











Chitosan-drug encapsulation as a potential candidate for COVID-19 drug delivery systems: A review

Onome Ejeromedoghene ^{a1*}  , Olayinka Oderinde ^{a1*}  , George Egejuru ^b  , Sheriff Adewuyi ^{c*}  

^a School of Chemistry and Chemical Engineering, Southeast University, Jiangning District, Nanjing, Jiangsu Province, 211189, PR China.

^b School of Public Health, Southeast University, Jiangning District, Nanjing, Jiangsu Province, 211189, PR China.

^c Department of Chemistry, College of Physical Sciences, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria.

¹ Authors OE and OO contributed equally to this work and should be regarded as the first author.

Abstract: Since the outbreak of COVID-19, the World Health Organization (WHO), Centre for Disease Control (CDC), and other health organizations around the world have coordinated the flow of information and given out preventive directives measures and guidelines to reduce the impact and spread of the disease. Meanwhile, bodies of scientists and researchers around the world are still working ceaselessly to study the virus, mode of transmission mechanisms, and are rapidly developing therapeutic antiviral drugs and vaccines. Thus, the urgent need for the fabrication of biocompatible and biodegradable composite materials as drug delivery vehicles for the efficient loading, targeted delivery, and controlled release of antiviral drugs to the target site is being inspired. Therefore, this review highlights the antimicrobial and antiviral activities of chitosan as well as the potency of a combined therapy via electrostatic/hydrogen bonding encapsulation onto the WHO suggested clinical trial drugs and possible chelation with metal ions to form new improved antiviral compounds as promising agents for targeted drug delivery.

Keywords: COVID-19, Biopolymeric chitosan, Antiviral agent, Drug delivery, Biocompatible, Antibiotics.

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Corresponding author. E-mail: oejeromedoghene@seu.edu.cn, Tel.: +86-18851663211.

INTRODUCTION

Since the outbreak of the novel coronavirus disease (COVID-19) which was first identified in December 2019 in China, the World Health Organization (WHO) has considered it as a global epidemic due to its current spread across different countries around the world (1,2). This infectious and deadly disease, formally referred to as Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-

CoV-2), is caused by a new virus possessing spherical particles resembling crown-shape and bearing proteins called spikes (see Figure 1), protruding from their surfaces that can cling and replicate on the host's cells, hence the general name, coronaviruses (3). The origin of COVID-19 is similar to SARS, also caused by a coronavirus (CoV) that occurred in the seafood and wet animal wholesale market in Wuhan, Hubei Province, China (4). The virus can easily be transmitted by close

contact between two or more people (via touching, handshake, and hugging), and aerosol transmission (i.e. respiratory droplets from the cough or sneezing of an infected person) (5,6). The clinical manifestation of the disease reported so far is a respiratory involvement, which is linked to mild flu-like illness, potential lethal acute respiratory distress syndrome, or fulminant pneumonia (7). However, other symptoms such as a cough, fever, sore throat, and in more severe cases, difficulty in breathing have also been reported to be attributed with early signs of the contraction of the disease which have an incubation period of about 5 to 14 days (see Figure 2) (8). Although the clinical manifestations of COVID-19 are subject to frequent changes, they include an asymptomatic carrier, ARD, and pneumonia of varying degrees of severity. However, the first asymptomatic cases were diagnosed based on positive viral nucleic acid test results, without any early COVID-19 symptoms, such as fever, gastrointestinal and respiratory symptoms, as well as no significant abnormalities on pulmonary radiograph (9,10).

The widespread of the disease with a corresponding daily increase in the number of confirmed cases and deaths globally have inspired the urgent quest for antiviral drugs to curb the menace. Several attempts are being made to treat and reduce severe infections. Chloroquine and other antiviral drugs which have been used globally for decades as an antimalarial drug and treatment of previous cases of viral infection respectively, and are enlisted as part of the WHO

