

Future prospective of nanophytovirology: A review

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Abstract

Plant viral diseases are threatening for sustainable agriculture and cause financial ruin for farmers. In recent scenario advanced nanoparticles are the best tool for the management of plant viral diseases in the field of agronomy. The application of nanotechnology for management of plant viral disease are called nanophytovirology. These nanoparticles are managing plant diseases by variety of ways like directly inhibiting viral replication, antiviral drug delivery agent, increase the viral disease resistance of plants and deactivating viruses. In this way nanoparticles offer a versatile approach for controlling viral infections, from early detection to treatment and prevention. Through antiviral assays researchers prove that different nanoparticles have shown excellent virucidal properties against various plant viruses. The present review discusses about a new field of nanophytovirology which focusing on the use of nanotechnology to manage not only detection of plant viral diseases but also its control.

Keywords: Nanophytovirology, sustainable agriculture, financial ruin, inhibiting viral replication, antiviral drug delivery agent, versatile approach

Introduction

Viruses are obligate intracellular parasites and multiply by using host machinery and ultimately cause many diseases in the plant. A global survey shows that approximately 50% of plant diseases and 20% of total yield losses are caused by 900 species of viruses (Gaur *et al.* 2011) ^[58] which often cause food security problems. Some important plant disease caused by viruses are tobacco mosaic disease, tomato yellow leaf curl, maize dwarf mosaic disease, leaf roll disease of potato, leaf curl of papaya, yellow vein mosaic disease of okra, cauliflower mosaic disease, etc. Due to increasing human population the demand of plant-based products is also increasing. Hence, the effective approaches are needed to control the plant viral diseases and their transmission supporter vectors i.e. insects, nematodes, fungi etc. For implementation of correct control procedure of viral diseases, virologists need complete knowledge about viral infection and their effects on host plants (McGee *et al.* 2017) ^[51] because of specificity of viruses varies greatly (Marwal *et al.* 2018) ^[4]. Some of them can colonize different species and some are specific or interact to specific cell machinery. It is necessary to focus on reduction in crop loss by controlling movement of viral pathogen from which infected to healthy plants rather than treating the infected plant (Sanfaçon, 2017) ^[69]. For beneficial of agriculture sectors, pioneers done development of nanoparticles which affects the viral pathogen in similar way as chemical pesticides but at very low concentration (Arts *et al.*, 2014, Das *et al.* 2014 and Marwal *et al.* 2018) ^[4, 12, 56]. Nano based diagnostic kit is only used as a carrier of active ingredients of pesticides but also increase the speed and power of detection of viral pathogen (San Miguel *et al.* 2016) ^[71].

Nanophytovirology is a leading age science which supports nanotechnology in detection and management of plant viral diseases at an initial phase and helping in plant protection from the epidemic diseases (Prajapat *et al.* 2013)^[61]. The present study mainly focuses and centric towards the various aspects of nanoparticles in plant virology which is called nanophytovirology.

Characteristics features of nanoparticles

Nanoparticles greatest advantage lies in their small size, greater surface area and strong reactivity: such efficient activity favours for vast application in plant (Das *et al.* 2014)^[12]. Nanoparticles are small solid, colloidal, polymeric particles with diameter lower than 1 μm . Since the size of nanoparticles are very small, they can be applicated intravenously.

The structure of nanoparticles is similar to microspheres and can be matrix-like (nanoparticles) and capsule-like (nanocapsules). For creation of nanoparticles different materials like inorganic materials, proteins, lipids etc. are used.

