Formulation strategy for hydroxychloroquine as inhaler dosage from as a potential for COVID-19 treatment

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Plagiarism statement

This article describes review conducted in the College of Pharmacy, University of Basrah between April 2020 and July 2020 under the supervision of Dr. Mohammed Sattar. I, Hanan Raed Abdalaziz, certify that I have written all the text herein and have clearly indicated by suitable citation any part of this article that has already appeared in any publication.

Signature: Date: 8/7/2020

Dedication

_To the example of dedication and sincerity …… my dear father

_To the women who taught me survive on matter how the circumstances change …… my dear mother

_To everyone who in lights the others with his knowledge …… my teachers

_To the honest and faithful always in his work ……. my supervisor

Hanan

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List of Abbreviations

WHO.....World Health Organization

FDA…….Food and Drug Administration

ADE…….Antibody Dependent Enhancement

GIT…….Gastro Intestinal Tract

USP…….United States Pharmacopeia

NF……National Formulary

P……..Plasmodium

COPD……..Chronic Obstructive Pulmonary Disease

CFC…….Chlorofluorocarbon

DPI……..Dry Powder Inhaler

HFAs……...Hydrofluoroalkanes

Abstract

COVID-19 is one of the major pathogen that targeted human respiratory. On March 11, 2020 the WHO declared it as a worldwide pandemic. Hydroxychloroquine is one of protocols has been used in treatment of patients with COVID-19, but it can cause systemic side effects particularly serious heart related side effects such as QT interval prolongation so on June 15, 2020 FDA banned hydroxychloroquine from use in treatment of COVID-19 so that the known and potential benefits of hydroxychloroquine cannot outweigh the known and potential risks. Accordingly, the possibility to formulate hydroxychloroquine as pulmonary drug delivery system has been reviewed to localize its action in the lungs and prevent systemic absorption and prevent its systemic side effects. The reviewed properties of HCQ greatly suggest the formulation as metered dose inhaler and nebulizer is applicable.

1 Introduction

1.1 Background

Coronavirus is one of the major pathogen that primarily target human respiratory system. Coronavirus causes an epidemic disease. Severe acute respiratory syndrome (SARS)-CoV in 2002-2003 and the Middle East respiratory syndrome (MERS)-CoV in 2012 are represent previous outbreaks of coronaviruses (CoVs), which have been previously characterized as agents that are a great public health threat. Until late December 2019, a cluster of patients was admitted to hospitals with an initial diagnosis of pneumonia of an unknown etiology. These patients were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China [1,2].

Early reports predicted the onset of a potential Coronavirus outbreak given the estimate of a reproduction number for the 2019 Novel (New) Coronavirus on Feb 11, 2020 the WHO named novel coronavirus as COVID-19 [3] , also International Committee on Taxonomy of Viruses (ICTV) named this novel coronavirus as SARS CoV-2 [4], on March 11,2020 the WHO declared it as a worldwide pandemic [5].

There are four genera of CoVs: (I) a-corona virus (alpha CoV), (II) β-coronavirus (beta CoV) probably present in bats and rodents, while (III) δ-coronavirus (Delta CoV), and (IV) γ-coronavirus (gamma CoV) probably represent avian species [6,7], COVID-19 has classified as a β CoV of group 2B by World Health Organization (WHO) [8]. Two different types of SARS-CoV-2 were identified by phylogenetic analysis of 103 strains of SARS-CoV-2 from China, designated type L and type S, (70 percent of the strains was L type while 30 percent was S type). During the early days of the epidemic in China, the L type was predominated, while lower proportion of strains was accounted outside of Wuhan than in Wuhan. [9]

The word corona means "crown " in Latin, this name come from the virus shape under the microscope, it consists of a core of genetic material surrounded by an envelope with protein spikes ,so it appears like a crown [10,11 12]. Coronaviruses are transmitted between animals and humans so coronaviruses have zoonotic origin [13,14].

1.2 DESCRIPTION OF THE COVID-19

The structure of corona Virus Disease 2019 (COVID-19) is similar to other CoVs. It is an enveloped, non-segmented, positive-sense single-stranded RNA virus genome in a size ranging from 29.9 kb [15]. The structure of COVID-19 consists from following: (I) a spike protein (S); is heavily glycosylated, it has two subunits; one subunit, S1, binds to a receptor on the surface of the host's cell, the other subunit, S2, fuses with the cell membrane. (II) a hemagglutinin-esterease dimer (HE); it enhances S protein –mediated cell entry and spread the virus through the mucosa. (III) a membrane glycoprotein (M); it may have two different conformations to enable it to promote membrane curvature and bind to nucleocapsid. (IV) an envelope protein (E): it facilitated assembly and release of the virus and it acts as ion channel activity, it is necessary for virus pathogenesis. (V) a nucleoclapid protein (N); it binds to RNA and it is heavily phosphorylated, it helps viral genome to replicate –transcriptase complex and subsequently package the encapsulated genome into viral particles, and (VI) a RNA ; is the genome of the virus, [16]. The SARS-CoV-2 genome has 50 and 30 terminal sequences (265 nt at the 50 terminal and 229 nt at the 30 terminal region), with a gene order 50-replicase open reading frame (ORF) 1a 1b-S-envelope(E)-membrane(M)-N-30 [16]. The structure assembly of the virus is illustrated in Figure 1.

