Current Trends in Biotechnology and Pharmacy
Vol. 19(3) 2536-2554, July 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.3.36

Pulmonary Route of Targeted Ivermectin Delivery to SARS-CoV-2 in Lungs of COVID-19 Patients Based on Nanotechnology Approach

Annas Binarjo[1]*

[1]Pharmaceutics and Pharmaceutical Technology Department, Faculty of Pharmacy, Universitas Ahmad Dahlan, Jln Prof. Dr. Soepomo, Umbulharjo-55164, Yogyakarta, Indonesia.

***Corresponding Author**: annas.binarjo@pharm.uad.ac.id

Abstract

Although it is evidenced to exhibit virucidal activity against SARS-CoV-2, ivermectin has not been recommended for COVID-19 therapy due to the negative result in clinical trials. It is predicted that oral administration of the conventional formulation is unsuccessful in achieving the minimum inhibitory concentration in the alveolar lining fluid of COVID-19 patients. The development of lung-targeting drug delivery systems needs to be performed. Several studies to develop the inhalation delivery of ivermectin have been published. This review aims to examine the potential of delivery carriers and technology to administer ivermectin via the pulmonary route to reach the minimum inhibitory concentration against SARS-CoV-2 revealed in *in vitro* studies. Nebulizer technology of solution, nanoemulsion, or nanomicellar formulation, as well as Dry Powder Inhaler of engineered particle powder, freeze-dried product of nanostructured lipid carriers or solid lipid nanocarriers, has the potential to deliver ivermectin, achieving alveoli in sufficient concentration equal to about 5 µM of *in vitro* result. This review can be a point of view in conducting research to develop ivermectin target-oriented drug delivery systems.

Keywords: inhalation, targeting, ivermectin, COVID-19, pulmonary, nanocarrier

Introduction

In 2020, about one year since the COVID-19 outbreak occurred, ivermectin was proven in an in vitro setting to inhibit the growth of SAR-CoV-2, the responsible virus of the disease (1). However, up to now, ivermectin has not been recommended for COVID-19 therapy due to the unavailability of a clinical trial verifying the benefit of the drug (2,3). The clinical outcomes agree with pharmacokinetics data showing that the maximum plasma concentration after oral administration of ivermectin at a dose of 600 µg/kgbw/d for 3 days falls under the concentration in in vitro studies (1,4). It is predicted that the therapeutic plasma concentration, which is equal to that in vitro studies, can be reached by increasing the dose to 1200 mg, about 100-fold higher than the maximum Food and Drug Administration-approved dose of ivermectin (5), by an assumption that alveolar, the main location of the SARS-CoV-2, and plasma show the same concentration in equilibrium.

Following the recommended dose, a giant tablet needs to be prepared, especially if the additional excipients are taken into account. In addition, the solubility of ivermectin in the gastrointestinal fluids limits the dissolved fraction of the administered dose, resulting in the drug being wasted in the feces. Moreover, the other barriers to oral administration of ivermectin,

as listed in Table 1, also prevent the conventional oral drug delivery from reaching alveoli in an adequate drug level to inhibit SARS-CoV-2. Therefore, although oral delivery is the most common route of administration due to its convenient and self-administration aspect, alternative routes should be considered.

Table 1. Barriers to oral delivery of ivermectin

Site	Process	Problem	Ref.
Gastroin-testinal medium	dissolution	The dissolution process is in non-sink condition and reaches saturation after 30 minutes at 4 or 12 µg/ml in water or PBS pH 6.8, respectively.	(6)
Gastric medium	degradation	More than 50% of ivermectin is degraded for 2 h in 0.1 M HCl.	(6)
Gastro-intestinal membrane	permeation	Ivermectin efflux is processed by P-gp.	(7,8)
Liver	biotransfor-mation	Ivermectin is first-pass metabolized by CYP3A4 in the liver.	(9)
Blood circu-lation	distribution	Ivermectin is strongly bound to albumin	(10)
Adipose tis-sue	distribution	Ivermectin is deposited intensively in adipose tissue	(11)

Inhalation (lung/pulmonary) drug administration promises the ability to deliver ivermectin to the target of action. This route of delivery eliminates all processes in Table 1 since ivermectin is transferred directly from the inhalation device to the lungs of patients. The mild pH, the unavailability of digesting enzymes, and the availability of natural surfactant in the alveolar lining fluid are beneficial for many drugs, including ivermectin, to reach the target. To develop this approach, a basic knowledge of the inhalation drug delivery route and carrier systems should be at hand as the basis for conducting the necessary research. This review discusses this delivery route as a system that has the potential to deliver ivermectin to reach alveoli at a sufficient concentration for inhibiting SARS-CoV-2 proliferation.

Preclinical and Clinical Trial of Ivermectin Administration

The success story of the in vitro test of ivermectin to reduce SARS-CoV-2 proliferation was continued by in vivo activity evaluation, the preclinical studies using animal models. The experiments were conducted using either SARS-CoV-2-infected animals or other coronaviruses showing the similarity with the COVID-19 responsible virus, for instance Mouse Hepatitis Virus (MHV). The benefit of ivermectin oral administration before SARS-CoV-2 infection to reduce the viral load was reported in the hamster model. This outcome was accompanied by other advantages, including a decrease in pulmonary disease, inhibition of inflammatory cytokines expression, and a reduction in the severity of pathological symptoms of COVID-19. The positive clinical outcome was also evaluated in the curative of COVID-19 hamsters, even though the viral load did not affect the ivermectin remedy. The reduction in viral load by ivermectin treatment was evaluated in MHV-infected mice (12–14)\. These in vivo data suggested that clinical trials are the right effort to improve ivermectin levels for the treatment of COVID-19.

Ivermectin has been administered orally in several clinical trials, as reported in clinicaltrials.gov. For searching the conducted clinical trials, the words: “Covid19”, “SARS-CoV2 Infection”, and “Oral ivermectin” were filled into the sections of “Condition/disease”, “Other terms”,

and “Intervention/treatment”, respectively. Up to now (June 25th, 2025), about 36 studies were conducted from April 24th, 2020, to August 15th, 2023. Of these trials, 16 studies have been completed, 1 study is still ongoing, while the others (approximately 53%) have been terminated, withdrawn, or have an unknown status. The study results were posted by 6 completed clinical trials, which were only 2 studies comparing the outcomes of oral ivermectin therapy versus placebo. It is concluded from these two studies that 400 µg/kg body weight as a single dose or daily for 3 days did not affect the time of recovery, negative PCR result, for instance, of mild to moderate COVID-19 patients (15,16). This data is in line with the barriers of ivermectin oral delivery as listed in Table 1.

Replacing the word “oral ivermectin” in the intervention section with “ivermectin inhaled” or “inhalation ivermectin” retrieved 3 trials. Only 2 ivermectin inhalation administrations were conducted, which have an “unknown” status. The completed trial did not deliver ivermectin by inhalation. This data suggested that the inhalation drug delivery system of ivermectin has not been studied much, making this topic interesting to discuss.

Administration Technology of Inhalation Drug Delivery System

Pulmonary administration promises a higher accumulation of ivermectin in the lung because the drug is delivered directly to the site of action. After reaching the surface of the lungs, the delivered drug may undergo absorption into the systemic circulation, but for ivermectin used to treat COVID-19, the drug is expected to remain in the lungs, so-called pulmonary (inhalation) drug delivery for local therapy. Although the pharmacokinetics is quite simple, the technology behind the deposition of the drug or drug in nanocarriers onto the surface of the alveoli deserves considerable attention. The final target of the technology is to achieve a high level of deposition of the internal phase of aerosol, either solid or liquid state.

