection, and release of antiviral agents [115,116]. Their properties can be manipulated in a variety of different ways so as to tune their functionality for particular applications. In this section, an overview of the various physicochemical and structural properties of nanoparticles is given:

- **Composition:** Nanoparticles can be fabricated from a variety of synthetic and natural ingredients, which may be organic or inorganic. In this article, we mainly focus on the creation of antiviral delivery systems from biocompatible materials including lipids, carbohydrates, proteins, phospholipids and surfactants, since these have advantages in terms of their high biocompatibility and low toxicity. The nature of the components used to construct the nanoparticles impacts their functional performance. For instance, the polarity of the components used (polar or non-polar) determines the type of antiviral agents that can be encapsulated. They also impact the chemical stability of antiviral agents, *e.g.*, many proteins have antioxidant properties that can protect chemically labile substances. In addition, nanoparticle composition can be selected to control the retention and release of antivirals. For instance, the location that antivirals are released within the human gut after oral ingestion depends on the nature of the particle matrix: starch is degraded in the mouth by amylase, proteins by proteases in the stomach and small intestine, lipids by lipases in the stomach and small intestine, and dietary fibers by bacterial enzymes in the colon [117,184].
- **Particle size:** Strictly, the definition of a nanoparticle is a particle that has at least one major dimension that is less than 100 nm. In practice, the term is often used more loosely to apply to any particles that have dimensions within the nanometer range, *i.e.*, 1 to 1000 nm [14]. When particles are in the nanometer range they often have different functional properties than larger particles. For instance, small nanoparticles may appear transparent because of weak light scattering, they may have strong resistance to gravitational separation because of the increased importance of Brownian motion, they may

have higher chemical reactivity because of their high specific surface area, and they may be able to penetrate biological barriers because of their small dimensions [113]. The size of nanoparticles can often be controlled by selecting different ingredients or processing operations to produce them, thereby allowing their functionality to be tuned.

- **Surface Characteristics:** The surface characteristics, such as electrical charge, hydrophobicity, chemical reactivity, and targeting, of nanoparticles can be manipulated by controlling the nature of the molecules that form their outer layer. For homogeneous nanoparticles, which are comprised of a single component (such as protein nanoparticles), the outer surface characteristics are determined by the nature of this component. For core-shell nanoparticles, which consist of a core of one substance surrounded by shell of another substance, the surface composition is determined by the nature of the shell material. For instance, for oil-in-water nanoemulsions, the surface characteristics are determined by the type of emulsifier used to coat the oil droplets [113]. The electrical charge on nanoparticles can be made positive, negative, or neutral by selecting different building blocks to assemble them. As an example, protein surfaces go from positive at pH values below their isoelectric point to negative at higher pH values. Alternatively, anionic, cationic, or non-ionic surfactants, or their mixtures, can be used to tune the electrical properties of surfaces. In some cases, it is possible to locate targeting molecules on the surfaces of nanoparticles so that they will become attached to viruses or specific tissues within the human body.

## 5. Nanoparticle delivery systems in antiviral therapy

Many different kinds of nanoparticle delivery systems can be fabricated from pharmaceutical and/or food grade ingredients, including microemulsions, nanoliposomes, nanoemulsions, solid lipid nanoparticles, biopolymer nanoparticles, and biopolymer nanogels (Fig. 1). In general, nanoparticles can be produced using two different approaches: *top-down approaches*, which use mechanical devices (such as homogenizers, sonicators, or milling devices) to breakdown bulk phases or large particles into small particles; *bottom-up approaches*, which use changes in solution or environmental conditions to promote the formation of small particles from molecules (such as crystallization, spontaneous emulsification, or anti-solvent precipitation) [87]. In practice, these approaches may be combined to create nanoparticles with new or improved properties. For instance, a nanoemulsion can be formed by homogenization and then the emulsifier-coated lipid nanoparticles can be coated by nano-laminated polymer layers using electrostatic deposition [112].

Many previous researchers have demonstrated the ability of nanoparticles to encapsulate and deliver antivirals (Table 3). Nanoparticles often have advantages for delivering antiviral to infected sites because they can overcome biological barriers due to their small dimensions and tunable surface characteristics [103,138]. For instance, antivirals released from nanoparticles can attach to viral receptors on the surface of the host cells or they can be released inside the cell, thereby disturbing the cycle of viral replication [39,40,106] (Fig. 2). (See Fig. 3.)

### 5.1. Micelles and microemulsions

Micelles and microemulsions are surfactant-based nanoparticles ( $d = 5\text{--}100\text{ nm}$ ), which can be used to design thermodynamically stable delivery systems for antivirals and other active agents [115,116]. These colloidal dispersions are typically produced using a bottom-up approach by mixing appropriate types and amounts of surfactant, oil, and water together under suitable environmental conditions, such as temperature, pH, and ionic strength [131]. They should form spontaneously because they are thermodynamically favorable, but some energy input is often needed to overcome kinetic energy barriers associated with inducing the individual components to form colloidal structures [114]. Micelles and oil-in-water microemulsions consist of small surfactant-rich