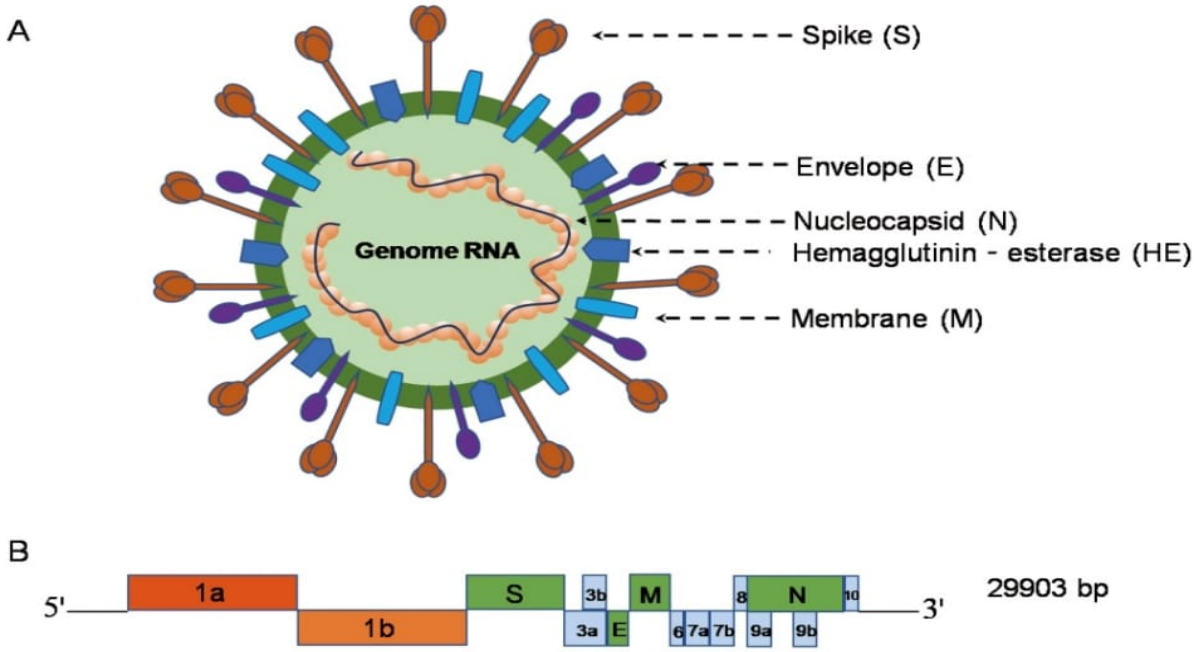


Figure 1 Structure of corona virus

1.1 PATHOGENESIS

1.1.1 Virus Entry and Spread

Respiratory droplet and contact are the major rote that SARS-CoV-2 is transmitted predominantly via it, and maybe it transmitted via fecal-oral rote [17]. Mucosal epithelium of upper respiratory tract (nasal cavity and pharynx) is the major site for primary viral replication, with further multiplication in lower respiratory tract and gastrointestinal mucosa [18], so this causes a mild viremia. controlled at this point and remain asymptomatic. Non-respiratory symptoms such as acute liver and heart injury, kidney failure, diarrhea are also has been reported in some patients [19,20,21] , Accordingly, it can be considered as adisease of multiple organ involvement.

Angiotensin-converting enzyme-2 (ACE-2) is the receptor for viral entry for COVID-19, the structure of the receptor-binding gene region is very similar to that of the SARS coronavirus [22]. Nasal mucosa, bronchus, lung, heart, esophagus, kidney,

stomach, bladder, and ileum are exhibit broad expression of ACE-2, and these human organs are all vulnerable to SARS CoV-2 [23]. However, the lungs seem to be particularly more vulnerable to SARS-COV-2 due to their large exposed surface area and because of alveolar epithelial type 2 cells seemingly act as a reservoir for virus replication [24], ACE-2, different from ACE-1 that is inhibited by ACE-1 inhibitors, such as enalapril and ramipril [25]. Briefly, for SARS-CoV-2, S protein is activated and cleaved by a host trans membrane serine protease TMPRSS2 into two separate polypeptides S1 and S2, the S1 subunit of the spike binds to the ACE-2 enzyme on the cell membrane and TMPRSS2 also acts on the S2 subunit, facilitating fusion of the virus to the cell membrane. The virus then enters the cell, at acidic pH, the membrane surrounding the virus fuses with the membrane of lysosome, allowing the nucleic acid of virus to cross lysosomal membrane and enter to cytoplasm where the virus replicates [26]. The envelope also has a crucial role in virus pathogenicity as it promotes viral assembly and release. Testicular tissues also recently showed to be affected by COVID-19. Additionally, it has been proposed by clinicians, implying fertility concerns in young patients [27]. The postulated pathogenesis of SARS-CoV-2 infection is demonstrated Figure 2.