- Polymeric nanoparticles are mostly made of natural or synthetic biocompatible and biodegradable polymers such as poly (lactic acid), poly ((lactic-co-glycolic acid), cyclodextrin, alginate, hyaluronic acid, poly (glycolic acid), and polycaprolactone. These polymers used for maximizes tissue compatibility and minimise cytotoxic effects. In nanoparticles, the active molecules can be dissolved, encapsulated, adsorbed or conjugated. As it was described for liposomes, the phagocytosis of nanoparticles can be avoided by the addition of hydrophilic components, like PEG chains, on their surface. These PEGylated nanoparticles are often labeled as stealth nanoparticles. The different polymeric nanoparticles (made from polylactide, poly (isobutyl cyanoacrylate), poly (ethylacrylate, methacrylate, and chlorotrimethyl methacrylate)) with incorporated acyclovir increased the activity and oral bioavailability of acyclovir.
- Lipid nanoparticles, made from lipids that are solid at body temperature can also be used for the delivery of therapeutic agents. There are two types of lipid nanoparticles: Solid lipid nanoparticles and nanostructured lipid carriers. Solid lipid nanoparticles are in stable solid state, have the ability to protect the ingredients with labile chemical properties, and can modulate the release of the drug. Nanostructured lipid carriers contain the solid lipid matrix and partly a liquid lipid phase content. Lipid nanoparticles have an advantage in comparison with liposomes and conventional emulsions due to their ability to overcome the problems of stability of the membrane that is associated with drug leaching. Lipid nanoparticles containing acyclovir *in vitro* showed greater activity than the free drug. Also, lipid nanoparticles with incorporated adefovir dipivoxil has been prepared for the therapy of hepatitis B infection.
- Protein nanoparticles prepared from albumin polymers have also been developed as delivery system for ganciclovir and for antisense oligonucleotides for therapy of Cytomegalovirus infections. Cationic gelatin nanoparticles increased the immunostimulatory effects of CpG oligonucleotides.

Forms of nanoparticles

Eminent scientists have synthesized nanoparticles in various forms i.e. quantum dots, metalloids, metallic oxides, nonmetals, carbon nanomaterials (Das *et al.* 2015)^[16], dendrimers, liposomes (Das *et al.* 2015)^[16], Virus-based

nanoparticles (VNPs) (Das *et al.* 2015 and Guenther *et al.* 2018) [16, 22].

Synthesis of nanoparticles

Nanoparticles can be synthesized either by chemical route or by green synthesis method taking in account the top down or bottom-up approach, whichever better feasible (Das *et al.* 2014) [12]. This can be further categorized into chemical, reduction, microemulsion, colloidal, sonochemical, electrochemical, microwave, solvothermal and microbial synthesis of nanoparticles (Das *et al.* 2016)

[17].

Primeval of nanophytovirology

Earlier so many techniques have been given by researchers for detection of virus and their carriers vectors in the host plant (crops, ornamental plants, weeds). These techniques for recognition and detection of viruses and carrier vectors, participate for a decisive role in virus disease management (Prajapat, 2013) [61]. Some most popular techniques are listed in Table 1.

Table 1: Some common techniques used during primeval of nanotechnology for plant virus and their carrier vector detection

S.No.	Name of Techniques	References
1.	Electron microscopy	Marwal <i>et al.</i> 2013 [60]
2.	Symptom determination	Marwal <i>et al.</i> 2013 [60] and Prajapat 2014 [63]
3.	Biotest	Marwal <i>et al.</i> 2013 [60], and Marwal <i>et al.</i> 2014 [54]
4.	Mechanical transmission	Sahu <i>et al.</i> 2015 [68]
5.	Seed transmission	Sahu <i>et al.</i> 2014 [67]
6.	Serological technique (enzyme-linked immunosorbent assay, phage display, tissue blot immunoassay (TIBA), lateral flow devices, immunocapture transmission electron microscopy (ICTEM))	Marwal <i>et al.</i> 2014 [54] and Nehra <i>et al.</i> 2019 [57]
7.	Restriction fragment length polymorphism (RFLP)	Chen and Adams 2001
8.	Thermostable amplification-based methods (PCR and reverse transcription-polymerase chain reaction (RT-PCR), multiplex PCR/RT-PCR, immunocapture PCR (IC-PCR), immuno-precipitation PCR (IP-PCR), nested PCR, multiplex nested PCR, real time PCR (qPCR), multiplex real time PCR, Co-operational PCR (Co-PCR))	Marwal <i>et al.</i> 2013 [60], Nehra <i>et al.</i> 2014 [54], Zhang <i>et al.</i> 2014 [77] and Nehra <i>et al.</i> 2018 [56]
9.	Isothermal amplification-based methods (helicase dependent amplification (HDA), recombinase polymerase amplification (RPA), nucleic acid sequence base amplification (NASBA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA))	Lamprey <i>et al.</i> 2013 [28] and Marwal <i>et al.</i> 2013 [60]
10.	Nucleic acid sequence hybridization techniques (in situ hybridization, microarray, lateral flow microarrays)	Elbeaino <i>et al.</i> 2014 [18] and Marwal <i>et al.</i> 2018 [50]
11.	Next Generation Sequencing (NGS), recombinant DNA approach	Boonham <i>et al.</i> 2008 [5]

Accessibility of the few above-mentioned diagnostic methods endow with a superior elasticity, increased sensitivity, and specificity for quick judgment of virus diseases. The accurate and reliable detection of the associated virus pathogens therefore forms the first line of defense in management of these diseases.