model list of potential medicines, have been suggested as potential candidates against the spread of the disease, however, their efficacy and safety concerns remain unclear (11,12). Also, the virtual screening and repurposing of some drugs such as Chromocarb, Ribavirin, Telbivudine, Vitamin B12, etc. approved by the Food and Drug Administrations (FDA), show a high docking score and high ligand efficiency and can be used to inhibit the COVID-19 main protease (13,14). Cortegiani et al. carried out a study using PubMed, EMBASE, and three trial registries to identify studies on the use of chloroquine in patients with COVID-19. The authors were able to unravel one narrative letter, one *in-vitro* study, one editorial, expert consensus paper, two national guideline documents, and 23 ongoing clinical trials. Early investigations inferred that chloroquine derivatives seem to be effective in limiting the replication of SARS-CoV-2 (the virus causing COVID-19) *in vitro* (15). Some other studies have proposed the efficacy of a single drug or combined drug therapy of Remdesivir, Lopinavir /Ritonavir, Favipiravir since they can interfere with the synthesis of viral mRNA targeting RdRp (16,17).

Experimental research is currently in top gear on the use of various antibiotics and antiviral agents, especially in combined therapy as a possible vaccine for COVID-19 (18). Herein, we project the potency of the combined therapy of encapsulating the clinical trial drugs with biocompatible and biodegradable chitosan (a biopolymeric cationic molecule with free OH and NH groups) as a potential drug delivery agent for COVID-19.

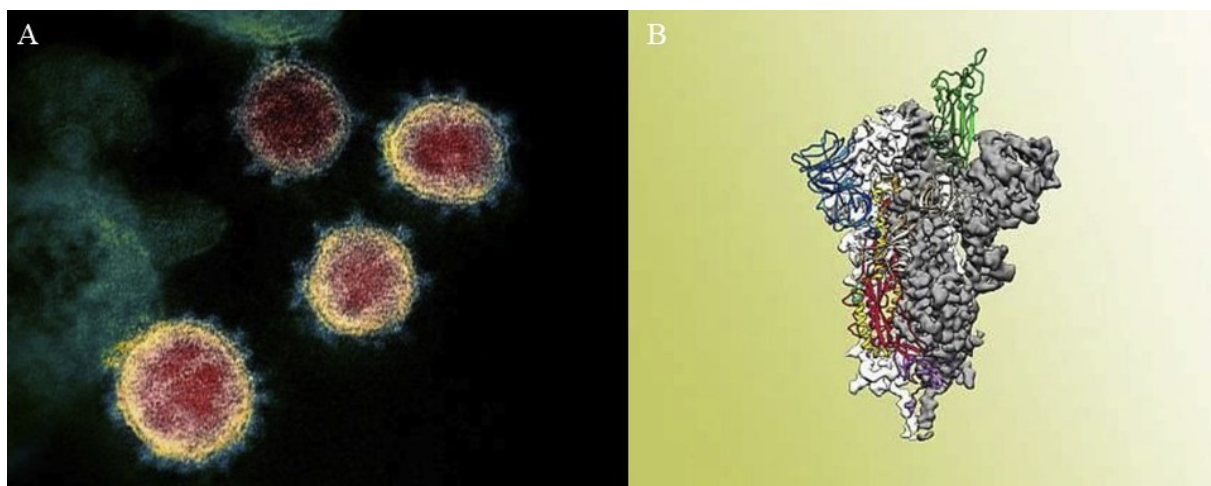


Figure 1. (A) The spikes on the outer edge of the virus particles give coronaviruses their name, crown-like, TEM image (B) Atomic-level structure of the SARS-CoV-2 spike protein. Reproduced with permission from Ref. (3). Copyright 2020 IMSS (Elsevier Inc.).

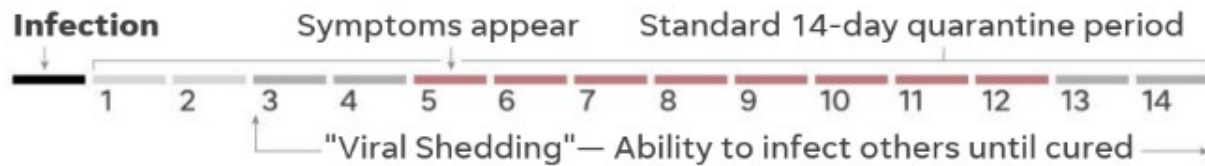


Figure 2. Incubation period of COVID-19 after an initial period of exposure to the virus to quarantine period. Reproduced from Ref. (8). Copyright USA Today.