At least, there are three technologies of aerosolization as illustrated in Figure 1. 1). Nebulization technology (nebulizer) converts the drug solution or suspension in a non-propellant solvent, preferably water, into the liquid aerosol (cloud) using various energy, including air jet and ultrasonic vibration. In this technology, the patient is in a passive state, allowing it to be adopted by critical patients, even in the state of fainting and coma. 2). A pressurized metered-dose inhaler produces droplets from a precise volume of liquid containing the drug as a solution or suspension using the kinetic energy of pressure derived from the boiling process of propellant. The propellant is a gaseous state material at room temperature and normal atmospheric pressure. The high pressure in the canister is sufficient to convert the material into a liquid state in which the drug can be dissolved or dispersed with or without additional solvent. Setting the canister in the auxiliary device causes the liquid dosage form of the drug to move into the metering chamber. Connecting the metering chamber to the atmosphere by pressing the actuator reduces the pressure, resulting in the rapid conversion of liquid propellant into a burst of gas containing drug-loaded liquid droplets. 3). Dry powder inhalers provide solid-state drugs or drugs in carriers that can be converted to dust using the patient’s inhalation energy or using additional energy (17).

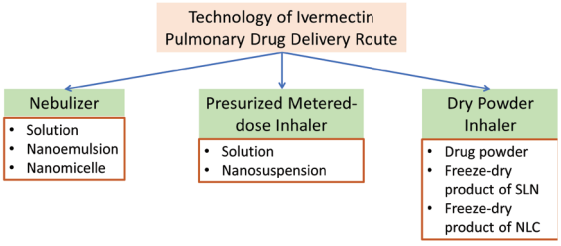

Figure 1. Classification of aerosolization technology and potential preparation for ivermectin inhalation drug delivery system.

In defining the advantages and disadvantages of each aerosolization technology, several points of view can be applied. Patients in an unconscious state (fainting) can only re-

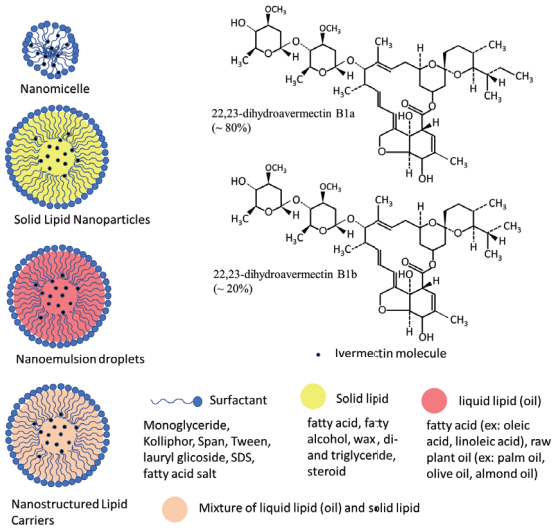

Figure 2. Schematic representation of potential nanocarriers as inhalation formulations. The molecular structure of ivermectin and examples of materials for nanocarrier preparation are also displayed.

ceive a nebulizer, since in pMDI, the patients should control the actuation and inhalation, while in DPI, patients have to expend more energy on inhalation to aerosolize the powder. Concerning the percentage of delivered to administered drug (in the formulation), jet (conventional) nebulizer shows the worst. The nebulizer administers the drug continuously, while the patient alternately inhales and exhales. While the patient exhales, the administered drug is wasted. This technology also requires more time to prepare the tools for dosing and to proceed with medication administration. Moreover, regarding the chemical stability of the drugs, DPI shows the best performance due to its solid properties. The liquid dispersed or dissolved drugs, as in nebulizer and pMDI, are easier to degrade than drugs in the solid state. Finally, based on portability and dose precision, pMDI is preferable. The metering chamber set up on the device ensures the dose of the drugs (18) the inhaler device, and the patient. However, the biggest single problem that accounts for the lack of desired effect or adverse outcomes is the incorrect use of the device due to lack of training in how to use the device or how to coordinate actuation and aerosol inhalation. This review summarizes the structural and mechanical features of aerosol delivery devices with respect to mechanisms of aerosol generation, their use with different formulations, and their advantages and limitations. A technological update of the current state-of-the-art designs proposed to overcome current challenges of existing devices is also provided.”,”container-title”:”Medical Devices (Auckland, N.Z..

The First consideration for preparing an inhalation formulation is the size of the generated aerosol droplets in relation to the target of deposition. Liquid droplets or particles larger than 5 µm fail to penetrate the filtering mechanism of the respiratory tract, especially in the curvature airways connecting the nasopharynx to the oropharynx, due to the inertial impaction mechanism [19]. This results in aerosol droplet deposition in the upper respiratory system. For targeted delivery of ivermectin to the lungs, the aerosol should be lower than 5 µm to access the alveoli and undergo two deposition mechanisms, namely gravitational sedimentation and diffusion deposition, which are determined by their size categories as discussed by Darquenne. In short, the small and large size categories experience diffusion deposition and sedimentation deposition, respectively, while the medium size categories were exhaled with expiration air, which is an unexpected condition [20]. The size limits of the three categories vary between formulation [21,22], which is likely dependent on other factors, including density and surface properties of the particles. Therefore, research should be conducted specifically. Moreover, the properties of generated droplets or particles are also determined by the aerosolization technology, as categorized in Figure 1. In the following sections, such methods of aerosolization are discussed with regard to carrier formulations applicable for each method. The description of carriers and examples of materials to prepare the carrier are displayed in Figure 2.

Nebulizer of ivermectin

An aqueous solution of nebulizer formulation is more efficient in delivering the drug than suspensions. However, since ivermectin exhibits poor water solubility, several attempts are required to develop aqueous solutions of ivermectin, including the introduction of co-solvents or complexing agents, and nanocarrier systems. Ivermectin solubility in water increased dramatically from about 0.005 mg/ml to 5 mg/ml (equal to about 5.7 mM) by introducing 10% propylene glycol or 20% N-methylpyrrolidone (23). Using this solution, the administered volume of nebulizer (V_{ad}) can be calculated using equation 1, in which the ivermectin concentration in the formulation (C_f) is 5700 µM, the targeted concentration is the effective virucidal concentration (C_{eff}, 5 µM (1)), the volume of alveolar lining fluid (V_{alf}) is 36 ml (24) and the administration efficiency for nebulizer (E_{ad}) is about 60% (25). The calculated volume of administration, i.e. 52.7 µl, is much lower than the regular nebulizer volume of about 3 ml (26). Therefore, due to toxicity issues, it is possible to reduce the co-solvent concentration, together with the consequence decrease in ivermectin concentration in the formulation and an increase in volume of application.

$$V_{ad} = \frac{V_{alf} C_{eff}}{C_f \cdot E_{ad} - C_{eff}} \qquad \textbf{(equation 1)}$$

The development of water-based nanocarrier dispersion formulations, such as nanoemulsion/microemulsion and nanomicelle dispersions, for instance, are potential systems nanocarriers, while nanoemulsion preconcentrate, namely Self-Nanoemulsifying Drug Delivery System (SNEDDS) showing a higher loading capacity than nanoemulsion, could not be used due to its high viscosity, making it difficult to be aerosolized. Several poorly water-soluble drugs have been successfully delivered to the lungs as nanoemulsion formulations, including ibuprofen, budesonide, docetaxel, and amphotericin B (27–30). The water solubility of drugs could also be enhanced by the introduction of various surfactants at concentrations above the critical micelle concentration. Nebulization of water-based nanomicelle dispersion has been performed to deliver budesonide, itraconazole, and amphotericin B to the surface of the alveoli (31–33). The formulation of ivermectin as a nanoemulsion as well as a nanomicelle dispersion increases its solubility, making it possible to prepare aqueous-based dosage forms at high concentration (34–36). Ivermectin could be easily dissolved in the oil phase of nanoemulsion, the droplets, due to its lipophilicity. In nanomicelle dispersion, there is not any oil introduced in the formulation. At a high concentration, above the critical micelle concentration, surfactant molecules produce micelles, where ivermectin can be incorporated in the micelle nucleus, which is a lipophilic zone constructed from the tails of surfactants. These events promise the possibility to prepare a water-based solubilized solution of ivermectin in the concentration of 5473 µM (36). To reach the antiviral concentration of 5 µM at a normal volume of SMV, 3 ml, about 65 µM of ivermectin in the dosage form is required. This concentration is much lower than the highest possible concentration of ivermectin, either in nanoemulsion or in nanomicelle preparations.