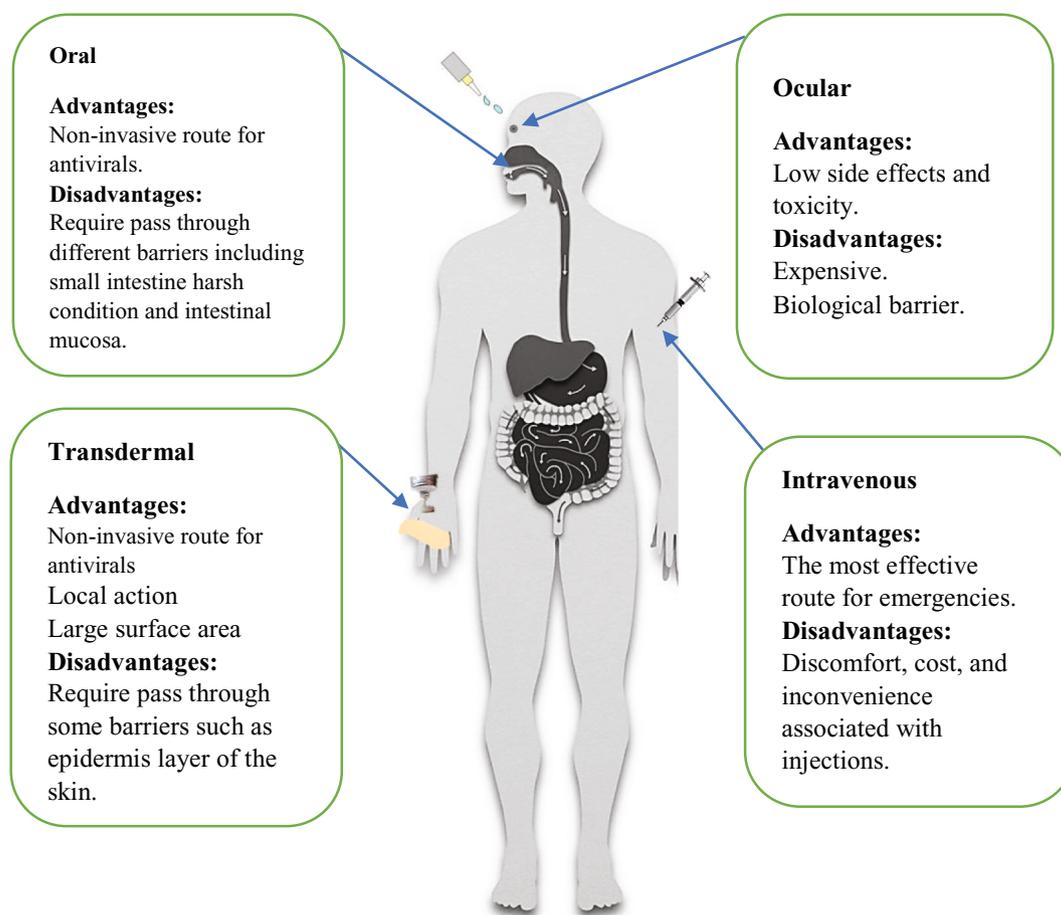


Fig. 1. Different antiviral delivery routes.

nanoparticles dispersed within water. The surfactants are organized so their hydrophilic head groups are in contact with water, whereas their hydrophobic tails form a non-polar core in the interior of the particle. The size, charge, and solubilization capacity of these particles can be controlled by using surfactants (and sometimes co-surfactants) with different head or tail groups to assemble them [65]. Hydrophobic antivirals can be incorporated into their interiors, whereas amphiphilic antimicrobials can be incorporated into their interfacial regions. Hydrophilic antivirals have to be encapsulated using reverse micelles or water-in-oil type microemulsions, which consist of surfactant-based nanoparticles dispersed in oil. In this case, the hydrophilic head groups of the surfactants form the core, whereas the hydrophobic tails form the shell. The core may also contain varying amounts of water molecules, depending on the optimum curvature of the surfactant monolayer and the composition of the system.

Micelles and microemulsions are commonly used as delivery systems for drugs administered through all major routes [44,161]. These systems are typically formulated with synthetic surfactants, which may be pharmaceutical and/or food grade.

The antiviral activity of microemulsions formulated from Tween 80, Span 20 (surfactants), oil, isopropyl myristate (oils), and ethanol/water (aqueous phase) have been studied [4]. These microemulsions were reported to exhibit antiviral activities against herpes simplex virus type 2 (HSV-2) using a cytopathic effect assay. The mean diameter of the microemulsion droplets in these systems was reported to be relatively small (4.7 nm), which may have enhanced their ability to interact with the viruses. In another study, W/O microemulsions containing a hydrophilic antiviral agent (acyclovir) were formulated from Tween 20, Span 20 (surfactants), isopropyl myristate, medium chain triglycerides

(oils), and water/dimethylsulfoxide (aqueous phase) [162]. In addition, ethyl alcohol, eucalyptus oil, and peppermint oil were also investigated as potential permeation enhancers. *In vivo* studies on mice showed that application of the optimized microemulsions could suppress the development of skin lesions caused by herpes simplex virus I infection. In a similar study, acyclovir encapsulated in W/O microemulsions formulated from Tween 20, Span 20 (surfactants), isopropyl myristate (oil), and water/dimethylsulfoxide (aqueous phase) was also shown to be effective in treating herpes skin lesions in a mouse model. It has been reported that the antiviral activity of a botanical extract (olive leaves extract) against herpes simplex virus was enhanced by encapsulating it within O/W microemulsions [92]. The authors attributed the antiviral activity of OLE to its ability to inhibit viral attachment to the host cell receptors.

It should be noted, that in many respects, microemulsions have similar characteristics to nanoemulsions, but there are important differences [114]. In particular, microemulsions are thermodynamically stable, whereas nanoemulsions are not. It should be noted that there is still a great deal of confusion about these terms in the literature and many researchers use the incorrect term for the kind of colloidal system they are actually working with. Most previous studies using microemulsions have focused on transdermal delivery, which is probably because of the high levels of synthetic surfactants required in their formulation.