MODE OF ACTION OF COVID-19

Coronaviruses (SARS-CoV) are single-stranded RNA viruses belonging to the family Coronaviridae, which can cause various diseases with enteric, respiratory, hepatic, and neurological symptoms with mild to serious infections in birds and mammals (19). Under an electron microscope, the coronaviruses were observed to be protruding to the periphery with a diameter of 60-160 nm. Each particle is enveloped containing a single-stranded positive-sense RNA (+ssRNA) genome of 27-32 kb with 5'-cap structure and 3'-poly A tail which interacts with the nucleoprotein (20). Thus, when the virus finds its way into the host, it infects the cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of the cell through its envelope-spike glycoprotein domain (21-23).

The entry of SARS-CoV into cells was initially identified to be accomplished by direct membrane fusion between the virus and plasma membrane. Therefore, once inside the cells, the viral RNA genome is released into the cytoplasm and is

translated into two polyproteins and structural proteins, after which the viral genome begins to replicate (see Figure 3) (24). The newly formed envelope glycoproteins are inserted into the membrane of the endoplasmic reticulum or Golgi, and the nucleocapsid is formed by the combination of genomic RNA and nucleocapsid protein. Then, viral particles germinate into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) (25). Eventually, the vesicles containing the virus particles fuse with the plasma membrane to release the virus (23).

Presently, nucleic acid testing and computed tomography (CT) scans are being used to diagnose and screen possible COVID-19 infected patients, with a number of reverse transcription polymerase chain reaction (RT-PCR) kits been designed to detect SARS-CoV-2 based on genes, with CT scans which involves many X-ray measurements taken at different angles at infected person's pulmonary region, are both used for accurate diagnoses as they can target as well as identify the specific viral agent (26-29).

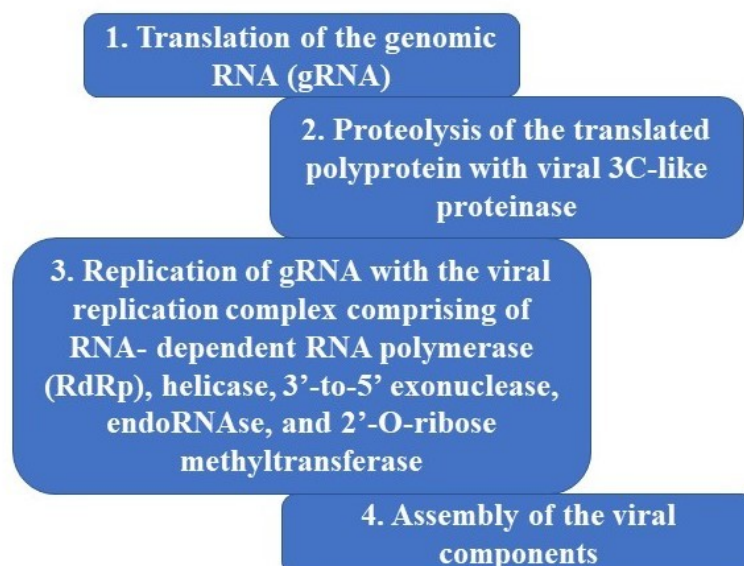


Figure 3. Replication processes involved in the life cycle of the coronavirus after entering the host cell. Adapted from Ref. (30,31) Copyright 2020 CC-BY-NC-ND 4.0 International license and 2015 Elsevier B.V.