Management of plant viral diseases

A number of methods has been devised for plant viral disease management (Makkouk 1978, Prajapat *et al.* 2011, Marwal *et al.* 2016 and Marwal *et al.* 2018) ^[30, 58, 41, 46].

- Destruction/killing of arthropod vectors like grasshopper, either via biological control or with traditional methods. This can help in reducing the viral populations. *Scymnus offmanni*, *Coccinella septempunctata*, *Propylaea japonica*, *Euseius cutalis*, etc. are some natural predators of whiteflies.
- Chemiecolological technique employs honeydews excreta of whitefly, which work as a kairomone to attract natural prey, i.e., *Encarsia formosa*. Such approach has been successfully used in the Mediterranean regions against whiteflies vector.
- Plant age is also crucial in vector population controlling.
- Field trial has been successful where young plants were covered with plastic bag (yellow polyethylene film) or grown under green house until maturing phase, limits the contact with arthropods vectors.
- Similarly masking the crop with living ground covers of perennial peanuts, cinquillo and coriander plants condensed the impact of incoming whitefly adults.
- An old age practice helps in diminishing the virus populations by destroying the weeds growing in the near vicinity of the crop fields, or even sowing the seeds a little later when the vector populations flourishes. Both help in viral disease management (Kayser *et al.* 1994) ^[26].
- The use of insecticides against various arthropod vectors is also helpful to a certain extent. Few of them are *Neonicotinoids*, *Buprofezin*, *Thiamethoxam* 70 WS, *Imidacloprid* 600 FS, *Imidacloprid* 70WS and *Carbosulfan* 25 DS, *Triazophos*, *Ethion*, *Imidacloprid*, *Acephate* 95 SG, *Spirotetramat*, *Diafenthiuron*, *Nitroguanidines*, *Thiamethoxam*, *Ryanodine* and *Pymetrozine*, quite lethal against whiteflies (Sanjaya *et al.* 2005) ^[70].
- Two remarkable technologies have come up as one the best solution against plant viruses, i.e., interference RNA (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR).
- RNAi-mediated virus resistance was reported against potato virus Y (PVY) in transgenic tobacco plants, against African cassava mosaic virus (ACMV), croton yellow vein mosaic virus (CYVMV) and many more (Marwal *et al.* 2012 and Sahu *et al.* 2014) ^[59, 67]. RNAi is usually associated with methylation of nuclear DNA corresponding to the transcribed region of the target RNA despite transcription levels of the transgene remains unaffected (Ali *et al.* 2015) ^[1].
- CRISPR-Cas (CRISPR Associated Systems) is an adaptive immune system in many archaea and bacteria that cleaves foreign DNA based on sequence complementarity. Virus based guide RNA (gRNA) delivery system for CRISPR/Cas9 mediated plant genome editing cause mutations in target genome locations and resulted in transgenic plants showing resistance against viruses (Worrall *et al.* 2018) ^[76].

Epoch of nanophytovirology

Plant diseases caused by viruses are the most difficult to manage (Khurana and Marwal 2016) ^[27], because they spread through vectors. Nanotechnological-based disease diagnosis and management for virus infecting crop plants is attaining magnitude with the increased spread of viruses and threats of their epidemics (Marwal and Gaur 2017) ^[44]. Therefore, there is a demand for an improved management of viruses employed by a series of strategies (Vardhan *et al.* 2012 and Nehra *et al.* 2016) ^[75, 55]. In-fact such practices relied on the ecology of the virus. Many approaches have been used to decrease crop losses due to viruses, only a few are effective in their management. Even understanding the plant mediated interactions between viruses and their carrier vector is quite important to tackle epidemiology of viral diseases (Vardhan *et al.* 2012 and Nehra *et al.* 2016) ^[75, 55]. Developments in nanofabrication and nanotechnology endow a crucial part in plant viral disease detection, simplicity in handling and are cost-effective as compared to other plant viral diagnostic methods.