## 5.2. Nanoliposomes

Nanoliposomes have been also been widely used for the encapsulation of antivirals for certain applications. They can be distinguished from regular liposomes by their relatively small dimensions:  $d < 200$  nm

**Table 3**  
Various nanoparticle-delivery systems for antiviral agents.

Nanoparticle	Production method	Antiviral	Delivery system benefits	References
SLNs	Lipid-based nanoparticles Emulsification and low-temperature solidification method	Acyclovir	Sustained release for acyclovir, which can be improved its antiviral activity against HSV. Loading ACV into SLN leads to improvement of bioavailability and reduction in toxicity.	[136]
SLNs	Using high pressure hot-homogenization technique.	Acyclovir	Obtained higher encapsulation efficiency, and relatively high loading capacity and predetermined <i>in vitro</i> drug release profile of acyclovir.	[19]
Nanoemulsion	Using high pressure homogenization technique.	Indinavir and lactoferrin	Significant brain penetration enhancement of indinavir and lactoferrin loaded nanoemulsions, which can lead to higher antiviral activity against HIV. Indinavir loaded nanoemulsions had the higher drug residence time in brain compared to lactoferrin.	[89]
SLNs	Solvent emulsification evaporation (SE) and double emulsion methods (DE)	Ritonavir	Higher encapsulation efficiency, improved antiviral activity, and modulated release of ritonavir. The <i>in-vitro</i> antiviral experiment showed ritonavir SLNs can actively maintain inhibition of HIV virus production.	[80]
SLNs	The hot – pressure homogenization technique	<i>Artemisia arborescens</i> essential oil	SLNs containing essential oils indicated high physical stability. <i>In vitro</i> antiviral assays showed that SLN incorporation did not affect the essential oil antiherpetic activity. SLN improved the oil accumulation into the skin.	[98,99]
LNCs	Hot homogenization method combined with high shear and ultrasonication	Lamivudine	Lamivudine loaded nanoparticles had low cytotoxicity and was physically stable for at least 45 days. Controlled release of lamivudine under gastric and plasma-simulated conditions. The optimized nanoparticle present suitable profiles for oral administration.	[31]
SLNs	Solvent diffusion method	Adefovir dipivoxil	About 15 wt% drug entrapment efficiency (EE) and 3 wt% drug loading (DL) could be reached in SLN loading ADV. The inhibitory effects of Adefovir dipivoxil loaded in SLN on hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and hepatitis B virus (HBV) DNA levels <i>in vitro</i> were significantly enhanced.	[180]
Chitosan nanoparticles	Biopolymer-based nanoparticles The ionic gelation technique.	Saquinavir	As compared to the soluble drug control, the saquinavir-loaded chitosan carriers caused superior control of the viral proliferation as measured by using two different viral strains, NL4-3 and Indie-C1, and two different target T-cells, Jurkat and CEM-CCR5. Chitosan nanoparticles loaded with saquinavir were characterized and they demonstrated superior drug loading potential with greater cell targeting efficiency leading to efficient control of the viral proliferation in target T-cells.	[145]
Chitosan nanoparticles	Using cross-linked chitosan with triphosphosphate (TPP)	Acyclovir	From the diffusion profiles, it was found that the amounts of ACV effectively diffused in 24 h were 30, 430 and 80 µg for the ACV solution, chitosan microparticle and chitosan nanoparticle respectively. Obtained sustained release of antivirals with moderate irritation and mild tissue damage.	[26]
Chitosan-g-HPβCD nanoparticles	The ionic gelation technique.	Efavirenz	Nanoparticles showed sustained Efavirenz release (99.03 ± 0.30% in 8 h) and followed Fickian diffusion mechanism. Nanoparticles showed 4.76 times greater permeability than un-encapsulated Efavirenz solution through porcine nasal mucosa. Development intranasal mucoadhesive EFV-NPs for the CNS targeting.	[15]
β-cyclodextrin-based nanoparticle	By cross-linking Hydroxypropyl-β-cyclodextrin (HPβCD) with diphenyl carbonate.	Dolutegravir sodium	Significantly higher concentration of Dolutegravir sodium in the CSF and higher CSF:Plasma ratio was achieved, which can be useful in treatment of HIV. High brain drug transport percentage (83.47%) from intranasal NPs confirms nose to brain transport of the drug. Gamma scintigraphy studies in rats revealed enhanced CNS uptake of drug from NPs.	[16]
Alginate nanoparticles	Emulsion solvent evaporation method	Zidovudine	A slow and sustained release of AZT in PBS (pH -7.4) was observed. Being biocompatible and showing significantly higher internalization efficiency <i>in vitro</i> cellular internalization studies.	[86]