ANTIVIRAL AGENTS FOR COVID-19

Chloroquine (see Figure 4a), also known as Resochin is a weak base that was first extracted from the bark of cinchona tree which had been used to treat fever and malaria. This compound, which is an amine acidotropic form of quinine is a lysosomotropic agent with rapid rate of absorption and distribution in bodily tissues; its metabolism is partially hepatic, giving rise to its main metabolite, desethylchloroquine and easily excreted in urine (32). Hydroxychloroquine (see Figure 4b) is the main derivative of chloroquine which differs by the presence of a hydroxyl group at the end of the side chain: the N-ethyl substituent is β -hydroxylated (33). Over the years, chloroquine and its derivatives have been used to treat autoimmune diseases, such as amebiasis that is occurring outside the intestines, rheumatoid arthritis, and systemic lupus erythematosus (34). More so, the sulfate and phosphate salts (see Figure 4c) of chloroquine have both been commercialized as antimalarial drugs (35). For example, Kashyap et al. formulated dextran nanoparticles bearing chloroquine diphosphate by solvent diffusion method yielding particle size below 70 nm with zeta-potential of -20.1 ± 3.2 mV. This was successfully used for drug delivery and overwhelmed drug resistance in *Plasmodium falciparum* parasites (36).

Studies have shown that chloroquine or its derivatives possess a broad spectrum of antiviral effects as potent inhibitors on a variety of viruses including the Ebola virus, Zika virus, and SARS-CoV-1, etc. (37–39). They are deployed as an antiviral drug because it can affect virus infection

in many ways depending on the part and extent to which the virus utilizes endosomes for entry (37). Thus, they can inhibit the *in vitro* replication of viruses which envelope and fuses with that of the acidified endosome (40), since the structure and mechanism of action is via acting as a weak base that can change the pH of acidic intracellular organelles including endosomes/lysosomes, essential for the membrane fusion (41,42). Additionally, since SARS-CoV-2 utilizes the similar surface receptor ACE2, it is believed that chloroquine can also interfere with ACE2 receptor glycosylation and prevents SARS-CoV-2 attachment to the target cells (43,44) The clinical safety profile of hydroxychloroquine is better compared with that of chloroquine (during long-term use) and allows higher daily dose with fewer concerns regarding drug-drug interactions (45). It also shows a high partitioning in tissue, including lung and brain, thus, offering a key clinical advantage in the case of COVID-19 (46).

Preliminary results from a small trial suggest that a combination of hydroxychloroquine and azithromycin is efficacious for reducing the viral load in patients with COVID-19 as tested on a 72-year-old woman admitted to a non-telemetry floor with a cohort of other COVID-19 patients (47). Meanwhile, Gautret et al. iterated that hydroxychloroquine treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin after an extensive study on 22 cases in France (48). Apart from chloroquine and derivatives, other therapeutic drugs reported for treating viral-related cases are listed in Table 1.

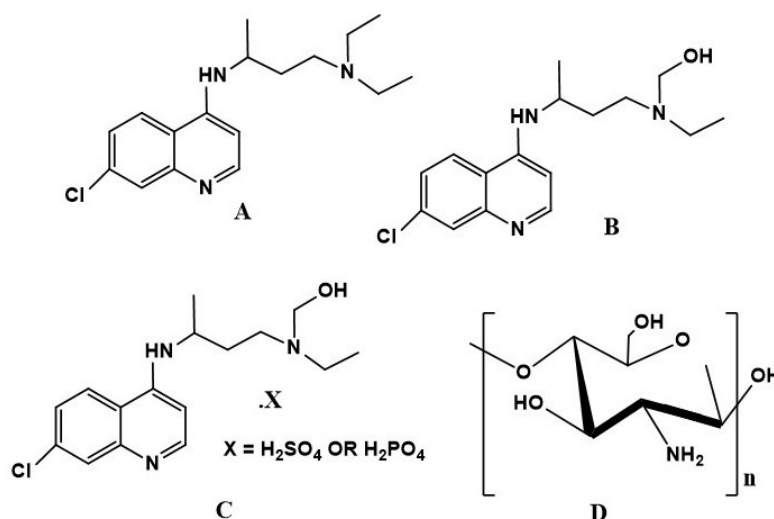


Figure 4. Structural formula of (A) chloroquine (B) hydroxychloroquine (C) hydroxychloroquine sulfate/phosphate (D) chitosan.

Table 1 Other therapeutic prospective antiviral drugs/treatments.