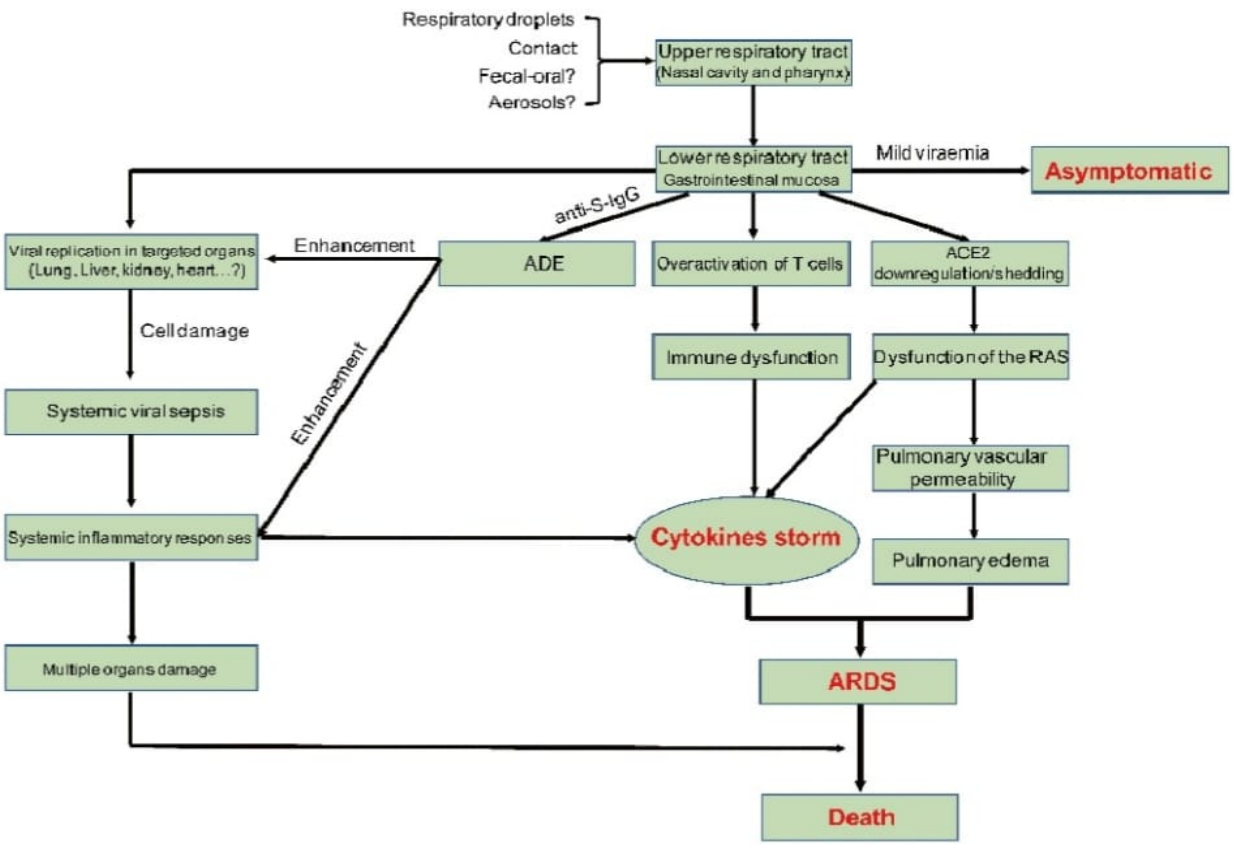


Figure 2: Diagrammatic representation of pathogenesis of SARS-CoV-2

1.1.2 Acute Respiratory Distress Syndrome (ARDS)

ARDS is a life-threatening lung condition that prevents enough oxygen from getting to the lungs and into the circulation, accounting for mortality of most respiratory disorders and acute lung injury [28]. Severe respiratory distress occurs in fatal cases of human SARS-Co, MERS-CoV, and SARS-CoV-2 infections, so mechanical ventilation require in these cases, and the histopathology findings also support ARDS [29,30]. Genetic susceptibility, and inflammatory cytokines were closely related to the occurrence of ARDS that implying by previous studies. More than 40 candidate genes including ACE2, interleukin 10 (IL-10), tumor necrosis factor (TNF), and vascular

endothelial growth factor (VEGF) among others have been considered to be associated with the development or outcome of ARDS [31]. Increased levels of plasma IL-6 and IL-8 were also demonstrated to be related to adverse outcomes of ARDS [28]. The above biomarkers suggest both a molecular explanation for the severe ARDS and a possible treatment for ARDS following SARS-CoV-2 infection.

1.1.3 Cytokine Storm

Clinical findings showed abundant inflammatory responses during SARS-CoV-2 infection, further resulting in uncontrolled pulmonary inflammation, likely a leading cause of case fatality. Many factors are responsible for aggressive inflammation caused by SARS-CoV-2 include rapid viral replication and cellular damage, virus-induced ACE-2 down regulation and shedding, and antibody dependent enhancement (ADE) [32]. Massive epithelial and endothelial cell death and vascular leakage were caused by initial onset of rapid viral replication, that trigger the production of abundant pro-inflammatory cytokines and chemokines [33]. Loss of pulmonary ACE-2 function has been proposed to be related to acute lung injury [34] because ACE-2 down regulation and shedding can lead to dysfunction of the renin-angiotensin system (RAS), and further enhance inflammation and cause vascular permeability. Persistent inflammation, ARDS, and even sudden death were reported by one issues for SARS-CoV that has been occurring in few patients, particularly those who produce neutralizing antibodies early, while most patients survive the inflammatory responses and clear the virus [32]. The above phenomenon also exists in SARS-CoV-2 infection. A possible underlying mechanism of antibody-dependent enhancement (ADE) has been proposed recently [32]. ADE, a well-known virology phenomenon, has been confirmed in multiple viral infections [35]. ADE can promote viral cellular uptake of infectious virus–antibody

complexes following their interaction with Fc receptors (FcR), FcR, or other receptors, resulting in enhanced infection of target cells [35]. The interaction of FcR with the virus-anti-S protein-neutralizing antibodies (anti-S-IgG) complex may facilitate both inflammatory responses and persistent viral replication in the lungs of patients [32].

1.1.4 Immune Dysfunction

Peripheral CD4 and CD8 T cells showed reduction and hyper activation in a severe patient. High concentrations of pro inflammatory CD4 T cells and cytotoxic granules CD8 T cells were also determined, suggesting antiviral immune responses and over activation of T cells [29]. Additionally, several studies have reported that lymphopenia is a common feature of COVID-19 [36,19], suggestive of a critical factor accounting for severity and mortality.

2 Hydroxychloroquine and COVID-19

Hydroxychloroquine (HCQ) is medication that has recently been making headlines as possible treatments for COVID-19. There are number of theories to explain the potential mechanism of action of HCQ against SARS-CO-2. Hydroxychloroquine inhibits terminal glycosylation of ACE-2, the receptor that SARS-CoV-2 target for cell entry. ACE2 that is not in the glycosylated state may less efficiently interact with the SARS-CoV-2 spike protein, further inhibiting viral entry. [37,38, 39]

Another potential mechanism involves the inhibition of viral release into the intracellular space, hydroxychloroquine is a weak base that diffuses in lysosome and raises the pH of lysosome causing impairment of function of lysosomal enzymes

thereby inhibiting release of the viral contents so in presence of hydroxychloroquine the viruses can no longer do this, thereby inhibiting release of the viral contents, and the cells are protected from infection [37,40,41,42]. HCQ may also block the production of interleukin -6 and other pro-inflammatory cytokines [43], which are key mediator of ARDS. Hydroxychloroquine have a crucial role in the early treatment of COVID-19 patients [44,45].

2.1 What is HYDROXYCHLOROQUINE ?

Hydroxychloroquine is a medication first approved to treat malaria, an infection caused by a parasite. It is similar in structure to chloroquine, which was first approved by the FDA in 1949. Hydroxychloroquine was approved in 1955[46], it is typically preferred over chloroquine because it has fewer side effects [47]. Side effects for both medications, include [46].

*GIT side effects (nausea ,vomiting ,diarrhea ,abdominal cramps ,reduced appetite) .

*Long QT or QT Prolongation (abnormal heart rhythm).

*muscle weakness or nerve pain.

*Hypoglycemia (low blood glucose).

*Retinopathy.

*Headache.

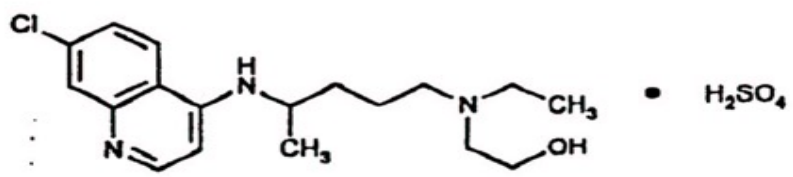
*Anemia and other blood problems.

*Worsening of seizures and other neurology problems.

*Worsening of psoriasis.

2.2 Description of hydroxychloroquine

Hydroxychloroquine present as salt (hydroxychloroquine sulfate), is a white or practically white, crystalline powder, freely soluble in water; practically insoluble in alcohol, chloroform, and in ether. The chemical name for hydroxychloroquine sulfate is 2-[[4-[(7-Chloro-4-quinolyl) amino]pentyl] ethylamino]ethanol sulfate (1: 1) [46,48] .The structural formula is :



The molecular formula is C18H26ClN3O.H2SO4. and molecular weight of hydroxychloroquine sulfate is 433.95, the brand name is plaquenil. Plaquenil (hydroxychloroquine sulfate) tablets contain 200 mg hydroxychloroquine sulfate, equivalent to 155 mg base, and are for oral administration [46,48].