Mode of action of nanoparticles against viral pathogen in plants

Several studies showed antiviral potential of the silver nanoparticles. Ag⁺ ions released from silver nanoparticles interact directly with biomolecules that contain phosphorus or sulfur, including proteins, DNAs, and RNAs. It seems that silver nanoparticles interfere with several stages of the viral replication cycle including the attachment of the viruses to the cell membrane and their entry into the cells, DNA and RNA replication, and protein synthesis. Some common actions are:

Direct Antiviral Action

Inhibition of viral replication: Nanoparticles can interfere with the virus's ability to replicate within the host plant cells, preventing the spread of infection. For example, silver nanoparticles are known for their antiviral and antimicrobial properties, and can be used to inhibit viral replication and protect plants from infection.

- **Disruption of viral particles:** Some nanoparticles, like silver and gold nanoparticles, can directly damage or inhibit the structure of viral particles, rendering them ineffective.

Delivery of Antiviral Agents

- **Nanocarriers for pesticides:** Nanoparticles can be used to encapsulate and deliver pesticides or other antiviral compounds directly to the site of infection, increasing their effectiveness and reducing off-target effects.
- **Gene delivery:** Nanoparticles can be engineered to deliver genetic material (e.g., RNAi) into plant cells to silence viral genes, preventing viral protein synthesis.

Enhancing Plant Defense Mechanisms

- **Activating plant defense genes:** Nanoparticles can stimulate the plant's natural defense mechanisms, triggering the production of proteins and other compounds that fight off viral infections. For example, chitosan nanoparticles can be used to induce plant defense responses.
- **Improving plant health:** Nanoparticles can enhance overall plant health, making them more resistant to viral infections and other stresses.

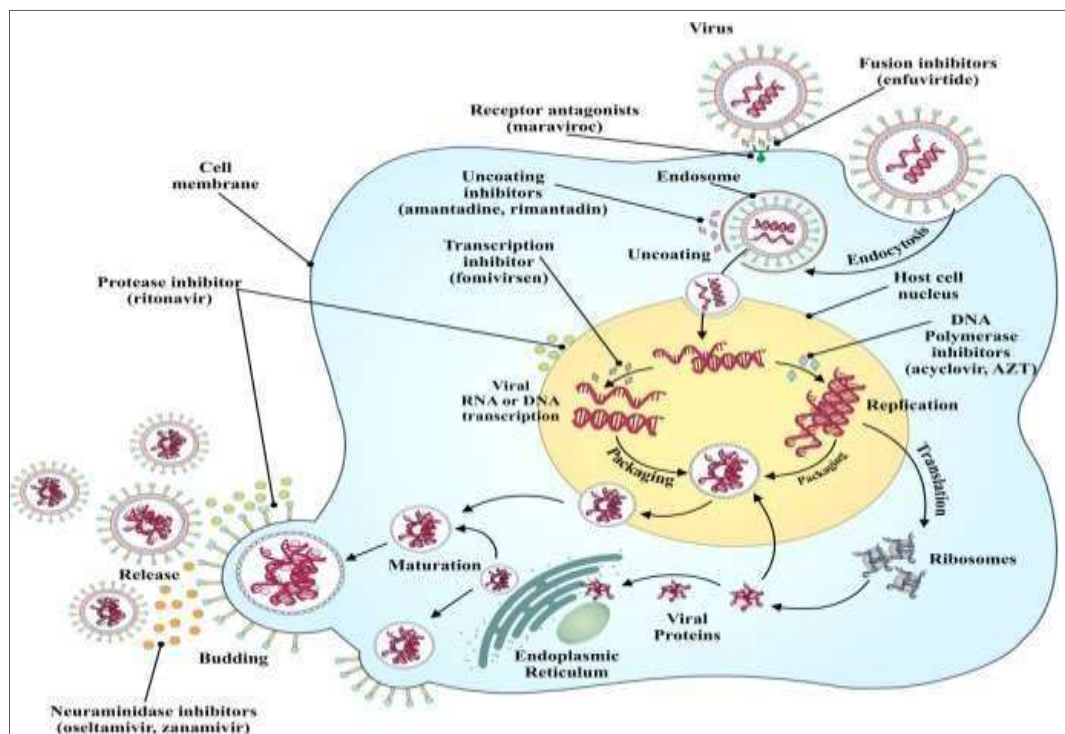


Fig 1: Mode of action of nanoparticles against viral pathogen in plants

Thus, advanced nanoparticles offer a powerful and versatile tool for managing plant viral diseases. Their ability to directly target viruses, deliver antiviral agents, and enhance plant defenses makes them a promising area of research and development for sustainable agriculture.