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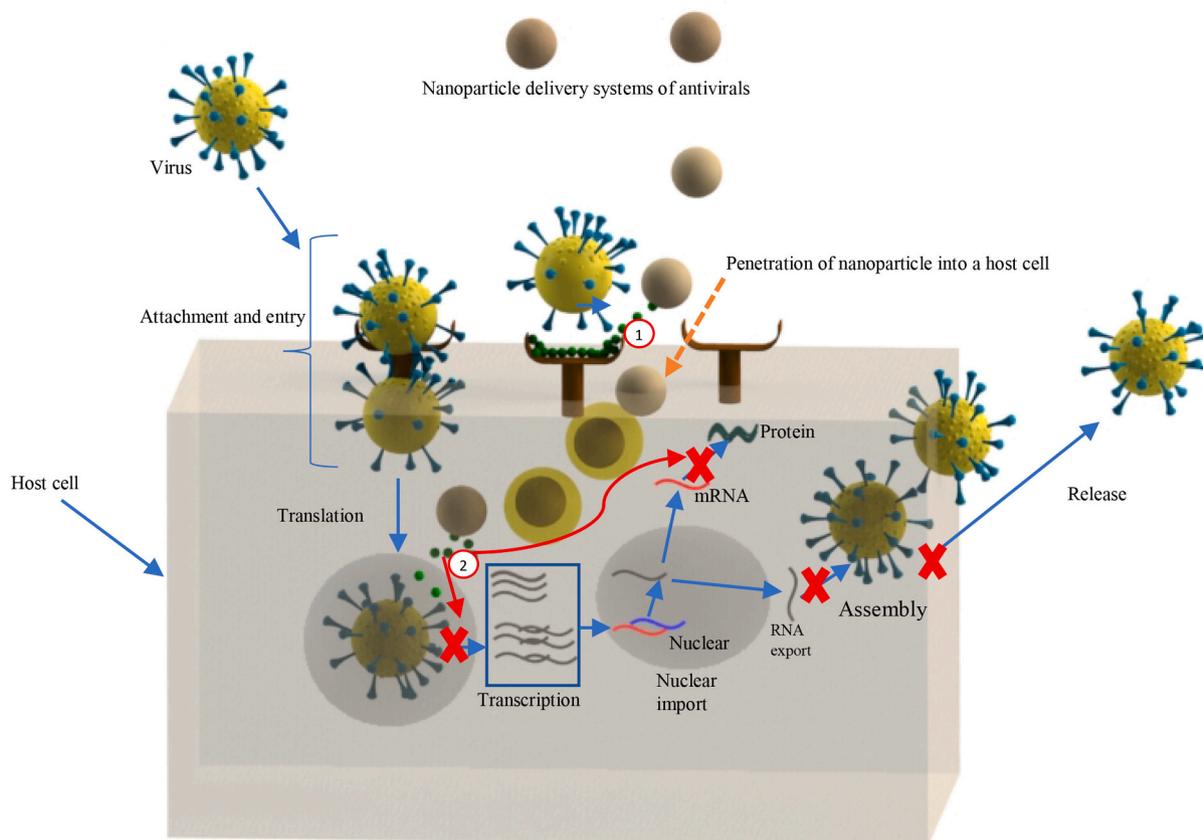
Table 3 (continued)

Nanoparticle	Production method	Antiviral	Delivery system benefits	References
Nano-liposome	Surfactant-based nanoparticles A rapid extrusion procedure	Enfuvirtide and protoporphyrin IX	Co- delivery of combining two clinically relevant entry inhibitors of HIV viruses, Enfuvirtide and protoporphyrin IX. Combination of the two entry inhibitors in the nanocarrier resulted in improved synergy against HIV-1 entry compared to combination in free form, strongly when immune-evasive formulations are used. Liposomes remain largely unexplored platforms for combination of viral entry inhibitors, with potential for improvement of current antiretroviral therapy drug safety and application.	[60]
Nano-liposome	The film method and sonication	Santolina insularis essential oil	Santolina insularis essential oil can be incorporated in high amounts in the prepared liposomes, which successfully prevented its degradation. Liposomal Santolina essential oil is nontoxic in the range of the concentration tested. Antiviral activity assays demonstrated that Santolina insularis essential oil is effective in inactivating HSV-1.	[171]
Nano-liposome	The film method and sonication	<i>Artemisia arborescens</i> L. essential oil	Liposomes containing essential oils were stable at least for six months. Antiviral assays demonstrated that the liposomal incorporation of <i>A. arborescens</i> essential oil enhanced its <i>in vitro</i> antiherpetic activity especially when vesicles were made with P90H.	[164]
Gel nanoemulsion	Low energy method.	Acyclovir	Acyclovir permeation of optimum gel nanoemulsion was about 2.8-fold higher than the uncoated Acyclovir. This can be used as an appropriate delivery system to use topically for the treatment of viral ophthalmic disease.	[108]
Microemulsion	Obtained from pseudoternary diagrams.	Acyclovir	A cationic charge-inducing agent, L-alanine benzyl ester, was added to the formulations to prepare positively-charged microemulsions. The presence of oleyl alcohol or oleic acid increased the flux but not the drug skin accumulation compared to a control suspension, while the use of the cationic charge-inducing agent had no influence on the formulation performance. Significantly optimizing drug targeting, maintaining the structure of the stratum corneum intact.	[139]
Microemulsion	Obtained from pseudoternary diagrams.	Zidovudine	Microscopic examination after <i>in vivo</i> skin irritation studies using mice suggested few histological changes in the skin of animals treated with the ME compared to the control group (hydrogel). Higher permeability of zidovudine through skin.	[30]
Nanoliposome	Lipid film hydration method.	Acyclovir	Promoted the prolonged contact between the drug and the absorptive sites in the nasal cavity, and facilitated direct absorption through the nasal mucosa.	[6]
Dextran and stearic acid nanoparticles	Hybrid nanoparticles Double emulsion solvent evaporation method.	Zidovudine	Higher cellular internalization of drug loaded hybrid nanoparticles. The results showed the feasibility and efficacy of the hybrid nanoparticles for effective delivery of zidovudine	[83]
Thiolated chitosan (TCS) core/shell nanofibers	A coaxial electrospinning technique.	Tenofovir	The core/shell NFs were 40–60-fold more bioadhesive than the pure PEO based nanofibers. The nanofibers were nontoxic and noninflammatory <i>in vivo</i> after daily treatment for up to 7 days. The TCS core/shell NFs are promising candidates for the topical delivery of HIV/AIDS microbicides such as tenofovir.	[122]
Lipid-coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles	A modified single-emulsion evaporation method.	Latency-reversing agents	Latency-reversing agents combination that displayed synergistic latency reversal and low cytotoxicity in a cell model of HIV and in CD4 <sup>+</sup> T cells from virologically suppressed patients. Selectively activated CD4 <sup>+</sup> T cells in nonhuman primate peripheral blood mononuclear cells as well as in murine lymph nodes, and substantially reduced local toxicity.	[28]
Polyvinylpyrrolidone (PVP)/stearic acid (SA)-polyethylene glycol (PEG) nanoparticles	Emulsification–solvent evaporation method.	Zidovudine	Significant improvement in cellular internalization. It is envisaged that nanoparticles composed of lipid and polymer moieties may constitute a preferred	[84]

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Table 3 (continued)

Nanoparticle	Production method	Antiviral	Delivery system benefits	References
Nanoparticles are composed of carboxy methyl cellulose-zidovudine core enclosed by a compritol (Comp)-polyethylene glycol shell.		Zidovudine	embodiment for anti-viral drug delivery for use in HIV/AIDS therapy. Higher encapsulation efficiency, nontoxic, targeted releases, effectively penetrate to the brain cells were achieved by hybrid nanoparticles.	[85]



**Fig. 2.** The mechanism of antiviral activities of nanoparticle-delivery systems of antivirals; Nanoparticle containing antivirals can inhibit viral replication in a host cells by: 1) released antivirals from nanoparticles block viral receptors, and 2) released antivirals from penetrated nanoparticles into a host cell prevent major viral replication steps including transcription, replication of phage DNA and synthesis of protein, and assembly.