Inactive Ingredients: Dibasic calcium phosphate USP, hypromellose USP, magnesium stearate NF, polyethylene glycol 400 NF, polysorbate 80 NF, corn starch, titanium dioxide USP, carnauba wax NF, shellac NF, black iron oxide NF [46,48]

2.3 How is it supplied ?

Plaquenil tablets are white, to off-white, film coated tablets imprinted "PLAQUENIL" on one face in black ink. Each tablet contains 200 mg hydroxychloroquine sulfate (equivalent to 155 mg base) [46,48].

Paquenil film-coated tablet should not crush or divide, It should dispense in a tight, light-resistant container as defined in the USP/NF. It must keep out of the reach of children [46,48]. Piaquenil should store at room temperature [20° to 25°C (68° to 77°F), allows excursions between 15° and 30°C (59° and 86°F)] [46,48]

2.4 Physiochemical properties of hydroxychloroquine

Physical prescription → solid.

Melting point → 90º c

Solubility → 0.02 g/L

pKa → 9.67

Log P → 3.87

[48]

2.5 Pharmacology OF HYDROXYCHLOROQUINE

2.5.1 Indication

Malaria

Hydroxychloroquine is indicated for the treatment of uncomplicated malaria due to *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax,* also it is indicated for the prophylaxis of malaria in geographic areas where chloroquine resistance is not reported [46].

Lupus Erythematous

Hydroxychloroquine is indicated for the treatment of chronic discoid lupus erythematosus and systemic lupus erythematosus in adults [46].

Rheumatoid Arthritis

Hydroxychloroquine is indicated for the treatment of acute and chronic rheumatoid arthritis in adults [46].

2.5.2 Mechanism of action

The exact mechanisms of hydroxychloroquine are unknown. It has been shown that hydroxychloroquine accumulates in lysosomes of malaria parasite, raising the pH of the vacuole [49]. This activity interferes with the parasite's ability to proteolyse hemoglobin, preventing the normal growth and replication of the parasite [49]. Hydroxychloroquine can also interfere with the action of parasitic heme polymerase, allowing for the accumulation of the toxic product beta-hematin [50]. Hydroxychloroquine accumulation in human organelles also raise their pH, which inhibits antigen processing, prevents the alpha and beta chains of the major histocompatibility complex (MHC) class II from dimerizing, It inhibits antigen presentation of the cell, and reduces the inflammatory response [49]. Elevated pH in the vesicles may alter the recycling of MHC complexes so that only the high affinity complexes are presented on the cell surface [49]. Selfpeptides bind to MHC complexes with low affinity and so they will be less likely to be presented to autoimmune T cells [49].

Hydroxychloroquine may also reduce the release of cytokines like interleukin-1 and tumor necrosis factor [49,51]. The raised pH in endosomes, prevent virus particles (such as SARS-CoV and SARS-CoV-2) from utilizing their activity for fusion and entry into the cell. Hydroxychloroquine inhibits terminal glycosylation of ACE2, the receptor that SARS-CoV and SARS-CoV-2 target for cell entry [37,40]. ACE2 that is not in the glycosylated state may less efficiently interact with the SARS-CoV-2 spike protein, further inhibiting viral entry [37].

2.5.3 Pharmacokinetics properties

Bioavailability: Rabid and complete absorption (hydroxychloroquine is 67-74% bioavailable [52]. Onset: May take 4-6 months to show response: peak response takes several months [46]. Peak plasma time :1-3 hours [46]. Distribution: hydroxychloroquine is 50% protein bound in plasma [52]. Metabolism: Metabolite: Desethylhydroxychloroquine and desethylchloroquine , Desethylhydroxychlorine is the major metabolite [46]. Elimination: Half-life :32-50 days [46]. Rote of elimination: 40-50% of hydroxychloroquine is excreted renally, while only 16-21%of a dose is excreted in the unchanged drug [52]. 5% of a dose is sloughed off in skin and 24-25% is eliminated through the feces [52] .

2.6 Why hydroxychloroquine has being mentioned with COVID-19 ?

Recent in vitro studies (studies done in a petri dish or test tube rather than in animal or humans) have shown that hydroxychloroquine has antiviral properties against SARS-CoV-2, the virus that causes COVID-19. In these studies, this medication worked by interfering with the chemical environment of human cell membranes. This blocked the virus from entering and multiplying inside the cells [53]. A medication working *in vitro* do not always mean that it will work once inside a human body.

Nonetheless, based on these early findings, hospitals worldwide have begun using hydroxychloroquine for patients with COVID-19. On March 28, 2020, the FDA issued an Emergency Use Authorization (EUA) that allows providers to request a supply of hydroxychloroquine for hospitalized patients with COVID-19 [54,55]. The EUA does not mean that the FDA has approved this medication for the treatment of COVID-19. The intent of the EUA is to help increase access to this medication by allowing doctors to request a supply from the Strategic National Stockpile for these specific cases [54,55].

2.7 Can Hydroxychloroquine be used to treat or prevent COVID-19 ?

Hydroxychloroquine has been used for many decades to treat malaria and, rheumatoid arthritis and lupus [56]. There is not enough medical data at this time to prove that hydroxychloroquine works for COVID-19, while some small studies suggest the medication may be helpful, other showed no benefets. There have been no studies showing that these medications work for prevention, and the FDA has issued a warning for serious heart-related side effects such as QT interval prolongation and ventricular tachycardia if hydroxychloroquine are taken outside of a hospital setting for COVID-19 [56] .

2.8 Is hydroxychloroquine safe ?

Hydroxychloroquine is generally considered safe when taken for conditions that they have been approved for. Hydroxychloroquine can cause side effects, it can also interact with other drugs and cause serious problems, but in these cases, the benefits outweigh the risks [56]. On April 24, 2020, the FDA issued a warning stating that using hydroxychloroquine for COVID-19 outside of a hospital setting can put people at risk of serious heart rhythm problems, particularly QT prolongation [56]. This is a well-known side effect of these medications. Because there is still a lot we don't know about COVID-19, it is unclear if COVID-19 itself increases this risk. Some hospitals in France, Brazil, and Sweden have had to stop using hydroxychloroquine for some coronavirus patients due to severe side effects.

3 PULMONARY DRUG DELVERY SYSTEM

Pulmonary drug delivery is not a new route of drug administration, this system was widely accepted in the ancient period for lung and other respiratory diseases. Ancient inhalation therapies included the use of leaves from plants, balsams and myhrr. However, around the turn of the 19th century, with the invention of liquid nebulizers, these early treatments developed into legitimate pharmaceutical therapies [57,58].