Drug	Chemical formula	Class of drug	Target	Ref.
Remdesivir	C ₂₇ H ₃₅ N ₆ O ₈ P	Adenosine analogue	Viral RNA chains	(49)
Lopinavir /Ritonavir	C ₃₇ H ₄₈ N ₄ O ₅ / C ₃₇ H ₄₈ N ₆ O ₅ S ₂	Protease inhibitors	HIV protease enzymes, SARS associated coronavirus	(50,51)
Favipiravir	C ₅ H ₄ FN ₃ O ₂	Prodrug anti-influenza	Viral RNA-dependent RNA polymerase	(52)
Ribavirin	C ₈ H ₁₂ N ₄ O ₅	Guanosine (ribonucleic) analog	Viral RNA synthesis and viral mRNA capping	(53,54)
Sofosbuvir	C ₂₂ H ₂₉ FN ₃ O ₉ P	HCV polymerase inhibitor	Inhibitor of viral RNA synthesis	
Galidesivir	C ₁₁ H ₁₅ N ₅ O ₃	Nucleoside analog	Broad-spectrum of RNA virus families	
Tenofovir	C ₁₉ H ₃₀ N ₅ O ₁₀ P	Antiretroviral prodrug	Nucleotide reverse transcriptase inhibitor	
Triazavirin	C ₅ H ₄ N ₆ O ₃ S	Non-nucleoside antiviral drugs	Influenza strains such as H5N1	
Teicoplanin	C ₈₈ H ₉₇ Cl ₂ N ₉ O ₃₃	Glycopeptide antibiotic	Viral spike protein	(55)
Atazanavir	C ₃₈ H ₅₂ N ₆ O ₇	Protease inhibitor	Active viral proteins	(30)
Interferons		Signaling proteins (cytokines)	Viral infections	(56,57)
Convalescent plasma		Antibody titer	Inhibiting the formation of inflammatory cytokine storms	(58,59)

CHITOSAN-ENCAPSULATED DRUG AS DRUG DELIVERY VEHICLES

Chitosan (see Figure 4d) is a naturally occurring polysaccharide that has attracted lots of scientific interest over the years especially in the aspect of drug delivery systems which is considered to be a hopeful and viable strategy for improving infectious disease treatment (60). Chitosan is a biodegradable and biocompatible deacetylated derivative of chitin made up of randomly distributed β -(1 \rightarrow 4)-2-amino-2-deoxy-D-glucopyranose which have been extensively exploited in bio-medical applications (61–63). The antimicrobial activity of chitosan has been extensively observed against a wide variety of microorganisms including fungal, algal, and bacterial strains (64–66). This has been achieved by taking advantage of the cationic charge and hydrogen bonding ability to stabilize biological structures such as DNA, RNA, and proteins in biological systems (67). Also, the higher antibacterial activity of chitosan over other types of disinfectants is demonstrated in its ability to chelate with metal ions to form new compounds and lower toxicity towards mammalian cells (68).

The skeletal framework of chitosan is made of free hydroxy and amine groups which can easily

hydrogen-bond with other molecules, while the free amine groups can be protonated in acidic medium and ionically bind with the reactive sulfate or phosphate groups of the drug molecules (see Table 1) via an encapsulation strategy to form new compounds (see Figure 5) (30). Therefore, this cationic biopolymer can act as an effective viral drug delivery agent since it can enhance the loading efficiency of DNA/RNA binding electrostatically, thereby reduces the rate of diffusion and promoting a more sustained release to target sites (69,70). Additionally, the existence of the outer polymer encapsulate could protect the DNA/RNA from innate biological degradation systems allowing more-effective transport across the cellular membrane to reach the target site in the nucleus (see Figure 6) (71). Apart from the successful encapsulation of chitosan for enhanced drug delivery (72), chitosan and its derivatives have been successfully utilized *in vitro* and *in vivo* for DNA and siRNA delivery systems because of their cationic charge, biodegradability and biocompatibility, as well as mucoadhesive, and permeability-enhancing properties (73). Additionally, the encapsulation of chitosan with other bulk- or nano-materials have been achieved for the effective delivery of gene (74), nucleic acid, (75) and as a non-viral vector in different clinical applications.