(although some researchers use a less strict definition of  $d < 1000$  nm). Nanoliposomes can be classified into two categories depending on their internal structures: multilamellar (onion-ring structure) and unilamellar (single ring) vesicles [124,125]. Each ring consists of a bilayer of phospholipid molecules with their non-polar tails forming the inner region and their hydrophilic head groups forming the outer layers.

Nanoliposomes can be produced using a variety of different fabrication methods [93]. In the laboratory, they are often prepared using a solvent evaporation approach that involves several steps: dissolve phospholipids in organic solvent; place organic solvent in a glass flask; remove organic solvent by drying (resulting in phospholipid bilayers forming on the flask surface); and, finally add water (causing the phospholipid bilayers to peel off the flask and form nanoliposomes). This approach is not suitable for large scale production of nanoliposomes, but other methods can be used. For instance, mechanical dispersion methods such as sonication, homogenization, or microfluidization [3]. Antivirals can be encapsulated by adding them to the phospholipids before nanoliposome formation or by incorporating them into the liposomes after their formation. Nanoliposomes can be used to deliver both lipophilic and hydrophilic antivirals because they contain both non-polar

(phospholipid tails) and polar (phospholipid heads) domains within the bilayer structures [49]. Because nanoliposomes are formed from phospholipids they tend to be non-toxic, biocompatible, potentially minimally immunogenic, and biodegradable, which means they are particularly suitable for oral administration, as well as by other routes. However, nanoliposomes have been formulated in ways to elicit an immune response in multiple applications [182], suggesting that potential immune reaction should be a consideration before their application. In addition, the surfaces of nanoliposomes can be functionalized by incorporating specific ligands, thereby enhancing their ability to deliver antivirals to specific targets. However, there are also some potential limitations to their widespread application such as relatively high costs and low physicochemical stability [3].

The ability of cationic nanoliposomes to improve the antiviral activity of suramin against noroviruses has been investigated [109]. The activity of this antiviral agent is normally limited by its low permeability through cell membranes and poor cell internalization. The authors used a mouse model to show that these hurdles could be overcome by using the nanoliposome delivery systems. In another study, PEGylated nanoliposomes ( $d = 181$  nm) were developed for targeted delivery of