Drugs are generally delivered to the respiratory tract for the treatment or prophylaxis of airways disease, such as bronchial asthma and cystic fibrosis by this route [59], so it presents several advantages over the administration of same drugs by other routes leading to the systemic delivery of such drugs [60]. Drug inhalation enables rapid deposition in the lungs and induces fewer side effects than does administration by other routes [60]. The lung also may be used as a route for delivering drugs having systemic activity, because of its large surface area, the abundance of capillaries and the thinness of the air-blood barrier. Studies have demonstrated the potential for delivering proteins and peptides such as insulin, vaccine, and growth hormone via the airways [59].

3.1 ADVANTAGES OF PULMONARY DRUG DELIVERY SYSTEM [61]

1-It is needle-free pulmonary delivery.

2-It having a very negligible side effects since the rest of the body not exposed to drug

3-The onset of action is very quick.

4-Degradation of a drug by the liver is avoided.

5-Avoid the first pass metabolism.

6-It requires low and fraction of oral dose

7-The dose needed to produce a pharmacological effect can be reduced.

8-Avoidance of gastrointestinal upset.

9-Bioavailability of smaller drug molecule is very good .

10-Bioavailability of larger drug molecule can be improved by means of the absorption enhancer.

11-Convenient for long-term therapy, compared to parenteral medication.

3.2 LIMITATIONS OF PULMONARY DRUG DELIVERY SYSTEM [61]

1. Improper dosing.

2. Stability of drug in vivo.

3. Some drug may produce irritation and toxicity.

4. Difficulty in producing optimum particle size.

5. Some drugs may be retained in lungs and clearance of the drug may be difficult.

6. Targeting specificity.

7. Difficult to transport.

8. Difficult to use

9. Drug absorption may be limited by the physical barrier of the mucus layer.

3.3 ANATOMY AND PHYSIOLOGY OF THE RESPIRATORY SYSTEM

The respiratory system works with the circulatory system to deliver oxygen from the lungs to the cells and remove carbon dioxide and return it to the lungs to be exhaled.

The human respiratory system consisted of two regions,

1. Conducting airway (peripheral region)

2. Respiratory region (central region)

The conducting airways are further divided into various types, i.e. nasal cavity, associated sinuses, nasopharynx, oropharynx, larynx, trachea, bronchi, and bronchioles [62]. Its function is humidification and filtration of inhaled air [61]. The conducting airways are mainly layered by two different epithelial cells termed ciliated epithelial cells and goblet which collectively form the mucociliary escalator [62]. As a protective mechanism of the body, the mucociliary escalator can effectively entrap insoluble inhaled particulates and sweep those particulates out of the lung. Mucus plays a significant part in mucociliary clearance and the major functional components of mucus are the mucin glycoproteins presented at a concentration of 1–5% [63],

The respiratory region consists of respiratory bronchioles, alveolar ducts, and alveolar sacs. The cellular population in the respiratory region mainly consists of alveolar epithelial type I (AT-I) and alveolar epithelial type II (AT-II) cells, AT-I cells are larger and thinner than AT-II cells, accounting for 90% of the alveolar surface area, [64,65]. By contrast, AT-II cells are much smaller covering only 3% of the alveolar surface area. The main role of AT-II cells is the synthesis and secretion of pulmonary surfactants, which form the surface- active film at the respiratory air–liquid interface [66,67]. As shown in Figure 3 [68].

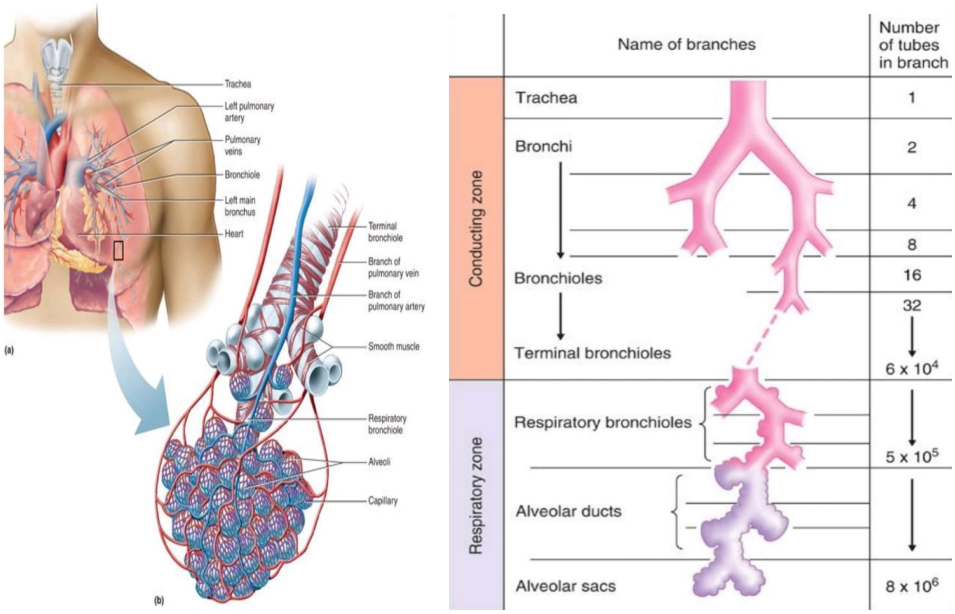


Figure 3: Physiological illustration of the respiratory system

3.4 FACTORS AFFECTING PARTICLE DEPOSITION IN THE LUNGS

3.4.1 Drug related factors:

Those might include; particle size, shape, density, charge and hygroscopicity [69]. All these factors control the drug deposition in the lung. Particle size and zeta potential are crucial factors which decide the efficacy and stability of the delivery system as particle size tend to control the drug deposition in the lung, where zeta potential helps in the stability management of the system [70], the size is defined by what is called the mass median aerodynamic diameter (MMAD). Particle in size range of 1-5 Mm are considered respirable (have significant lung deposition) [69]. Particle shape and density determine the proportion of inhaled particles that deposit in deep lung alveoli versus major airways [69]. Particle with low density is prefer in pulmonary drug delivery [71].

Hygroscopicity is the property of some substances to absorb and exhale humidity depending on setting in which they are found. This means that they can get larger or smaller in size upon entering into the airway, with the consequent modification in the deposition pattern compared to what was initially expected. The diameter that a particle reaches after hygroscopic growth depends on its initial diameter, the intrinsic properties of the particle, and the environmental conditions in the airways. In general, it is considered that hygroscopic growth does not have much of an effect in particles with MMAD less than 0.1 Mm; meanwhile it is very intense in particles with MMAD lager than 0.5 Mm [72,73].

3.4.2 Physiological factors:

a-Absorption barriers [60] : include –

*pulmonary surfactant: This covers the alveolar surface to a thickness of 10-20 nm.