Pressurized metered dose inhaler (PMDI)

The PMDI formulation contains propellant as the main component and additional excipients, including co-solvents and surfactants. Co-solvent is needed to increase drug solubility in the propellant if solution formulation is expected. However, if solution formulation in the expected concentration is difficult (or impossible) to achieve, an alternative formulation, namely a suspension, may be selected. In this case, a surfactant is needed as a dispersing agent. The low solubility of surfactants in safer propellant, namely hydrofluoroalkane (HFA), causes co-solvents to be needed in suspension formulations (37,38).

Micronized ivermectin can be formulated into PMDI suspension using HFA propellant,

aided by ethanol as a co-solvent in a concentration of about 18% and 0.055% oleic acid as a surfactant. Unfortunately, there is not any sufficient information regarding droplet size. However, the high nasopharyngeal deposition compared to the lungs indicates that the droplet is likely to be above the optimal size (39). Two arguments are proposed to support this prediction: (a) As mentioned in the material, the ivermectin particles contained in the droplet are in micrometer size; (b) The addition of ethanol to HFA reduces the vapor pressure of the propellant, thereby reducing the atomization force. Consequently, the initial droplet size increases, resulting in a larger residual phase containing oleic acid and ivermectin once the propellant evaporates (38). Therefore, to achieve optimal droplet size, ivermectin particles should be reduced to nanosized before being suspended in PMDI propellant.

Previous studies formulated ivermectin as PMDI solutions using various propellants, including two types of HFA and alkanes containing 3-5 carbon atoms. Solutions at concentrations of up to 1714 µM are likely achieved due to the high co-solvent concentrations of ethanol and isopropanol, i.e. up to 50% (40). However, it is predicted that the low portion of propellant leads to a high droplet size and, in turn, the product may only be able to accumulate ivermectin in the nasal and nasopharyngeal regions, not in the lungs.

Dry powder inhalers (DPI)

Pulmonary administration of ivermectin powder without nanocarrier systems is possible to reach an effective antiviral concentration of the drug in alveolar lining fluid. The ivermectin water-solubility of 5 mg/L (35) is equal to about 5.7 µM, which might be increased by naturally occurring endogenous surfactants available in alveolar lining fluid, namely dipalmitoyl phosphatidylcholine (41). However, the powder properties of ivermectin might hinder the selection of such a formulation. Ivermectin particle size ranges from 90-250 µm (42), which is preferred for deposition in the upper respiratory region. Particle engineering, for example jet milling, ball milling, spray drying of pastas or solutions, or controlling the crystallization process, can be applied to reduce the size to the optimal aerodynamic size of 1-5 µm. These methods have been successfully applied to other drugs, for example reducing ibuprofen from 110 µm to 1.7-5.1 µm by jet milling (43) and Vinca rosae leaf particles from about 2007 µm to 0.75 µm in size by ball milling (44). Crystallization of griseofulvin by pumping a drug solution in acetone into water containing an immersed vibrator probe yielded 4.6 µm fine particles (45). Ivermectin can be micronized by these techniques to produce the desired size. However, often the fine particles of hydrophobic substances form aggregates, resulting in poor respirable properties. Leucine can be added to modify the surface properties and surface energy of fine particles, hindering the aggregation (46). During the preparation of this manuscript, a spray drying process of ivermectin solution in isopropyl alcohol reduced the particle size from 49.7 µm to 0.88 µm. The crystal habitus of the column was also converted to a sphere, which is a better shape for inhalation administration. Co-processing with pre-treated lactose, suspended in a solution of ivermectin in isopropyl alcohol, at an ivermectin percent ratio of 5%, produced larger particles in the optimal range for inhalation. In an in vitro evaluation, about 10 mg of formulation containing about 5 mg of ivermectin was successfully delivered. Only about 20% of ivermectin remained in the capsule and device, which indicated that about 400 µg of ivermectin was possibly dispersing and saturating the alveolar lining fluid, achieving the minimum inhibitory concentration (MIC) (47). In another study, L-leucin was used as a co-processing agent in the spray drying technique at a concentration of 10%, and the resulting properties were compared to products without co-processing. The particle sizes of the products obtained were in the range of 1.7 – 2.2 µm, either with or without co-processing. However, co-processing with L-leucin increases the product yield by reducing product deposition on the wall of the

spray dryer, thereby increasing the dry powder in the collector (48).

An alternative approach, as an addition to particle engineering, nanocarrier systems can be selected. For DPI, the nanocarriers must be solid, and solid lipid nanoparticles (SLN) can be selected due to their high capacity to load ivermectin. Nanostructured lipid carrier (NLC) might show higher drug loading and release capabilities, but the wet properties of the lipid mixture need to be considered. Typically, SLN and NLC are formulated and administered as liquid colloidal dispersions for oral, topical, or injection, but actually it is also possible to use them in pulmonary application (49). For inhalation delivery as DPI, the freeze drying process of SLN and NLC colloidal dispersions with the addition of cryoprotectant (50–52) might produce respirable solids. Several lipids can be formulated as lipid components of SLN inhalation powder, including stearic acid, palmitic acid, cholesteryl myristate, and Compritol®, which were used successfully to deliver the antituberculosis drugs rifampicin and ethambutol previously (53–56). Regarding NLC, a mixture of solid lipid stearic acid and liquid lipid oleic acid can be formulated as a dry inhalation formulation to deliver ciprofloxacin (57). It is possible to replace such loaded drugs with ivermectin.

Several SLN and NLC formulations of ivermectin were successfully prepared using various lipids, including the solid lipid palmitic acid and Precirol®, and the liquid lipid mygliol (58,59). Unfortunately, only the SLN findings could be evaluated regarding the critical issue, namely loading capacity (LC) and drug release. LC is the mass of drug loaded (incorporated) in a certain mass of lipid nanocarrier, which could be converted into mass percent. In short, LC is the concentration of the drug loaded in the nanocarrier. This definition is the same as the definition in the referred article, but a slight recalculation was performed due to a different equation (59). Showing an LC of 11.76%, 1.4 mg of SLN equivalent to 158 µg of ivermectin is needed to reach a concentration of 5 µM in 36 ml of alveolar lining fluid.

SLN is converted to a flowable and inhalable powder by freeze-drying or spray drying with the addition of cryoprotectants. This addition causes the drug concentration in the inhalable material to be smaller, which results in an increase in the amount of solid material that must be administered. Usually, the freeze-drying process using selected cryoprotectant increases the particle size due to cryoprotectant deposition on the surface of dried SLN. The addition of cryoprotectant is expected to increase the physical stability of the nanocarrier, especially to reduce the nanocarrier aggregation, leading to particle size enhancement. To obtain the most effective lung deposition, as discussed above, DPI formulations are prepared with refined drug composition in an ideal size of 1-5 µm or below 50 nm (60) adhering to the coarse host.

In general, SLNs are too small particles for DPI formulations since their diameters range from 50-500 nm (61). Particles in this size range are able to access the lungs but show low deposition on the surface of the alveolar lining fluid. The freeze-drying process with the addition of cryoprotectants can increase the range of particle sizes close to ideal conditions for inhalation. This size shift is influenced by the type and concentration of cryoprotectant, and also the type of lipid as the main component of SLN. For instance, while the particle size of freeze-drying product of Dynasan® SLN is independent of the addition of some cryoprotectant (62), SLN containing Compritol® produced freeze-dried particles whose size is determined by the type and concentration of the cryoprotectant. Introducing cryoprotectants glucose, mannose, and maltose in the SLN to a cryoprotectant mass ratio of 1:2 to 1:3 in the freeze-drying process, produced fine particles in the range of 1-2 µm in diameter, while without cryoprotectant, the freeze-drying process produced particles diameter of about 2.5 µm.