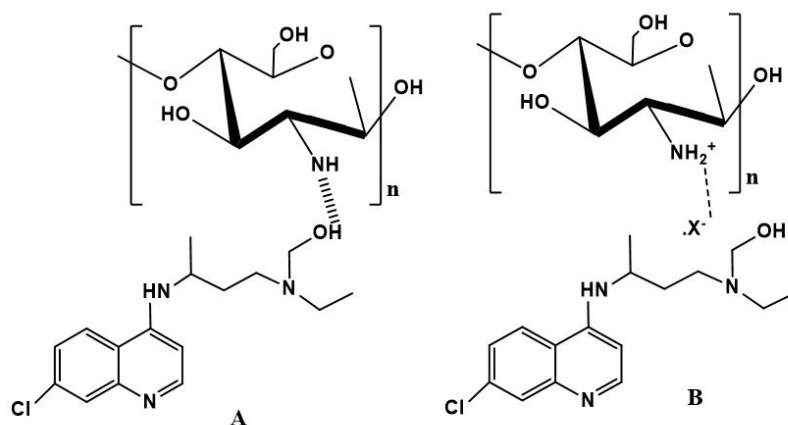


Figure 5. Possible interaction between chitosan and chloroquine or other drug molecules via (A) hydrogen bonding (B) ionic bonding with the available OH or ionic group in the drug's skeletal framework.

Existing studies have reported the efficacy of chitosan–tripolyphosphate conjugated chloroquine nanoparticle as an *in vivo* anti-malarial and antioxidant agent (76). For example, Magalhães et al. prepared chitosan (polycationic)/*Sterculia striata* (polyanionic) polysaccharides nanocomplex by polyelectrolyte complexation method and subsequently employed as a potential chloroquine drug release agent (77). Also, polymeric based iron nano-chloroquine phosphate was designed to encapsulate drug molecule for the delayed or slow release of drug in biological systems (78). These studies clearly show the potency of the nanodrug delivery technology as a more prospective treatments strategy than using only chloroquine or its derivatives alone to combat the parasite infection, oxidative stress as well as inflammation and DNA/RNA damage (60). However, using a combination of structural and molecular modeling approaches, Fantini et al. was able to show that chloroquine, which is one of the drugs currently under investigation for SARS-CoV-2 treatment, could bind sialic acids and gangliosides with high affinity. Thus, the team identified a new type of ganglioside-binding domain at the tip of the N-terminal domain of the SARS-CoV-2 spike (S) protein. This domain (aa 111-158), which is fully conserved among clinical isolates worldwide, may

improve the attachment of the virus to lipid rafts and facilitate the contact with the ACE2 receptor, with an inference that in the presence of chloroquine or its derivate, the viral spike is no longer able to bind gangliosides (79). Moreover, hydroxychloroquine, being less toxic, has an N -hydroxyethyl side chain in place of the N -diethyl group of chloroquine. This modification makes hydroxychloroquine more soluble than chloroquine, making it more open for easy attachment with other compounds like chitosan. In the same vein, hydroxychloroquine encapsulated chitosan increases the pH and could confer antiviral effects (80), thereby offering a modulating effect on activated immune cells and altering the glycosylation of the cellular receptors of coronaviruses (81,82). Furthermore, very limited studies have reported the encapsulation of other viral drugs on polymeric substrates as drug delivery vehicles to target sites. The success of Ravi et al. on the oral delivery of Lopinavir loaded nanoparticles on a hydrophobic derivative of pullulan acetate with a nanoparticle size distribution of ~197 nm, high entrapment efficiency (~75%), monodispersive nature (PDI < 0.2) and stability for 3 months opens promising adventure for higher encapsulated drug delivery than a drug-free system (83).