Diagnosis of viral particles by nanophytovirology

Some methods of diagnosis of viral particles by nanophytovirology are listed in Table- 2

Table 2: Diagnosis of viral particles by nanophytovirology

S. No.	Name of Method	Procedure	References
1.	By using Microcantilevers	They are in the micrometer range, but their tip end is in the nanometer scale and is widely used for various biosensing applications. Microcantilevers works in two different modes, i.e., straining and resonating mode, both are helpful in identifying viruses. Resonating mode is like QCM, whereas straining mode relies on the changes in electrical resistance whenever a virus particle bound to the surface. The major limitation is the low performance of device in the liquid medium, hence the sample need drying before application. For virus particle detection nanowire employed transistors have been devised. An immuno-biosensor was developed for the detection of Plum pox virus in plum (<i>Prunus domestica</i>) and tobacco (<i>Nicotiana benthamiana</i>) leaves sap, where gold electrodes were modified with 1,6- hexanedithiol, gold nanoparticles, anti-PPV IgG polyclonal antibody and BSA. Nanowires can be engaged against the target virus, and when encountering a charged virus capsid, a depletion or gain of charge in the nanowires is thus recorded as a simple conductance change. In a similar instance a	Hsyden <i>et al.</i> 2003

		lithographically patterned nanowire electrodeposition (LPNE) technique was used to develop a label-free chemiresistive sensors based on a polypyrrole (PPy) nanoribbon conjugated with anti-viral antibodies against cucumber mosaic virus (CMV)	
2.	By using biosensor	In yet another instance nano-biosensor was developed against plasmodio- phoromycete <i>Polymyxa betae</i> which is responsible for the carrier of beet necrotic yellow vein virus (BNYVV) and caused the deadly disease rhizomania in sugarcane plants.	Vardhan <i>et al.</i> 2012 ^[75]
3.	By preparation of pesticides and chemical fertilizers by using nanoparticles and nanocapsules	Nanotechnology benefits agriculture sectors and diminish environmental pollution. This is carried out by manufacturing of pesticides and chemical fertilizers using nanoparticles and nano-capsules and has the capability to control or delayed the delivery and absorption of pesticides and chemical fertilizers with lower dose. "Nano-5" is a marketed product pesticide to control several plant viruses. It was found effective at dilution of 1:500 against Mosaic, ringspot, transitory yellowing, tristeza virus, exocortis viroid by spraying "Nano-5" onto the surface of leaves and apply to the roots once every 3 days. It was reported that chitosan nanoparticles have the ability to induce resistance in host crops against few viruses, for example mosaic virus of alfalfa, snuff, peanut, potato, and cucumber were targeted. Similarly, gold nanoparticles showed antiviral effects against Bean mild mosaic virus in beans, barley yellow mosaic virus in barley and tobacco necrosis virus in tobacco plants. Peoples also claimed silver nanoparticles application made the host plant resistance against sun-hemp rosette virus (infecting bean plants) and bean yellow mosaic virus (causing disease in faba bean crops)	Chartuprayo <i>o n et al.</i> 2013 ^[6]
4.	By using specific antibodies	Specific antibodies against conjugated with Cadmium-Telluride QDs against the glutathione-S-transferase protein's (GST). The developed nano-biosensor showed enough fluorescence resonance energy transfer (FRET) to detect the plasmodiophoromycete <i>Polymyxa betae</i> . Cadmium-Telluride QDs conjugated to antibodies were also developed against citrus tristeza virus (CTV) using the similar approach.	Marwal <i>et al.</i> 2014 ^[54]
5.	RNAi technique	This technique also employed in coupling with nanotechnology, a remarkable study was carried out to show resistance against cucumber mosaic virus (CMV) and pepper mild mottle virus (PMMoV). In this wonderful approach dsRNA was loaded onto LDH (Layered double hydroxides) nanoparticles, called BioClay and were sprayed on the challenged plants. Plants showed resistance against the above-mentioned viruses for 20 days as compared to controlled ones.	Elbeshehy <i>et al.</i> 2015 ^[19]
6.	By using nanoparticles	Earlier fluorescent dyes were used straight away for staining the viruses, now nanoparticles and quantum dots (QDs) have been developed which helps in carrying the detection tags (dyes or anti-viral antibodies) and are quite efficient in	Nehra <i>et al.</i> 2016 ^[55]
	and quantum dots	identifying the viruses, which are also helpful as labeling and imaging agents. Such fluorescent tags are easily detected in flow cytometry enabled devices.	