As previously discussed, without the addition of cryoprotectant, 1.4 mg of freeze-dried

ivermectin SLN is required for a single dose. Since the ratio of refined drug to coarse host in DPI formulation is 1:1000 to 1:25 (63), about 140 mg of coarse host particle is required, resulting in the total mass of the dosage form being higher than that of a normal DPI preparation. A 1.4 mg of fine drug powder or nanocarrier is above the normal potency of DPI, which is usually in the µg range. Therefore, several approaches should be conducted to overcome this problem as discussed by Farkas and coworkers and briefly listed in Table 2 (64). Novel formulations containing submicron drug particles (less than 1 µm) and growth-enhancing excipient (EEG) are prepared by, for instance, the spray drying method. The concentration of the drug in this formulation is higher than that of conventional DPI (host-drug particle system). These fine particles are able to pass through the upper respiratory tract, namely the mouth to throat, and access the alveoli. The water uptake by the EEG due to the high humidity of the alveoli induces the growth of particles to a larger size in the lungs, preventing them from the exhalation process (65).

Table 2. Strategy for administering high-dose DPI formulation. Conventional formulation means host-particle formulation. Passive DPI uses the patient's inspiratory airflow to generate aerosol, whereas active DPI uses another source of force.

Method	Device	Formulation	Aerosolization force
Multiple dosing of the DPI formulation	conventional	conventional	active
Loading multiple capsules in the device	conventional	conventional	passive
Single capsule containing a large mass of formulation	in development	conventional	passive
Novel formulation without a host. Submicron particles containing mostly active substance and a small amount of excipient-enhancing growth	in development	in development	active/passive

The drug release of ivermectin from SLN formulation is quite slow due to the lipophilic nature of ivermectin. However, it is still faster than the dissolution rate of ivermectin suspension in the same instrumentation setting. This is probably due to the large particle size of the ivermectin suspension. Complete release of loaded ivermectin takes about one day, during which only about 50% of ivermectin dissolves from the ivermectin suspension (66). In the in vivo setting, the rate of drug release may be faster due to the enzymatic destruction of the nanoparticles (67). In addition, being loaded in SLN does not necessarily mean reduced antiviral activity compared to unbonded drugs. In some cases, loaded drugs show similar antiviral activity compared to free drugs (68) and even higher (69). Therefore, the effect of slow release of ivermectin from SLN on antiviral activity should be assessed prior to in vivo development.

In the aforementioned discussion regarding the various technologies of inhalation administration, nanocarriers primarily play a role in aiding the formulation in reaching the minimum concentration necessary to achieve the MIC of ivermectin for SARS-CoV-2. Additionally, nanocarriers also contribute to concentrating the drug in the alveoli by several mechanisms. The surface of nanocarriers, including nanodroplets, SLN, and NLC, contains the hydrophilic part of surfactant components, which is in agreement in polarity with the aqueous film covering the surface of mucus (70) resulting in a high spread ability of nanocarriers. While the hydrophobic nature (and the particle charge) of ivermectin is hidden in the core of nanocarriers, the surface of nanocarriers could be managed easily in positive charge showing a higher alveolar cell and alveolar surfactant interaction (71). The second mechanism involves the alveolar clearance, a process by which xenobiotics

are eliminated from the alveoli. The alveolar bloodstream absorbs soluble xenobiotics rapidly and delivers them to the systemic circulation. Whereas, the insoluble xenobiotics are engulfed by the macrophage (72). Since nanocarrier is a phase, i.e. insoluble, it is cleared from the alveoli by macrophage engulfment. This mechanism can be hindered by surface modification, for instance by mixing natural or synthetic pulmonary surfactant, i.e. phosphatidylcholine-rich surfactant (73,74), with the ivermectin nanocarriers. Moreover, chemical-physical properties of the drug product preparation can be tailored by nanocarrier systems, for instance surface modification.

Nanocarrier Modification to Increase Lung Concentration

Since SARS-CoV-2 of COVID-19 patients is concentrated mainly on the alveolar surface, the inhalation delivery system of ivermectin is intended for local therapy. Therefore, it is the purpose of this drug delivery system to retain nanocarriers in the lung, instead of allowing the nanocarriers to be absorbed by the blood perfusing the lung. Several surface modifiers, as listed in Table 3, can be attached onto the nanocarriers to reduce drug absorption by blood circulation.

Table 3. Potential substances as targeting agents for the alveoli

Class of molecule	Substance	Mechanism	Reference
Carbohydrate	mannose, mannan, sulfated carbohydrate, galactose, dextran, amylopectin	Interaction with the alveolar macrophage*)	(75)
Protein	fibronectin, collagen		
Glycoprotein	Laminin		
Phosphate	Diacethylphosphate		
Peptide	Amino acid chain: CGSPGWVRC	Interaction with the lung endothelial cell	(76)
heteropolysaccharide	high-methoxyl pectins	Adhesion to the pleural surface	(77)
	Aminated PVA	In situ polymerization	(78)
	Glutaraldehyde		

*) These targeting substances are effective for non-pulmonary administration in which the drug is on the way to the alveoli. For pulmonary administration, these are not effective since the particle engulfment by alveolar macrophages eliminates the particle from the alveoli.

In the following section, several in vivo studies of the inhalation delivery of ivermectin are discussed. None of them successfully developed a system to deliver ivermectin, reaching an effective lung concentration. Therefore, the application of nanocarriers' surface modifiers as listed in Table 3 for ivermectin delivery via pulmonary needs to be conducted.

In Vivo studies of ivermectin inhalation delivery

An ivermectin solution in ethanol was administered using oxygen flow nebulizing the solution to 0.5 – 2 µm in size. The droplets were inhaled by female rats at a dose of about 120 mg/kg, and plasma and lung concentrations of ivermectin were determined. The plasma concentration of three rats euthanized at 72, 125, and 168 hours showed the same ivermectin level of 30 ng/ml, whereas in the lungs, the concentration decreased over time, resulting in lung to plasma ratios of 11, 6, and 3, for 72, 125, and 168 hours, respectively [79]. Since the virucidal activity of ivermectin against Sars-Cov-2 in the in vitro test was about 4 µg/ml, the administration did not reach the expected concentration.

In other research, PMDI formulation of ivermectin suspension containing propellant 134a, ethanol, and oleic acid was administered in a pig model at a dose of about 0.2 mg/kg BW. Ivermectin levels in nasopharyngeal, lungs, and plasma were determined at 2, 4, and 6 hours after treatment and were found to be below the effective concentration for inhibiting SARS-Cov-2 [39]. The highest ivermectin concentration was achieved in the nasopharynx, indicating that the droplets may be above the optimal inhalable size for pulmonary delivery.

The safety evaluation of inhalation formulations as nebulizer and PMDI, as discussed above, shows that there were not any signs of toxicity in the lungs [39,79], thereby there is a space for dose enhancement. Although the concentration is below the minimum level of virucidal activity against Sars-Cov-2, inhalation delivery, as exemplified above, has potential for lung targeting due to its high lung-to-plasma ratio. The targeted concentration may be achieved not only by increasing the dose due to the low level of toxicity, but also by optimizing the droplet size. For this purpose, along with the development of preparation methods, the nanocarrier material selection also plays a critical role.