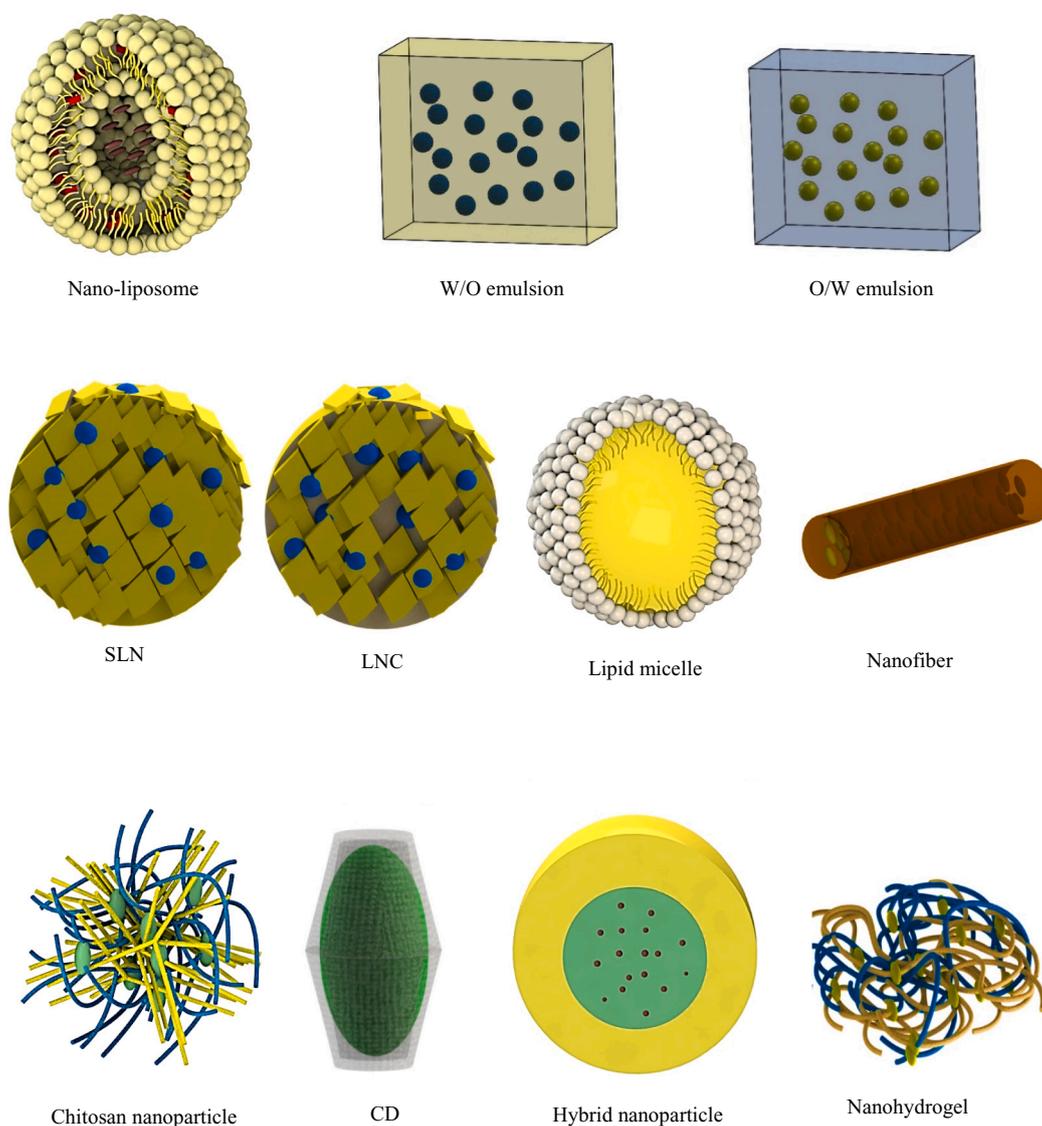


Fig. 3. Different nanoparticle-delivery systems of antivirals.

interferon alpha-2b (IFN  $\alpha$ -2b) to inhibit human papilloma virus (HPV) [81]. A study showed that IFN  $\alpha$ -2b loaded nanoliposomes had better penetration through the sheep vaginal tissue than the free form of IFN  $\alpha$ -2b. When the surfaces of the nanoliposomes was functionalized with PEG, they could more easily penetrate deeper into the epithelium. This phenomenon was attributed to the presence of the neutral PEG molecules, which inhibit the adhesive interactions of the nanoliposomes with mucus, thereby allowing them to pass through more easily. In another study, antibody-coated nanoliposomes ( $d = 100\text{--}230$  nm) were developed to increase the efficacy of an antiviral agent (dapivirine) against HIV [175]. These nanoliposomes could inhibit HIV through two complementary mechanisms: (i) the virus neutralizing effects of the antibodies (Vhhs); (ii) the antiviral activity of the dapivirine.

### 5.3. Nanoemulsions

Nanoemulsions are another kind of colloidal system that has great potential for the encapsulation, protection and delivery of antivirals. Nanoemulsions can be distinguished from conventional emulsions by their relatively small droplet dimensions ( $< 200$  nm) [113]. Unlike microemulsions, they are thermodynamically unstable colloidal dispersions [114]. They are typically formed from oil, water, emulsifier,

and (sometimes) other stabilizers. Nanoemulsions are classified according to the structural organization of the oil (O) and water (W) phases, with the oil-in-water (O/W) and water-in-oil (W/O) types being the most common. However, more sophisticated types can be formulated for specialized applications, including oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) nanoemulsions.

The type of nanoemulsion used for a particular application depends on the nature of the active ingredient to be encapsulated. For instance, water-soluble compounds can be encapsulated inside the water droplets in W/O emulsions, whereas oil-soluble compounds can be encapsulated inside the oil droplets in O/W emulsions. Nanoemulsions are typically formed using mechanical devices known as homogenizers, including sonicators, microfluidizers, and high pressure valve homogenizers [113]. These devices generate intense disruptive forces that intermingle and breakup a mixture of oil, water and emulsifier to form tiny droplets. Nevertheless, nanoemulsions can also be formed without the use of mechanical devices using low-energy methods, such as spontaneous emulsification, emulsion phase inversion, or phase inversion temperature methods [113]. For instance, for the spontaneous emulsification method a mixture of oil and surfactant is titrated into water, which leads to the spontaneous generation of small oil droplets. The selection of an appropriate homogenization method and emulsifier is critical for the

creation of stable nanoemulsions with the required functional performance.

Nanoemulsions can be used to deliver antiviral agents by all major administration routes. Initially, we provide some examples of the development of nanoemulsion-based delivery systems for enhancing the efficacy of topical antiviral formulations. The antiviral activity of coumestrol, a natural compound isolated from soybeans, alfalfa, or red clover, against Herpes Simplex Virus (HSV-1 and HSV-2) has been increased by encapsulating it within nanoemulsions [9]. These formulations consisted of coumestrol-loaded oil droplets dispersed within a hydrogel (hydroxyethylcellulose) matrix. The nanoemulsions used to fabricate these systems were prepared using the spontaneous emulsification method by titrating a mixture of coumestrol, oil, phospholipids, and ethanol into an aqueous solution containing a non-ionic surfactant. Overall, the authors reported their coumestrol-loaded nanoemulsions were effective for the topical treatment of herpes simplex. A similar nanoemulsion-based delivery system was shown to be effective at increasing the antiviral activity of another natural compound (genistein) against herpes after topical application [10]. Encapsulation of curcumin, a natural antiviral agent that operates by various mechanisms, within O/W nanoemulsions has been shown to increase its effectiveness at topical treatment of lesions caused by human papillomavirus (HPV) infection [51]. W/O/W nanoemulsions have also been shown to be effective for the topical administration of an antiviral drug (acyclovir) that is effective against herpes, which was attributed to their ability to increase its permeation into the skin [157].

Nanoemulsions have also been developed for the delivery of antiviral agents through the nasal route. The HIV-1 virus is known to be present within the human central nervous system but is difficult to treat because of the low permeability of anti-HIV drugs through the blood-brain barrier (BBB). To overcome this problem, researchers have encapsulated a hydrophobic antiviral agent (saquinavir mesylate), a protease inhibitor, in O/W nanoemulsions fabricated using the spontaneous emulsification method [107]. The optimized nanoemulsion formulation was shown to increase the permeation of the antiviral agent into sheep nasal mucosa, and to increase the amount reaching the brain after intranasal administration to sheep. This study highlights the potential of nanoemulsion-based delivery systems administered nasally for treating viral infections in the central nervous system.