7.	By using surface phenomenon resonance (SPR)	This is also an optical based technique which employs diagnosis of viruses by change in refractive index on a metal surface. In this gold nanoparticles are conjugated with anti-viral antibodies adsorbed on a glass substrate and are sensed by SPR. Quartz-crystal microbalance (QCM) is a well-known, commercially accessible mass sensor technique generally employed in quantitative measurement of the thickness of thin films. The principal is that the exterior part of the quartz-crystal device (microchip: dimensions in nanometer) is typically coated with anti-viral antibodies against the targeted plant viruses. Now when a virus is encountered on the quartz-crystal surface there results an increase in the mass, thereby resonant frequency decreases, the change in the frequency before and after of the chip is measured subsequently. SPR and QCM based nano-biosensor has been manufactured successfully for the detection of orchid viruses, tobacco mosaic virus (TMV), cymbidium mosaic virus (CymMV), odontoglossum ringspot virus (ORSV), etc.	Shojaei <i>et al.</i> 2016 ^[72]
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It is clear from the above discussion that “nanophytopathology” represent an attractive advancement, owing to their potential advantages for the plant disease management against deadly crop viruses.

Challenges for applying nanophytopathology

The application of nanoparticles, particularly in plant disease management, need specific structural and physicochemical features, and any slight variations to their planned properties can hinder the function and performance of designed nanoparticle conjugate (Mitter *et al.* 2017) ^[53]. Hence, numerous factors related to nanoparticle synthesis are quite important for the development of an effective virus detection assay. Further, the methods employed in making/synthesizing nanoparticles seem easy and quick for large scale production but getting the final product in uniformity (shape and size) remains challenging. Nanoparticles itself cannot detect the viruses solely and thus need additional biomolecule specific in sensing the pathogen. In comparison to nanoparticles, biomolecules are quite delicate to severe chemical and physical alterations (high temperature, high salt concentrations, reducing agents) which might can harmfully affect their reactivity and specificity (Spicer *et al.* 2018) ^[73]. Therefore, such procedures demand for proper optimization steps aimed at in detecting viruses.

But when employing/using nanoparticles and its conjugates for the application in virus disease management, their biosafety and toxicity on human health and environment is yet another a major challenge. For example, nano-pesticides might get inhaled by the workers during treatment process. Similarly, other nano-composites might get deposit on the leaves or flowers, can affect animals, birds, honey bees, etc. They may clog the stomatal pores and might hinder the penetration of pollen grains on stigma. If get inside the plant system, nanoparticle might affect plant metabolism and can cause similar effect on humans as well. Cellular toxicity can be induced by nanoparticles (NPs) that lead to toxic side effects such as enhanced ROS generation, disruption of redox homeostasis, lipid peroxidation, impaired mitochondrial function, and membrane damage. Due to their long persistence and greater reactivity nano-pesticides may contaminate water and soil system (Jazayeri *et al.* 2016) ^[25]. Regardless of these developments in nanotechnology, there are some unsolved problems concerning the detection of many plant viruses due to their low titer in the plants, their uneven distribution, the existence of latent infection and lack of validated sampling protocols.

Conclusion

It is expected that usage of innovative nanomedicine will have the great positive effects in the treatment and eradication of infectious diseases. Nanoparticles could improve the efficacy of antiviral drugs and reduce the adverse side effects of the drug. These characteristics of nanoparticles are relevant in antiviral therapy in which the high dose of a drug is needed and the drugs are often very expensive. Usage of nanoparticulate carriers can reduce the frequency of the drug intake and the time of treatments, enhance the effectiveness of the approved antiviral therapeutics and overcome their limitations, such as low bioavailability. The main objectives of the future research of antiviral therapy will be the finding the new technologies for the characterization of nanomaterials, the development of highly biocompatible and biodegradable nanocarrier systems that have no cytotoxicity, the development of nanocarriers that effectively target specific sites of viral infection and thus have reduced drug toxicity in other tissues, and the design of nanomolecules that, besides their role in delivery, possess the intrinsic antiviral therapeutic characteristics (dendrimers and metal nanoparticles).

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