Excipients for ivermectin lung targeted nanocarrier preparation

As previously discussed, SLN and NLC are selected as carriers for ivermectin pulmonary administration as a DPI, whereas nanoemulsion droplets and nanomicelles are prepared for nebulizer formulations. In this subsection, the effect of the material used for nanocarrier formulation on nanocarrier properties is discussed. Amphiphiles (surface active agents/surfactants) are necessary components of these carriers to stabilize nanoemulsion droplets and lipid nanoparticles by reducing surface tension and constructing monomolecular membranes covering the lipoidal phase [80]. Based on the charge of the hydrophilic part, surfactants are classified into four groups, namely nonionic, anionic, cationic, and amphoteric surfactants. In general, ionic surfactants generate positive or negative zeta potentials of structured nanocarriers due to the localization of the cationic or anionic hydrophilic group of the surfactant on the surface of the nanocarrier. As a result, electro-repulsion between the nanocarriers appears, inhibiting the flocculation or aggregation instability during storage [81]. However, the toxicity issue of ionic surfactants, in particular cationic surfactants except the naturally occurring pulmonary surfactant dipalmitoyl phosphatidylcholine [82–87], has resulted in these types being rarely used for oral formulation nowadays [88]. Therefore, nonionic surfactants producing near-zero zeta potential are preferred for nanocarrier formulation.

In the selection of nonionic surfactants, one should consider a measure of surfactant hydrophilicity, namely the hydrophilic-lipophilic balance (HLB). The HLB value of nonionic surfactants is available elsewhere [89]. The HLB value of the surfactant mixture can be calculated as the sum of the product of the mass fraction and the HLB value of each surfactant. In order to achieve a stable lipid nanocarrier in the desired size, e.g. less than 200 nm, using as low as possible surfactant concentration, the HLB of the surfactant or mixture of surfactants should be equivalent to the O/W required HLB (rHLB) of the composed oils or lipids [80].

The second component of SLN is solid lipid, where the drugs are dissolved. Several groups of lipid phase of SLN are well known, including fatty acid, fatty alcohol, wax (esterified fatty acid with fatty alcohol), di-and triglyceride (glycerol di- and tri fatty acid ester), steroid (for instance cholesterol) as well as natural fats containing a mixture of various glyceride and fatty acid (for instances cocoa butter and goat fat) [90–93]. The lipid types used for SLN formulation determine the physical and biological properties of SLN. One of the important physical properties of SLN is the loading or encapsulation capacity (LC) calculated as the drug concentration in the lipid nanocarrier (usually

as mass percent). Researchers use several methods to study the effect of lipid compounds on LC, either by directly comparing the LC or a parameter affected by LC of a drug in a particular nanocarrier containing various lipids. The last method is exemplified in retinol SLN formulation as follows. By evaluating the chemical degradation of retinol at room temperature, in which the rate of degradation is inversely related to LC, it is concluded that the LC of SLN composed of glyceryl behenate>cetyl palmitate II (amorphous)>tripalmitate>cetyl palmitate I (crystalline) [94]. The first method was applied to measure the LC of nitrendipine by destruction drug drug-loaded SLN using a chloroform/methanol mixture, followed by drug quantification using liquid chromatography. The result showed that the LC of nanocarrier composed of glyceryl tripalmitate>cetyl palmitate>glyceryl monostearate [95]. In another study formulating methotrexate loaded SLN, it was revealed that if the fatty acid chain length is the same, namely stearic acid, the LC of methotrexate loaded in SLN cored by glyceryl monostearate>glyceryl tristearate>stearic acid [96]. Moreover, if the core of SLN is composed only of fatty acids, the longer methylene chain of fatty acids produces a higher LC. For instance, in the encapsulation process of enrofloxacin, the drug LC was ordered by stearic acid C-18>palmitic acid C-16>myristic acid C-14 [97].

The LC is influenced by (a) the solubility of the drug in molten lipid (about 5 degrees above the melting point) and (b) the crystal packing and crystallinity of the selected lipid under the storage conditions. The solubility of drugs in a given solvent, including the liquid lipids, which can be predicted using the Hansen solubility parameter, is the physico-chemical property of the drug [98,99]. Thus, by choosing a particular lipid or mixture of lipids, based solely on solubility theory, the LC cannot be modified. However, it is mentioned previously that SLN containing amorphous cetyl palmitate showed a higher LC than SLN in the same composition but in the crystalline form of cetyl palmitate [94]. Therefore, the crystal packing of lipids influenced by the preparation method determines the LC of the drug in SLN. Josep and co-workers predicted that nanocarriers containing a lipid or lipid mixture result in higher drug loading when applying the metastable crystalline form [100]. However, selecting metastable polymorph of lipid or lipids mixture as SLN core to increase LC, for instance by faster crystallization using lower temperature in the SLN preparation [101], is avoided due to the instability issue, i.e. the metastable polymorph (or amorphous form) of SLN core transform to stable one during storage triggers the expulsion of the loaded drug [102,103]. Therefore, it is preferred to increase crystal spacing by mixing several lipids and processing the crystallization slowly to achieve a stable form. Additionally, the SLN cores produced by lipid mixtures may contain more crystal defects (low crystallinity), where more drug can occupy these sites, resulting in a higher LC [90,103–105]. That explains why Compritol® 888 ATO (the mixture of glyceryl mono-, di-, and tribehenate) shows a higher drug loading than Imwitor® 900 (glyceryl monostearate) and Dynasan® 116 (glyceryl tripalmitate) [105].

Liquid lipids are frequently added to solid lipids as a core of nanocarriers to increase the drug loading due to the high availability of crystal defects and the amorphous part of the lipid mixture [106]. The nanocarrier, which is termed as nanostructured lipid carrier (NLC), lies between nanoemulsion droplets (ND) containing only liquid lipid in the core and SLN. Several classes of liquid lipid for ND and NLC include fatty acid (oleic acid, linoleic acid, linolenic acid, palmitoleic acid), ester fatty acid with glycerol (triglyceride: glyceryl tricaprylate, glyceryl trioleate, glyceryl-1-oleate-2,3-dicaprilate), and raw plant oil (palm oil, rice bran oil, virgin coconut oil).

Lung target-oriented delivery using injectable formulation: a glance

A class of carrier systems to load and deliver drugs to the target of action is cellular

carriers, for instance, blood cells, which can only be administered as an injection. White blood cells (WBC), the immune cells, traverse the inter-biological compartment freely to reach the area of inflammation (107), which could be part of the body infected by microorganisms, including SARS-CoV-2. Therefore, loading of ivermectin in the WBC, in particular neutrophil and monocyte, is expected to increase drug delivery to the inflamed lungs (108,109). The phagocytic ability of WBC against particulates is explored in the drug loading process. The drug that is expected to be loaded or bonded is dispersed together with WBC to process the engulfment of the drug nanoparticle by the cellular carrier (110).

A different method is applied for utilizing red blood cells (RBC) as a cellular carrier to increase the lung distribution of active substance, namely RBC-hitchhiking. Instead of loading the drug into the cell, the drug nanocarrier is adsorbed on the surface of the RBC (111). Attaching a homing device, for instance IgG or anti-ICAM-1, on the surface of RBC-nanocarrier hitchhiking system increases the lung-to-liver/spleen ratio compared to the corresponding antibody-modified nanoparticle formulation (112). It is possible for several ivermectin-nanocarrier systems, as aforementioned above, to be adsorbed on the surface of RBC to increase lung distribution.