Nanoemulsion-based delivery systems can also be used to deliver antiviral agents *via* the intravenous route. O/W nanoemulsions have been shown to be effective at increasing the amount of a hydrophobic antiviral agent (Indinavir), another protease inhibitor, reaching the brain when administered intravenously [142]. This effect was attributed to the ability of the nanoemulsions to increase the amount of the antiviral agent that was incorporated into the lipoproteins that carry hydrophobic substances around the bloodstream, as well as because the formulation contained known efflux inhibitors (Tween 80). Nanoemulsions have also been shown to be effective at delivering monoclonal antibodies *via* intravenous injection in a humanized mouse study [134].

Finally, nanoemulsions may also be suitable for the oral delivery of antiviral agents. Encapsulation of efavirenz, a hydrophobic antiviral agent that operates by inhibiting a reverse transcriptase enzyme, within O/W nanoemulsions increased its antiviral activity against HIV [158]. The efavirenz was incorporated into a formulation that included a mixture of surfactants, oils, permeation enhancers, and efflux inhibitors so as to increase its oral bioavailability. After ingestion, an O/W nanoemulsion containing small efavirenz-loaded lipid droplets was spontaneously formed. These droplets would be rapidly digested in the small intestine, thereby releasing the antiviral agent into mixed micelles that could transport it to the epithelium cells where it is absorbed.

#### 5.4. Solid lipid nanoparticle and nanostructured lipid carriers

In many respects, the structure of solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) is similar to that of O/W

nanoemulsions, except that the lipid phase inside the nanoparticles is solid rather than liquid [128,176]. In the case of SLNs, the lipid phase is completely solidified into a highly regular crystalline structure, whereas for NLCs the lipid phase is only partially solidified and has a more irregular crystalline structure. SLNs are therefore typically formed from pure lipids with a well-defined melting point, whereas NLCs are often formed from mixtures of lipids that have a broad melting range. Hydrophobic bioactive components are accommodated within the solidified lipid matrix of SLNs and NLCs. The presence of a solidified lipid phase can slow down molecular diffusion processes, which may inhibit the degradation or release of the bioactive components if designed correctly. However, the highly regular crystalline lipid phase in SLNs can lead to bioactive expulsion or particle aggregation (due to particle morphological changes) [176]. These problems can be overcome using NLCs since the irregular crystalline structure can accommodate more of the bioactive compounds [127,176].

SLNs and NLCs have been used for the encapsulation and delivery of various types of antiviral agent. For instance, SLNs ( $d = 167$  nm) have been used to deliver atazanavir, an antiviral that works as a protease inhibitor, to the brain using a cell culture model of the blood-brain barrier [34]. Encapsulation of the antiviral increased the amount accumulating in the endothelial cells, which may lead to improvements in its ability to tackle HIV-encephalitis. Both SLNs and NLCs have been investigated for their ability to increase the ocular bioavailability of the antiviral agent acyclovir [160]. The NLCs were shown to have a higher encapsulation efficiency and efficacy than the SLNs, which was attributed to the less regular lipid crystalline structure. SLNs have also been developed as topical delivery systems for an antiviral essential oil (*Artemisia arborescens*) [98,99]. The authors showed that the essential oil could be successfully encapsulated within the SLNs. In an *ex-vivo* experiment with pig skin, the authors showed that the nanoemulsions increased the accumulation of the antiviral agent on the surface of the skin, but reduced its penetration into the skin (when compared to a pure oil formulation). They also showed that encapsulation of the antiviral agent did not adversely impact its *in vitro* activity against Herpes Simplex Virus-1 (HSV-1). Taken together, the authors proposed that the SLNs would be a good formulation for antiviral agents intended for dermal applications. Ritonavir has been encapsulated in SLNs and shown to exhibit sustained release properties and antiviral activity using an *in vitro* anti-HIV-1 model [80].

El-Gizawy, El-Maghraby et al. [54] designed a SLN-based system for brain targeting of acyclovir. The SLNs were formulated from a variety of lipids and surfactants, and the nanoparticles were coated with chitosan to make them cationic. The authors showed that the acyclovir-loaded SLNs could deliver the antiviral agent to the brain through the BBB and that they remained in circulation for a longer time than the free form of the drug. This increased efficacy was partly attributed to the ability of the surfactants in the formulation (Pluronic®F68 and Tween 80) to block the action of the P-glycoprotein efflux system, as well as to facilitate their transport across the BBB. The cationic chitosan molecules at the surfaces of the SLNs may have enhanced their transport through the BBB by interaction interacting with anionic domains on the membranes of the brain endothelial cells.

#### 5.5. Biopolymer particles: nanoparticles and nanogels

A wide variety of biopolymer-based nanocarriers can be produced from proteins, polysaccharides, and their complexes [110]. Many of these nanocarriers can be used as antiviral delivery systems. Typically, these colloidal dispersions consist of small particles that contain a network of crosslinked biopolymer molecules. When the particles are predominantly composed of biopolymers they are referred to as nanoparticles, but when they are predominantly composed of solvent they are referred to as nanogels. Biopolymer particles can be fabricated through a variety of bottom-up and top-down methods, including injection-gelation, antisolvent precipitation, emulsion-templating,

thermodynamic incompatibility, molding, fragmentation, and coacervation approaches [117,118]. The composition, dimensions, morphology, and surface characteristics of the biopolymer particles can be modulated by selecting appropriate ingredients and fabrication methods, which enables them to be tailored for specific applications.