*Epithelial surface fluid: a thin fluid layer called the mucus blanket ,5 Mm in depth, covers the wall of the respiratory tract. This barrier serves to trap foreign particles for subsequent removal and prevents dehydration of the surface epithelial by unsaturated air during inspiration.

*Epithelium: the type of epithelium of upper respiratory tract is pseudostratified, ciliated, columnar epithelium while alveolar surface is mainly composed of single layer of squamous epithelial cells type 1 and alveolar cells and cuboidal type 2 alveolar cells.

*Interstitium: The lung interstitium is the extracellular and extracellular space between cells in tissues. In order for molecules to be absorbed from air spaces to the blood, it must pass through the interstitium.

*Vascular endothelium: The endothelium is the final barrier to molecule being absorbed from the airspace into the blood.

b-Clearance of inhaled particles from the respiratory tract:

*Mucociliary clearance : The respiratory tract possesses serious of defences against inhaled materials because of its constant exposure to outside environment. The lung has an efficient self- cleaning mechanism known as the mucociliary escalator. The mucous and cilia have significant role in this type of clearance [74]. Mucociliary clearance present predominantly in conducting airways as explain above [62].

*Alveolar macrophage clearance: Alveolar macrophages play a key part in the clearance of inhaled particles deposited in the respiratory region of the lung .The phagocytic behavior of alveolar macrophages in the lung is particle size ,surface charge and surface modification dependent [75].

Drug clearance of drug particles /droplets from the lung depends on whether particles are deposited in the central or in the peripheral part of the lung as mucociliary clearance in the conducting airways is faster compared with the slow macrophage clearance in the alveolar space [76,77,78] In addition, particles deposited in the central conducting are cleared faster than particles deposited in the peripheral conducting airways, as mucociliary clearance increases from peripheral to central conducting airways . Mucociliary clearance clears particles in these airways to the mouth –throat region, where drug may subsequently be swallowed and absorbed from the GIT so mucociliary clearance reduces pulmonary bioavailability of inhaled drugs [79].

c-Breathing patterns: breathing patterns affect particle deposition. Optimal aerosol deposition occurs with slow, deep inhalations to total lung capacity, followed by breath holding prior to exhalation [80].

3.4.3 Device-related factors:

These include efficiency of spray, size, and size uniformity of sprayed droplets, location of spray generation in the context of patient's anatomy, width of spray zone, and the speed of the aerosol [69].

3.5 FORMULATING AND DELIVERING THERAPEUTIC INHALATION AEROSOLS

There are currently three main types of aerosol generating device for use in inhaled drug therapy: pressurized metered dose inhalers, dry powder inhalers and nebulizer.

3.5.1 METERED DOSE INHALER

The metered- dose inhaler, called an MDI for short, is a pressurized inhaler that delivers medication by using a propellant spray. It is composed of four essential components: the base formulation (drug, propellant, excipients, etc) the container, the metering valve and actuator (or mouthpiece). It is a drug delivery device which provides the fine droplets of a medicament having the particle size of fewer than 5 micrometers. It is used for the treatment of respiratory diseases such as asthma and COPD. They can be given from suspension or solution. In case of suspensions formulations, the substances that are insoluble in the propellant and solvent are dispersed in the suitable propellant vehicle. Particle size, the solubility of active ingredient and surfactants or dispersing agents are the important factors to be considered in formulating MDI suspension formulations. Solution formulations of MDI consist of the active ingredient dissolved in a pure or mixture of propellants. Solution aerosol is relatively easy to formulate provide the ingredients are soluble in the propellant-solvent system. MDIs contains the propellant like chlorofluorocarbons and hydrofluroalkanes. They consist of a micronized form of the drug in a propellant under

pressure with surfactants to prevent clumping of drug crystals. Lubricants for the valve mechanism and other solvents are the other constituents. When the device is actuated, the propellant gets exposed to atmospheric pressure, which leads to aerosolization of the drug. As it travels through the air, the aerosol warms up leading to evaporation of the propellant that reduces the particle size to the desirable range [61] as shown in Figure 4. The main disadvantages of this device include inhalation techniques and patient co-ordination required, high oral deposition, maximum dose of 5 Mm and limited range of drugs available [81].

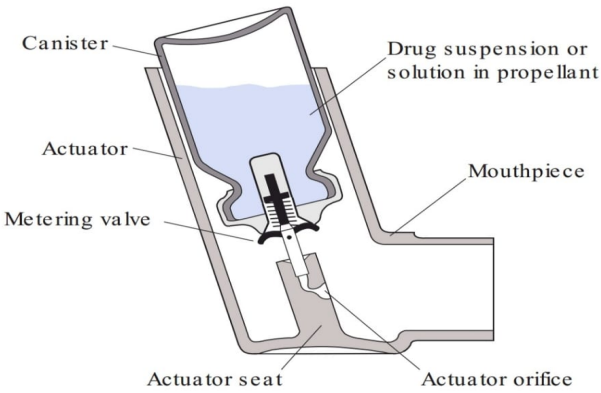


Figure 4: Metered Dose Inhaler

3.5.2 DRY POWDER INHALER

It is a versatile system that requires some degree of dexterity. The name itself indicates that formulation id solid form. It is a bolus drug delivery device that contain the solid drug in a dry powder mix that fluidized when the patient inhales. It contains the active drug alone or has a carrier powder mixed with the drug to increase the flow properties of a drug. Dry powder inhaler has a greater stability, ease of handling, and relatively cheap when compared to metered dose inhaler. There is no need for harmful

propellent like CFC [61]. The main disadvantages of this device include respirable dose dependent on inspiratory flow rate, humidity may cause powders to aggregate, dose lost if patient inadvertently exhales into the DPI [81]. They can be designed for a single or multi-dose purpose [61].

Single unit dose devices:

Single-dose powder inhalers are devices in which a powder containing capsule is placed in a holder. The capsule is opened within device and powder is inhaled. Spinhalar, rotahalar and cyclohalar are examples on this devices type [61].

Multi dose device;

The multi-dose device uses a circular disk that contains either four or eight powder doses on a single disk. The doses are maintained in separate aluminum blister reservoirs until just before inspiration. Turbohalar and dischalar are examples on this devices type [61]. As in Figure 5 [83-61].