Conclusion

Ivermectin, an established anthelmintic agent, is being investigated for the potential treatment of COVID-19, a new disease caused by SARS-CoV-2 infection that emerged in 2019. Preclinical studies in animal models have demonstrated that this drug shows promising outcomes in preventing and curing SARS-CoV-2 infection, consistent with in vitro test results indicating the drug's ability to reduce the proliferation of SARS-Cov-2. However, the lack of clinical trial evidence has prevented the WHO and some local and regional health authorities from issuing approval for the use of ivermectin in the treatment of COVID-19. The research to level up ivermectin from in vitro-in vivo anti-SARS-CoV-2 evidenced drug to be formally used in COVID-19 patients should be conducted, particularly the development of inhalation drug delivery, involving nanocarrier systems. The research should focus on the development of ivermectin nanoemulsion and nanomicelle dispersion in water for nebulizer and pressurized metered dose inhalers (PMDI) administration, expanding to foster Dry Powder Inhalers (DPI) formulation containing freeze-drying product of ivermectin-SLN or -NLC. Additionally, size-specific spherical ivermectin powder prepared by particle engineering also has the potential to be aerosolized by DPI technology. This systematic research will trigger the success of ivermectin-targeted delivery to SARS-Cov-2 in the lungs of COVID-19 patients.

Competing interests:

The authors declare that they have no competing interests

Funding:

The authors declared that this study received no financial support.

Authors' contributions:

AB contributed to all the work concerning this manuscript

References

1. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res. 2020;178:104787.
2. Ivermectin (Internet). COVID-19 Treat. Guidel. (cited 2023 Aug 18). Available from: https://www.covid19treatmentguidelines.nih.gov/therapies/miscellaneous-drugs/ivermectin/
3. Marcolino MS, Meira KC, Guimarães NS,

Motta PP, Chagas VS, Kelles SMB, et al. Systematic review and meta-analysis of ivermectin for treatment of COVID-19: evidence beyond the hype. BMC Infect Dis. 2022;22:639.

4. Smit MR, Ochomo EO, Waterhouse D, Kwambai TK, Abong'o BO, Bousema T, et al. Pharmacokinetics-Pharmacodynamics of High-Dose Ivermectin with Dihydroartemisinin-Piperaquine on Mosquitocidal Activity and QT-Prolongation (IVERMAL). Clin Pharmacol Ther. 2019;105:388–401.

5. Chaccour C, Hammann F, Ramón-García S, Rabinovich NR. Ivermectin and COVID-19: Keeping Rigor in Times of Urgency. Am J Trop Med Hyg. 2020;102:1156–7.

6. Sznitowska M, Pietkiewicz J, Stokrocka M, Janicki S. Dissolution test for ivermectin in oral veterinary paste. Pharm. 2004;59:814–5.

7. Kiki-Mvouaka S, Ménez C, Borin C, Lyazrhi F, Foucaud-Vignault M, Dupuy J, et al. Role of P-glycoprotein in the disposition of macrocyclic lactones: A comparison between ivermectin, eprinomectin, and moxidectin in mice. Drug Metab Dispos Biol Fate Chem. 2010;38:573–80.

8. Lacher SE, Skagen K, Veit J, Dalton R, Woodahl EL. P-Glycoprotein Transport of Neurotoxic Pesticides. J Pharmacol Exp Ther. 2015;355:99–107.

9. Kern C, Müller P, Chaccour C, Liechti ME, Hammann F, Duthaler U. Pharmacokinetics of ivermectin metabolites and their activity against Anopheles stephensi mosquitoes. Malar J. 2023;22:194.

10. Klotz U, Ogbuokiri JE, Okonkwo PO. Ivermectin binds avidly to plasma proteins. Eur J Clin Pharmacol. 1990;39:607–8.

11. Chaccour C, Hammann F, Rabinovich NR. Ivermectin to reduce malaria transmission I. Pharmacokinetic and pharmacodynamic considerations regarding efficacy and safety. Malar J. 2017;16:161.

12. Uematsu T, Takano T, Matsui H, Kobayashi N, Ōmura S, Hanaki H. Prophylactic administration of ivermectin attenuates SARS-CoV-2 induced disease in a Syrian Hamster Model. J Antibiot (Tokyo). 2023;76:481–8.

13. Arévalo AP, Pagotto R, Pórfido JL, Daghero H, Segovia M, Yamasaki K, et al. Ivermectin reduces in vivo coronavirus infection in a mouse experimental model. Sci Rep. 2021;11:7132.

14. de Melo GD, Lazarini F, Larrous F, Feige L, Kornobis E, Levallois S, et al. Attenuation of clinical and immunological outcomes during SARS-CoV-2 infection by ivermectin. EMBO Mol Med. 2021;13:e14122.

15. Naggie S, Boulware DR, Lindsell CJ, Stewart TG, Gentile N, Collins S, et al. Effect of Ivermectin vs Placebo on Time to Sustained Recovery in Outpatients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2022;328:1595–603.

16. Chaccour C, Casellas A, Blanco-Di Matteo A, Pineda I, Fernandez-Montero A, Ruiz-Castillo P, et al. The effect of early treatment with ivermectin on viral load, symptoms and humoral response in patients with non-severe COVID-19: A pilot, double-blind, placebo-controlled, randomized clinical trial. EClinicalMedicine. 2021;32:100720.

17. Myers TR. The science guiding selection of an aerosol delivery device. Respir Care. 2013;58:1963–73.

18. Ibrahim M, Verma R, Garcia-Contreras L. Inhalation drug delivery devices: technology update. Med Devices Auckl NZ. 2015;8:131–9.

19. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. Lancet Respir Med.

2014;2:497–506.

20. Darquenne C. Aerosol Deposition in Health and Disease. J Aerosol Med Pulm Drug Deliv. 2012;25:140–7.

21. Jabbal S, Poli G, Lipworth B. Does size really matter?: Relationship of particle size to lung deposition and exhaled fraction. J Allergy Clin Immunol. 2017;139:2013-2014. e1.

22. Perinel S, Leclerc L, Prévôt N, Deville A, Cottier M, Durand M, et al. Micron-sized and submicron-sized aerosol deposition in a new ex vivo preclinical model. Respir Res. 2016;17:78.

23. Komer G. Avermectin formulation (Internet). 1998 (cited 2023 Aug 22). Available from: https://patents.google.com/patent/US5773422A/en

24. Fronius M, Clauss WG, Althaus M. Why Do We have to Move Fluid to be Able to Breathe? Front Physiol. 2012;3:146.

25. Park HM, Chang KH, Moon S-H, Park BJ, Yoo SK, Nam KC. In vitro delivery efficiencies of nebulizers for different breathing patterns. Biomed Eng OnLine. 2021;20:59.

26. Force C-C of the T, Boe J, Dennis JH, O'Driscoll BR, Force M of T, Bauer TT, et al. European Respiratory Society Guidelines on the use of nebulizers: Guidelines prepared by a European Respiratory Society Task Force on the use of nebulizers. Eur Respir J. 2001;18:228–42.

27. Nesamony J, Shah IS, Kalra A, Jung R. Nebulized oil-in-water nanoemulsion mists for pulmonary delivery: development, physico-chemical characterization and in vitro evaluation. Drug Dev Ind Pharm. 2014;40:1253–63.

28. Amani A, York P, Chrystyn H, Clark BJ. Evaluation of a Nanoemulsion-Based Formulation for Respiratory Delivery of Budesonide by Nebulizers. AAPS PharmSciTech. 2010;11:1147–51.

29. Asmawi AA, Salim N, Ngan CL, Ahmad H, Abdulmalek E, Masarudin MJ, et al. Excipient selection and aerodynamic characterization of nebulized lipid-based nanoemulsion loaded with docetaxel for lung cancer treatment. Drug Deliv Transl Res. 2019;9:543–54.

30. Nasr M, Nawaz S, Elhissi A. Amphotericin B lipid nanoemulsion aerosols for targeting peripheral respiratory airways via nebulization. Int J Pharm. 2012;436:611–6.

31. Gilani K, Moazeni E, Ramezanli T, Amini M, Fazeli MR, Jamalifar H. Development of respirable nanomicelle carriers for delivery of amphotericin B by jet nebulization. J Pharm Sci. 2011;100:252–9.