Chitosan nanoparticles are one of the most popular biopolymer-based nanoparticles that have been used for targeting delivery of antivirals, because these provide high drug encapsulation efficiency, prolonged-release, and low cytotoxicity. Donalisio, Leone et al. [52] loaded acyclovir in chitosan-based nanoparticles for treatment of HSV-1 and HSV-2. These nanoparticles successfully improved the topical delivery of acyclovir through the skin by increasing its permeability compared to its free form. *In vitro* studies showed higher antiviral activity of acyclovir-loaded nanoparticles against both HSV strains. Russo, Gaglianone et al. [151] showed that chitosan nanoparticles could deliver an antiviral agent (foscarnet) to lung fibroblasts (HELFL) cells infected by the human cytomegalovirus (HCMV). The authors showed that the antiviral activity of foscarnet was enhanced by encapsulation in the chitosan nanoparticles, which was related to the ability of the chitosan to prolong the circulation and enhance the mucoadhesion of the nanoparticles.

The ability of PLGA-TPGS nanoparticles to overcome the barriers associated with the ocular delivery of the antiviral agent acyclovir have been investigated [5]. Here, PLGA (poly(lactic-co-glycolic acid)) is a biodegradable polymer and TPGS (D- $\alpha$ -Tocopherol polyethylene glycol 1000 succinate) is a synthetic surface-active material that releases vitamin E to cell membranes. An ocular irritation study using rabbits showed that the acyclovir-loaded nanoparticles were non-irritant and non-toxic to the eyes. The authors also reported that encapsulation of the antiviral agent increased its ocular bioavailability compared to the free form. In another study, Ayoub, Jasti et al. [12] showed that the antiviral agent entecavir could be encapsulated in PLGA nanoparticles and its release could be controlled by manipulating the formulation. The authors also used an *in vivo* study to show that the bioavailability of the entecavir was increased after encapsulation, which could lead to an effective approach for treating the hepatitis B virus.

Protein-based nanoparticles can also be used to effectively encapsulate and deliver antivirals. Suwannoi, Chomnawang et al. [167] produced acyclovir-loaded bovine serum albumin (BSA) nanoparticles to treat ocular herpes viral infections. This study reported that encapsulation increased the transport of the acyclovir through the multilayers of the corneal epithelial cells, which was related to the small size of the nanoparticles. In another study, Fodor-Kardos, Kiss et al. [61] showed that interferon-beta-1a (IFN- $\beta$ -1a) could be successfully incorporated into biopolymer-based nanoparticles fabricated from BSA and pegylated PLGA polymers. Cyclodextrins (CDs) are a family of cyclic oligosaccharides that have been also used to encapsulate antivirals within their hydrophobic cores [141].

### 5.6. Hybrid nanoparticles

Hybrid nanoparticles can be used to create antiviral delivery systems that combine the beneficial attributes of different individual nanoparticles. For example, hybrid nanoparticles can be formulated by trapping smaller particles inside larger particles, or by forming core-shell structures, which can lead to improved encapsulation, protection, or release properties [32,183].

Alginate and stearic acid-polyethylene glycol (SA-PEG) were used in the formulation of a nanoparticle delivery system for zidovudine [82]. In this system, the zidovudine and alginate were used as core materials, while the SA-PEG was used as a shell. This combination led to the formation of a dendritic structure with internal voids and channels. An *in vitro* study showed that the hybrid nanoparticles were nontoxic, had good blood compatibility, and could deliver the antiviral agent to model brain cells. This type of hybrid nanoparticle may therefore be suitable for tackling HIV infections. In another study, core-shell hybrid

nanoparticles were developed to encapsulate and deliver zidovudine. These nanoparticles consisted of a zidovudine-carboxymethylcellulose (CMC) core surrounded by a glyceryl behenate/polyethylene glycol (PEG) shell. The presence of the lipid (glyceryl behenate) in the shell prevented the burst release of the antiviral agent, thereby resulting in a more sustained release. Moreover, the presence of these lipids also improved the permeability of the nanoparticles through the cell membrane, thereby improving the bioavailability of the zidovudine.

Ramanathan, Jiang et al. [146] investigated the capability of hybrid nanoparticles composed of a hydrogel-core and a lipid-shell for improving the antiviral activity of tenofovir disoproxil fumarate (TDF) and maraviroc (MVC) toward HIV. A sustained release of both MVC and TDF was obtained by incorporation of them within these lipogel nanoparticles. These hybrid nanoparticles were shown to be effective for antiviral delivery *via* the vaginal route using an *in vivo* female mouse model. Cao, Jiang et al. [27] developed core-shell hybrid nanoparticles for targeting the delivery of tipranavir (TPV). These nanoparticles were composed of a lipid shell and a PLGA core, with tipranavir incorporated into the core and  $\alpha$ 4 $\beta$ 7 mAb was attached to the shell. The presence of the  $\alpha$ 4 $\beta$ 7 mAb increased the *in vivo* biodistribution of nanoparticles to mouse small intestines, as well as their ability to attach to gut-homing T cells, which should improve their antiviral activity.

## 6. Conclusion

Nano-enabled delivery systems can be designed to enhance the efficacy of many antiviral agents by overcoming physicochemical and biological barriers, such as low solubility, poor stability, matrix interactions, low bioavailability, untargeted release, undesirable side effects, and development of antiviral resistance. Many kinds of delivery systems are available for this purpose, such as micelles, microemulsions, nanoliposomes, nanoemulsions, solid lipid nanoparticles, biopolymer nanoparticles, and biopolymer nanogels. Each of these delivery systems has its set of advantages and disadvantages for certain applications. It is therefore important to select the most appropriate one and then optimize its formulation. At present, many researchers do not try to rationally identify the most appropriate delivery system – they simply select one type of delivery system they are familiar with and then investigate its potential. There is currently a lack of systematic studies that compare different kinds of delivery systems for a particular application to identify the most suitable one. This area would certainly benefit from the creation of standardized methods to systematically test antiviral agents against specific viruses using different kinds of delivery systems. These methods could then be used to compare the relative merits of different systems.

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