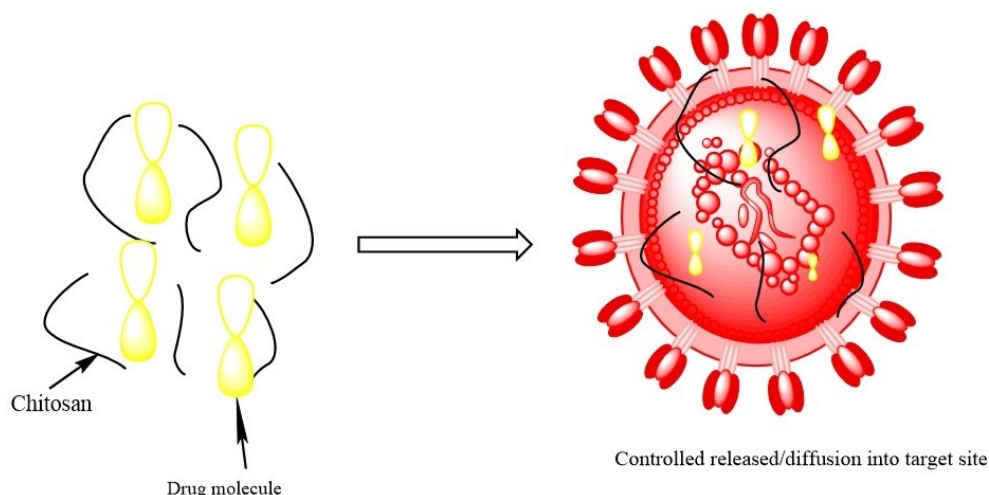


Figure 6. Controlled release of chitosan encapsulated drug and diffusion into the target site in the nucleus of the host.

Delivery of Chitosan Loaded Anti-Viral Drugs/ Vaccines

Antiviral drugs, either target specific or broad-spectrum are a class of drug molecules used for treating viral infections or inhibiting the development of a viral pathogen. Studies have shown that the pharmacokinetics of Triazavirin, $C_5H_4N_6O_3S$, can be improved via encapsulation with amino acids and quaternary amine compounds. Thus, Kozhikhova et al. (84) reported the stability and high mucoadhesive activity of charged liposome with modified chitosan coating as a nanocarrier for the delivery of Triazavirin via an underlying interaction between liposomes surface, positive chitosan coating, and negatively charged mucin secreted from epithelial cells, while Cánepa et al. (85) reported a novel chitosan-based nanocarrier for the oral delivery of interferon- α (IFN α -2b) prepared by ionotropic gelation and encapsulating approximately 100 % of the drug, with the antiviral potency of the nanocarrier showing maximum inhibitory efficiency against Human Lymphotropic-T Virus type 1 at 0.3 MIU to CF1 in mice. Also, Donalisio et al. (86) formulated chitosan nanosphere via a modified nanoemulsive template method for the delivery of acyclovir into Vero cell line infected with Herpesviruses, HSV-1, and HSV-2 strains. The acyclovir-loaded chitosan nanosphere displayed remarkable antiviral activity with no anti-proliferative activity, no signs of cytotoxicity and a drug loading capacity was recorded to be about 8.5 % with an *in vitro* release of approximately 30 % after 6 h. Additionally, Ulla et al. (87) examined the morphology and drug loading performance of a copolymerized chitosan and starch nanohydrogel functionalized with phthalic-anhydride and hexamethylenetetramine via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide catalyzed coupling, respectively. The promising antiviral and antimicrobial agents with

flexible and biocompatible functionalities revealed an increased drug loading efficiency from 65% to 80 and 85% for hydrophobic- anionic drugs. Furthermore, a novel nanoparticulate system for based on foscarnet-chitosan nanoparticles was prepared by Russo et al. (88) for the delivery of foscarnet (antiviral agent for herpesvirus DNA polymerase). It was reported that the foscarnet released from nanoparticles was non-toxic and also maintained the antiviral activity of the free drug when tested *in vitro* against lung fibroblasts (HELFI) cells infected with HCMV strain AD-169.