32. Pellosi DS, d'Angelo I, Maiolino S, Mitidieri E, d'Emmanuele di Villa Bianca R, Sorrentino R, et al. In vitro/in vivo investigation on the potential of Pluronic® mixed micelles for pulmonary drug delivery. Eur J Pharm Biopharm Off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV. 2018;130:30–8.

33. Moazeni E, Gilani K, Najafabadi AR, Reza Rouini M, Mohajel N, Amini M, et al. Preparation and evaluation of inhalable itraconazole chitosan based polymeric micelles. Daru. 2012;20:85.

34. Guan W, Tang L, Wang Y, Cui H. Fabrication of an Effective Avermectin Nanoemulsion Using a Cleavable Succinic Ester Emulsifier. J Agric Food Chem. 2018;66:7568–76.

35. Lo P-KA, Williams JB. Solubilization of ivermectin in water (Internet). 1982 (cited 2021 Nov 18). Available from: https://patents.google.com/patent/EP0045655A2/en

36. Dong J, Song X, Lian X, Fu Y, Gong T. Subcutaneously injected ivermectin-loaded mixed micelles: formulation, pharmacokinetics and local irritation study. Drug Deliv. 2016;23:2220–7.

37. Mogalian E, Myrdal PB. Pharmaceutical Solvents for Pulmonary Drug Delivery. In: Augustijns P, Brewster ME, editors. Solvent Syst Their Sel Pharm Biopharm (Internet). New York, NY: Springer; 2007 (cited 2022 Dec 8). p. 427–41. Available from: https://doi.org/10.1007/978-0-387-69154-1_14

38. Myrdal PB, Sheth P, Stein SW. Advances in Metered Dose Inhaler Technology: Formulation Development. AAPS PharmSciTech. 2014;15:434–55.

39. Errecalde J, Lifschitz A, Vecchioli G, Ceballos L, Errecalde F, Ballent M, et al. Safety and Pharmacokinetic Assessments of a Novel Ivermectin Nasal Spray Formulation in a Pig Model. J Pharm Sci. 2021;110:2501–7.

40. Vega JC. Delivery of aerosolized micromolar composition concentrations (Internet). 2021 (cited 2022 Dec 9). Available from: https://patents.google.com/patent/WO2021247283A1/en

41. Bernhard W, Hoffmann S, Dombrowsky H, Rau GA, Kamlage A, Kappler M, et al. Phosphatidylcholine molecular species in lung surfactant: composition in relation to respiratory rate and lung development. Am J Respir Cell Mol Biol. 2001;25:725–31.

42. Rolim LA, dos Santos FCM, Chaves LL, Gonçalves MLCM, Freitas-Neto JL, da Silva do Nascimento AL, et al. Preformulation study of ivermectin raw material. J Therm Anal Calorim. 2015;120:807–16.

43. Han X, Ghoroi C, To D, Chen Y, Davé R. Simultaneous micronization and surface modification for improvement of flow and dissolution of drug particles. Int J Pharm. 2011;415:185–95.

44. Hussain K, Qamar A, Bukhari NI, Hussain A, Shehzadi N, Qamar S, et al. Impact of Particle-Size Reduction on the Solubility and Antidiabetic Activity of Extracts of Leaves of Vinca rosea. Turk J Pharm Sci. 2019;16:335–9.

45. Dalvi SV, Dave RN. Controlling Particle Size of a Poorly Water-Soluble Drug Using Ultrasound and Stabilizers in Antisolvent Precipitation. Ind Eng Chem Res. 2009;48:7581–93.

46. Chang RYK, Chan H-K. Advancements in Particle Engineering for Inhalation Delivery of Small Molecules and Biotherapeutics. Pharm Res (Internet). 2022 (cited 2022 Dec 9); Available from: https://doi.org/10.1007/s11095-022-03363-2

47. Albariqi AH, Ke W-R, Khanal D, Kalfas S, Tang P, Britton WJ, et al. Preparation and Characterization of Inhalable Ivermectin Powders as a Potential COVID-19 Therapy. J Aerosol Med Pulm Drug Deliv. 2022;35:239–51.

48. Saha T, Sinha S, Harfoot R, Quiñones-Mateu ME, Das SC. Manipulation of Spray-Drying Conditions to Develop an Inhalable Ivermectin Dry Powder. Pharmaceutics. 2022;14:1432.

49. Naseri N, Valizadeh H, Zakeri-Milani P. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Structure, Preparation and Application. Adv Pharm Bull. 2015;5:305–13.

50. Khan AA, Mudassir J, Akhtar S, Murugaiyah V, Darwis Y. Freeze-Dried Lopinavir-Loaded Nanostructured Lipid Carriers for Enhanced Cellular Uptake and Bioavailability: Statistical Optimization, in Vitro and in Vivo Evaluations. Pharmaceutics. 2019;11:97.

51. Mozaffar S, Radi M, Amiri S, McClements DJ. A new approach for drying of nanostructured lipid carriers (NLC) by spray-drying and using sodium chloride as the excipient. J Drug Deliv Sci Technol. 2021;61:102212.

52. Alihosseini F, Ghaffari S, Dabirsiaghi AR, Haghighat S. Freeze-drying of ampicil-

lin solid lipid nanoparticles using mannitol as cryoprotectant. Braz J Pharm Sci. 2015;51:797–802.

53. Maretti E, Costantino L, Buttini F, Rustichelli C, Leo E, Truzzi E, et al. Newly synthesized surfactants for surface mannosylation of respirable SLN assemblies to target macrophages in tuberculosis therapy. Drug Deliv Transl Res. 2019;9:298–310.

54. Maretti E, Costantino L, Rustichelli C, Leo E, Croce MA, Buttini F, et al. Surface engineering of Solid Lipid Nanoparticle assemblies by methyl α-d-mannopyranoside for the active targeting to macrophages in anti-tuberculosis inhalation therapy. Int J Pharm. 2017;528:440–51.

55. Maretti E, Rustichelli C, Romagnoli M, Balducci AG, Buttini F, Sacchetti F, et al. Solid Lipid Nanoparticle assemblies (SLNas) for an anti-TB inhalation treatment-A Design of Experiments approach to investigate the influence of pre-freezing conditions on the powder respirability. Int J Pharm. 2016;511:669–79.

56. Nemati E, Mokhtarzadeh A, Panahi-Azar V, Mohammadi A, Hamishehkar H, Mesgari-Abbasi M, et al. Ethambutol-Loaded Solid Lipid Nanoparticles as Dry Powder Inhalable Formulation for Tuberculosis Therapy. AAPS PharmSciTech. 2019;20:120.

57. Almurshedi AS, Aljunaidel HA, Alquadeib B, Aldosari BN, Alfagih IM, Almarshidy SS, et al. Development of Inhalable Nanostructured Lipid Carriers for Ciprofloxacin for Noncystic Fibrosis Bronchiectasis Treatment. Int J Nanomedicine. 2021;16:2405–17.

58. Ahmadpour E, Godrati-Azar Z, Spotin A, Norouzi R, Hamishehkar H, Nami S, et al. Nanostructured lipid carriers of ivermectin as a novel drug delivery system in hydatidosis. Parasit Vectors. 2019;12:469.

59. Guo D, Dou D, Li X, Zhang Q, Bhutto ZA, Wang L. Ivermection-loaded solid lipid nanoparticles: preparation, characterisation, stability and transdermal behaviour. Artif Cells Nanomedicine Biotechnol. 2018;46:255–62.

60. Malamatari M, Charisi A, Malamataris S, Kachrimanis K, Nikolakakis I. Spray Drying for the Preparation of Nanoparticle-Based Drug Formulations as Dry Powders for Inhalation. Processes. 2020;8:788.