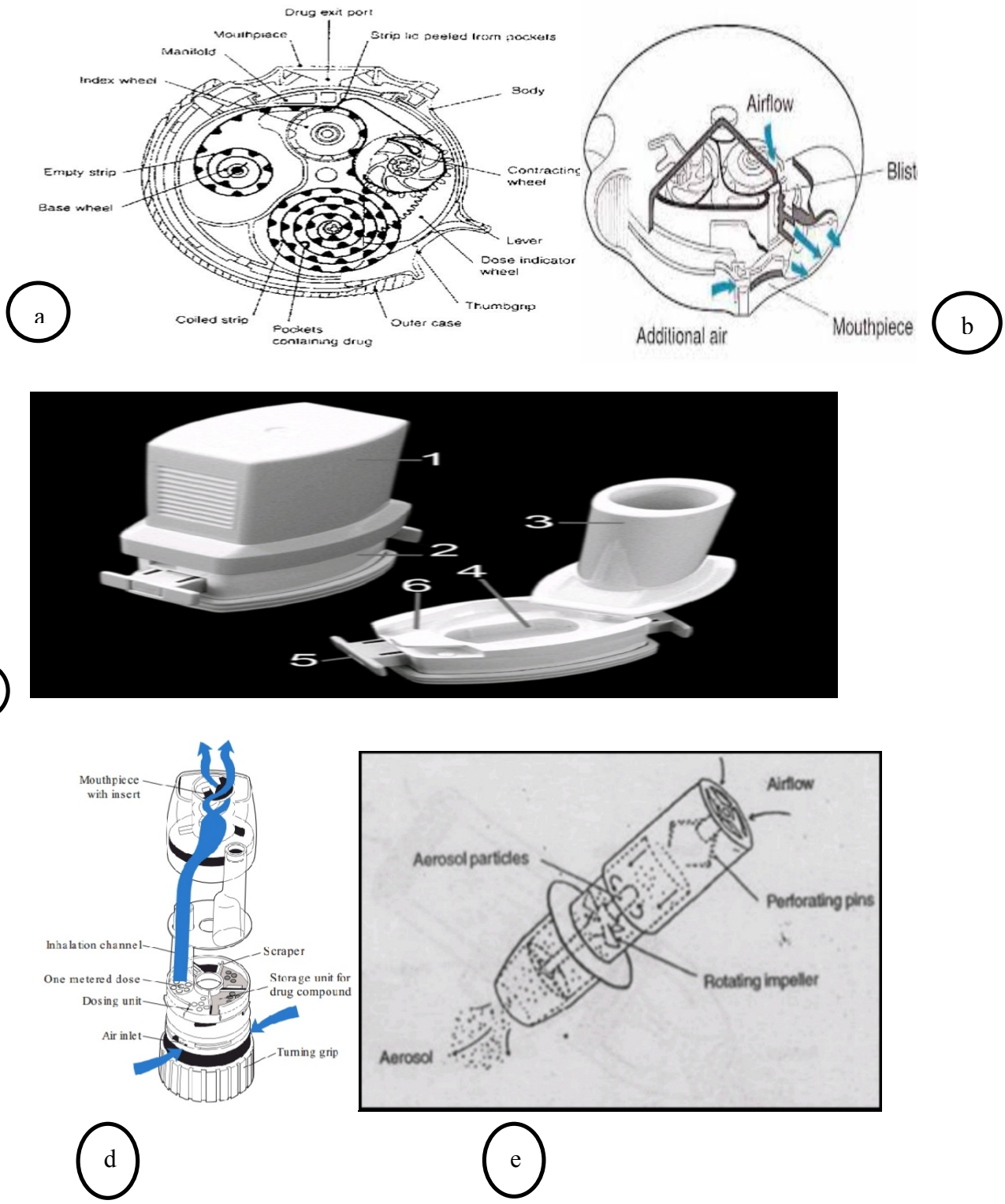


Figure 5: -[a-a schematic diagram of Diskus dry powder inhaler ,b-across sectional representation of devic, c-Cyclohaler dry powder inhaler{1-cap; 2-base;3-mouthpiece;4-capsule chamber; 5- buttonattached to pins for piercing capsule;6-air inlet channel}, d-the Turbothalar, e-Sinthaler

3.5.3 Nebulizer

The nebulizer is widely used as aerosolizing drug solution or suspensions for drug delivery to the respiratory tract and is particularly used for the treatment of a hospitalized patient. It is commonly used in treating cystic fibrosis, asthma, and another respiratory disease. There are two types of the nebulizer, namely jet and ultrasonic [61] as in Figure 6

Jet nebulizer; In jet nebulizer, the liquid is converted and sprayed into fine droplets by use of compressed gas, for the prevention of exits of a large droplet from the device the baffles are used in a jet nebulizer [61].

Ultrasonic nebulizer; In ultrasonic type, aerosol droplets are produced through high-frequency vibrations of a piezoelectric crystal, for that the ultrasound waves are formed in it [61]. The main disadvantages of nebulizer include time consuming, bulky, nonportable, contents easily contaminated, expensive and drug wastage[81].