In an attempt to improve the antiviral effectiveness of nucleoside reverse transcriptase inhibitors (NRTIs) and lessen possible side effects, Yang et al. (89) developed a novel nanosized water-soluble chitosan-O-isopropyl-5'-O-d4T monophosphate conjugate. The *in vitro* drug release studies of the conjugate system at pH 1.1 and pH 7.4 in MT4 cell line show outstanding anti-HIV effect and low cytotoxicity. Yan et al. (90) explored the potentials of nanosized polyelectrolyte (PEC) complexes constructed by simple mixing of negatively charged curdlan sulfate (CRDS) into positively charged chitosan in aqueous solutions. The spherical PEC with negative zeta potential (-38 mV) were successfully loaded into zidovudine (an antiretroviral medication) and exhibited favorable drug loading efficiency at controlled pH. On another hand, Cazorla-Luna et al. (91) reported as-prepared chitosan-based mucoadhesive vaginal tablets in combination with pectin and locust bean gum, for the sustained release of tenofovir against HIV infection. The obtained results show that the tablet's formulation containing chitosan/pectin has the most homogeneous tenofovir dissolution profiles and stayed attached to the vaginal mucosa for up to 96 h, hence a promising self-protection choice for women against sexually-transmitted

HIV. Furthermore, Wu et al. (92) investigated the nanocomplexation of polyelectrolytes comprising of chitosan, chondroitin sulfate, and zinc(II) ions followed by encapsulation onto tenofovir, with the study revealing that the nanoparticles were noncytotoxic to human peripheral blood mononuclear cells and could reduce HIV-1 infection with an inhibitory concentration, IC_{50} of $4.35 \mu\text{mol}\cdot\text{L}^{-1}$ to $1.95 \mu\text{mol}\cdot\text{L}^{-1}$ when employed as drug carriers.

Vaccination is the most effective method of inhibiting and controlling viral infections. Viral infections can be mainly categorized into three based on the infection nature, namely: (a) acute infections caused by antigenically stable viruses, e.g. yellow fever, measles, mumps; (b) acute infections caused by rapidly mutating viruses e.g. influenza; and (c) chronic infections such as HIV and HCV. (93–95) Antiviral vaccines, on the other hand, are biological preparations containing an agent that mimics the viral pathogen, offering an active acquired immunity to a specific viral infection. Biopolymeric chitosan has shown the potentials of augmenting the immune responses with established safety and nontoxicity record in animals and humans as a vaccine against respiratory syncytial virus (RSV) infection (96). Spinner et al. (97) examined the sublingual vaccination of mice with split-flu vaccine formulated with methylglycol chitosan (MGC) and synthetic toll-like receptor 4 agonist (CRX-601) alone or in combination. The obtained results revealed that MGC and/or CRX-601 adjuvanted split-flu vaccines administered sublingually prompted a better mucosal and a corresponding systemic immune response to flu vaccine when delivered intramuscularly. Also, an adjuvanted influenza chitosan nanovaccine based on poly(I:C) could induce the proliferation of antigen-specific IFN γ secreting T-helper/memory and $\gamma\delta$ T cells, have been developed and tested in young swine against swine influenza virus (98). In another approach, El-Sissi et al. (99) prepared an adjuvanted vaccine formulation based on chitosan (CS) or chitosan nanoparticles (CNP, prepared by ionic gelation technique) loaded with Rift Valley Fever Virus (RVFV) inactivated antigen. These formulations were observed to be superior to the RVFV-Alum vaccine as the former strongly boosted the phagocytic activity of peritoneal macrophage, neutralization of antibodies titer against RVFV and IgG values against RVFV nucleoprotein.

CONCLUSION

Chitosan is an abundant natural biopolymer that has been effective as an anti-microbial agent and has been successfully applied for the treatment of many ailments. Studies have shown that its effective application as a non-viral carrier for gene

delivery, nucleic acid, and drug delivery systems. The cationic biopolymer possesses free hydroxyl and amine functional groups that can easily be encapsulated to the existing antiviral agents via electrostatic interaction or hydrogen bonding to form new compounds with milder side effects and enhanced ability to be delivered to the target sites. The numerous binding sites and chelating ability of the polymer may also be exploited for the fabrication of organometallic complexes, coordination compounds, and nanocomposites for possible deployment as an effective drug delivery agent against the deadly COVID-19.

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CONFLICTS OF INTEREST

The authors hereby declare no conflicting interest.

AUTHORS' CONTRIBUTIONS

Onome Ejeromedoghene: Conceptualization, writing - original draft preparation, Funding acquisition; Olayinka Oderinde: Writing - original draft preparation, Funding acquisition; George Egejuru: Writing - review and editing; Sheriff Adewuyi: Supervision, critical review and editing.

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