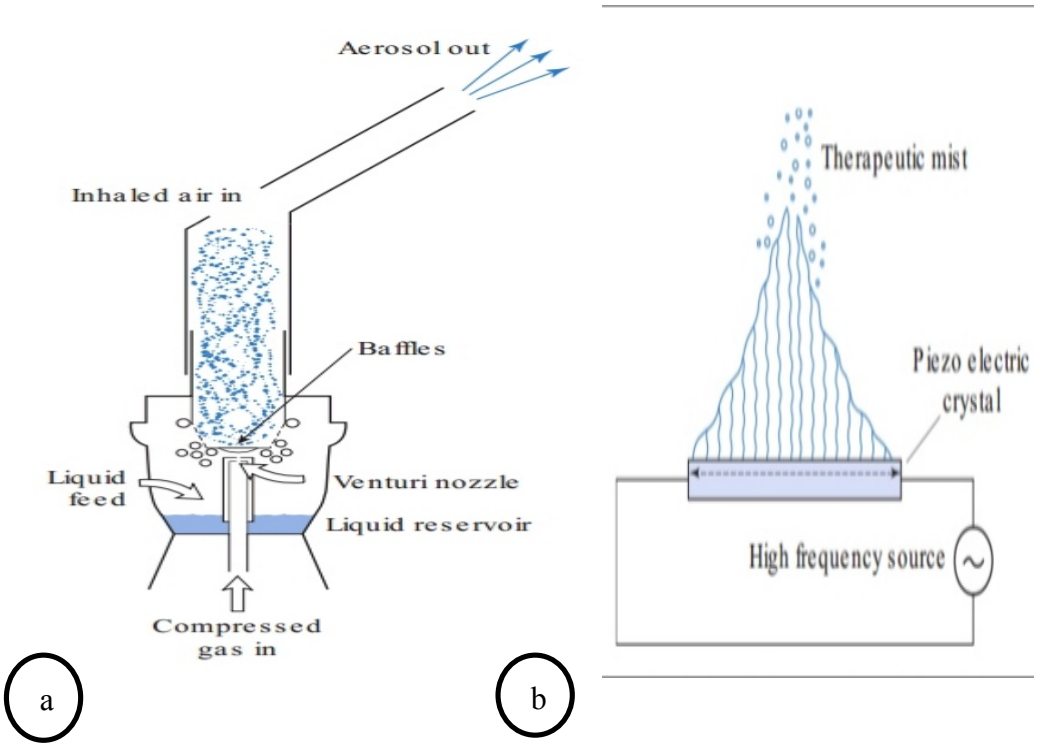


Figure 6: a-ultrasonic nebulizer, b-jet nebulizer

4 HYDROXYCHLOROQUINE AS PULMONARY DRUG DELIVERY SYSTEM

4.1 Can hydroxycloroquine be formulated as pulmonary drug delivery system to treat COVID-19?

Hydroxychlroquine medication can cause systemic side effects particularly serious heart related side effects such as QT interval prolongation and ventricular tachycardia that reportedly by ongoing reports [56], one of these reports published on May 1, 2020 in which the result was that patients who were hospotilized and receiving hydroxychloroquine for COVID-19 frequently experienced QTs prolongation [85], in addition to several new reports of methemoglobinemia in COVID-19 patients [55]. Some hospitals have had to stop using hydroxychloroquine for some COVID-19 patient due to severe side effects. In June 15 2020, FDA banned hydroxychloroquine from use in treatment of COVID-19 due to its severe side effects so that the known and potential benefits of hydroxychloroquine cannot outweigh the known and potential risks [55].

So in this review we are studying the possibility to formulate hydroxychloroquine as pulmonary drug delivery system to localize its action in the lungs and prevent systemic absorption and prevent its systemic side effects.

Firstly the pre-formulation studies for hydroxychloroquine as pulmonary drug delivery system must be determine which include :-

1-Physical description; hydroxychloroquine present in solid state [48].

2-Solubility; 0.02 mg/ml (very slightly soluble in water)[48[.

3-Enthalpy of vaporization; 83 KJ/mol [48].

4-Meting point; 90 °c [48].

5-pKa; 9.67 [48].

6-log p; 3.87 [48].

7-Hgroscopicity; hydroxychloroquine does not hygroscopic material [48].

8-Stability; hydroxychloroquine undergoes photolysis and oxidation [86].

Accordingly, it has been suggested that hydroxychloroquine formulation as MDI and nebulizer.

From pre-formulation studies many properties of hydroxychloroquine have been concluded. Firstly hydroxycloroquine itself is very slightly soluble in water so it is advisable to formulate it as salt (hydroxychloroquine sulfate), this salt is freely soluble in water ,also hydroxycloroquine undergoes oxidation so anti-oxidant must be added to prevent or minimize oxidation.

4.2 HYDROXYCHLOROQUINE SULFATE MDI

According to the above properties and preformulation data, it has been suggested that the hydroxychloroquine sulfate might be formulated as solution form MDI and as nebulizer.

The suggested Hydroxychloroquine sulfate MDI formula:

Active ingredient	hydroxychloroquine sulfate *
The solvent	water*
Co-solvent	ethanol*
Surfactant	soy lecithin*
Solubilized antioxidant	ascorbic acid*
*Propellants	-Norflurane (Propellant HFA-134a).

The suggested Hydroxychloroquine sulfate nebulizer formula:

*Active ingredient	hydroxychloroquine sulfate
*Solvent	water
*Co-solvent	ethanol
*Surfactant	soy lecithin
*Isotonicity modifier	sodium chloride
*Buffer	sulfuric acid

4.3 QUALITY CONTROL AND TENSTING OF MDI AND NEBULIZE

The FDA has provided several guidelines related to attribute testing of pulmonary drug delivery system.[87]. As shown in Table 1.

.

Table 1: Summary of FDA required tests for MDI and nebulizer

Attribute Test	Nebulizer Solution	Pressurized MDI
Drug product specifications		
Description-	+	+
Appearance ,color and clarity of the formulation		
-Identification	+	+
-Assay	+	+
Impurities and degradation products-	+	+
Preservatives and other stabilizing excipients -	a	a
Valve delivery /pump delivery-	-	+
Spray content uniformity-	-	+
Spray pattern-	-	+
Droplet size distribution-	+	-
Particle size distribution-	+	+
Particulate matter-	+	b
Microbial limits-	c	d
Weight loss-	+	+
Net content-	+	+
Number of doses-	-	+
Leachables-	+	+
PH-	+	-
Osmolality -	+	-
Viscosity-	-	-
-Aerodynamic particle size distribution	+	+
Leak rate-	-	+
Drug product characterization studies		
Priming and repriming (multi-use products)-	-	+
Effect of resting time (multi-use products)-	-	+
Temperature cycling -	+	+

Effect of moisture -	-	+
In vitro dose proportionality (for multiple strengths)-	+	+
Drug deposition on mouthpiece-	+	+
Cleaning instructions	+	+
Device ; -	-	+
Performance should be studies for in-use factors		
Effect of dosing orientation-	-	+
-Profiling of sprays near container exhaustion	-	+
-Effect of varying inspiratory flow rate	-	+
-Effect of storage on particle size	-	+
-Plume geometry	-	+
Preservative effectiveness and sterility maintenance	-	-
Microbial challenge	-	+
Characterization of nebulizer specified in the labeling	+	-
Photostability	+	+
Stability of primary (unprotected) package -	+	-

Note:
a-Applicable for all excipients used to stabilize the product , such as antioxidant and antimicrobial preservatives.

b-Particle size distribution for drug substance ;for pressurized MDIs where the drug is suspended.

c-Nebulizer solutions should be sterile .

d-HFAs do not support microbial growth.

5 CONCLUSIONS AND FUTURE STUDIES

Hydroxychloroquine is not preferred to be used systemically for treatment of patients with COVID-19 due to its systemic side effects particularly serious heart related side effects such as QT interval prolongation. Accordingly, the possibility to formulate hydroxychloroquine as pulmonary drug delivery system has been reviewed to localize its action in the lungs and prevent systemic absorption and prevent its systemic side effects. All preformulation studies with all related possible formulations have been discussed. The reviewed properties of HCQ greatly suggest the formulation as metered dose inhaler and nebulizer is applicable. The next step for future work will be the study of HCQ compatibility with the suggested excipients. Furthermore, the study of toxicity and bioactivity on animals must be done, then clinical studies on human must be conducted in a way that include three phases (phase I on 20-80 healthy volunteers, phase II on 100-300 patients and phase III on 1000-3000 patients).

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