61. Musicanti C, Gasco P. Solid Lipid Nanoparticles - SLN. In: Bhushan B, editor. Encycl Nanotechnol (Internet). Dordrecht: Springer Netherlands; 2012 (cited 2021 Nov 20). p. 2471–87. Available from: https://doi.org/10.1007/978-90-481-9751-4_249

62. Schwarz C, Mehnert W. Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). Int J Pharm. 1997;157:171–9.

63. Grasmeijer F, Hagedoorn P, Frijlink HW, Boer AH de. Drug Content Effects on the Dispersion Performance of Adhesive Mixtures for Inhalation. PLOS ONE. 2013;8:e71339.

64. Farkas DR, Hindle M, Longest PW. Characterization of a New High-Dose Dry Powder Inhaler (DPI) Based on a Fluidized Bed Design. Ann Biomed Eng. 2015;43:2804–15.

65. Son Y-J, Worth Longest P, Hindle M. Aerosolization characteristics of dry powder inhaler formulations for the excipient enhanced growth (EEG) application: Effect of spray drying process conditions on aerosol performance. Int J Pharm. 2013;443:137–45.

66. Gamboa GVU, Palma SD, Lifschitz A, Ballent M, Lanusse C, Passirani C, et al. Ivermectin-loaded lipid nanocapsules: toward the development of a new antiparasitic delivery system for veterinary applications.

Parasitol Res. 2016;115:1945–53.

67. Vlasova II, Kapralov AA, Michael ZP, Burkert SC, Shurin MR, Star A, et al. Enzymatic Oxidative Biodegradation of Nanoparticles: Mechanisms, Significance and Applications. Toxicol Appl Pharmacol. 2016;299:58–69.

68. Javan F, Vatanara A, Azadmanesh K, Nabi-Meibodi M, shakouri M. Encapsulation of ritonavir in solid lipid nanoparticles: in-vitro anti-HIV-1 activity using lentiviral particles. J Pharm Pharmacol. 2017;69:1002–9.

69. Zhang X, Miao J, Li M, Jiang S, Hu F, Du Y. Solid lipid nanoparticles loading adefovir dipivoxil for antiviral therapy. J Zhejiang Univ Sci B. 2008;9:506–10.

70. Seadler BD, Toro F, Sharma S. Physiology, Alveolar Tension. StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2025 (cited 2025 Jun 28). Available from: http://www.ncbi.nlm.nih.gov/books/NBK539825/

71. Wang F, Liu J, Zeng H. Interactions of particulate matter and pulmonary surfactant: Implications for human health. Adv Colloid Interface Sci. 2020;284:102244.

72. Pant AB. Alveolar Clearance. Dict Toxicol (Internet). Springer, Singapore; 2024 (cited 2025 Jun 28). p. 47–47. Available from: https://link.springer.com/rwe/10.1007/978-981-99-9283-6_117

73. Waring AJ, Jung GC-L, Sharma SK, Walther FJ. Lung Surfactant Protein B Peptide Mimics Interact with the Human ACE2 Receptor. Int J Mol Sci. 2023;24:10837.

74. Carregal-Romero S, Groult H, Cañadas O, A-Gonzalez N, Lechuga-Vieco AV, García-Fojeda B, et al. Delayed alveolar clearance of nanoparticles through control of coating composition and interaction with lung surfactant protein A. Biomater Adv. 2022;134:112551.

75. Costa A, Sarmento B, Seabra V. Targeted Drug Delivery Systems for Lung Macrophages. Curr Drug Targets. 2015;16:1565–81.

76. Giordano RJ, Edwards JK, Tuder RM, Arap W, Pasqualini R. Combinatorial Ligand-directed Lung Targeting. Proc Am Thorac Soc. 2009;6:411–5.

77. Zheng Y, Pierce AF, Wagner WL, Khalil HA, Chen Z, Servais AB, et al. Functional Adhesion of Pectin Biopolymers to the Lung Visceral Pleura. Polymers. 2021;13:2976.

78. Joglekar MM, Slebos D-J, Leijten J, Burgess JK, Pouwels SD. Crosslink bio-adhesives for bronchoscopic lung volume reduction: current status and future direction. Eur Respir Rev. 2021;30:210142.

79. Chaccour C, Abizanda G, Irigoyen-Barrio Á, Casellas A, Aldaz A, Martínez-Galán F, et al. Nebulized ivermectin for COVID-19 and other respiratory diseases, a proof of concept, dose-ranging study in rats. Sci Rep. 2020;10:17073.

80. Martin A, Bustamante P, Chun AHC. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Science. 3rd ed. Philadelpia: Lea & Feriger; 1993.

81. Van Tran V, Loi Nguyen T, Moon J-Y, Lee Y-C. Core-shell materials, lipid particles and nanoemulsions, for delivery of active anti-oxidants in cosmetics applications: challenges and development strategies. Chem Eng J. 2019;368:88–114.

82. Hwang T-L, Aljuffali IA, Lin C-F, Chang Y-T, Fang J-Y. Cationic additives in nanosystems activate cytotoxicity and inflammatory response of human neutrophils: lipid nanoparticles versus polymeric nanoparticles. Int J Nanomedicine. 2015;10:371–85.

83. Vlachy N, Touraud D, Heilmann J, Kunz W. Determining the cytotoxicity of catanionic surfactant mixtures on HeLa cells. Colloids

Surf B Biointerfaces. 2009;70:278–80.

84. Zhang Y, Li X, Yu H. Toxicity of nanoparticle surface coating agents: Structure-cytotoxicity relationship. J Environ Sci Health Part C. 2016;34:204–15.

85. Zhang Y, Newton B, Lewis E, Fu PP, Kafoury R, Ray PC, et al. Cytotoxicity of organic surface coating agents used for nanoparticles synthesis and stability. Toxicol In Vitro. 2015;29:762–8.

86. Wang S, Lu W, Tovmachenko O, Rai US, Yu H, Ray PC. Challenge in understanding size and shape dependent toxicity of gold nanomaterials in human skin keratinocytes. Chem Phys Lett. 2008;463:145–9.

87. Franco-Belussi L, Jones-Costa M, Salla RF, Souza BFS, Pinto-Vidal FA, Oliveira CR, et al. Hepatotoxicity of the anionic surfactant linear alkylbenzene sulphonate (LAS) in bullfrog tadpoles. Chemosphere. 2021;266:129014.

88. Hauss DJ. Oral lipid-based formulations. Adv Drug Deliv Rev. 2007;59:667–76.

89. Nollet M, Boulghobra H, Calligaro E, Rodier J-D. An efficient method to determine the Hydrophile-Lipophile Balance of surfactants using the phase inversion temperature deviation of CiEj/n-octane/water emulsions. Int J Cosmet Sci. 2019;41:99–108.

90. Duan Y, Dhar A, Patel C, Khimani M, Neogi S, Sharma P, et al. A brief review on solid lipid nanoparticles: part and parcel of contemporary drug delivery systems. RSC Adv. 10:26777–91.

91. Salminen H, Stübler A-S, Weiss J. Preparation, characterization, and physical stability of cocoa butter and tristearin nanoparticles containing β-carotene. Eur Food Res Technol. 2020;246:599–608.

92. Jain SK, Chourasia MK, Masuriha R, Soni V, Jain A, Jain NK, et al. Solid Lipid Nanoparticles Bearing Flurbiprofen for Transdermal Delivery. Drug Deliv. 2005;12:207–15.

93. Attama AA, Schicke BC, Paepenmüller T, Müller-Goymann CC. Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: Characterization. Eur J Pharm Biopharm. 2007;67:48–57.

94. Jenning V, Gohla S. Comparison of wax and glyceride solid lipid nanoparticles (SLN®). Int J Pharm. 2000;196:219–22.

95. Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: Influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm. 2007;335:167–75.

96. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, et al. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomedicine Nanotechnol Biol Med. 2009;5:184–91.

97. Xie S, Zhu L, Dong Z, Wang X, Wang Y, Li X, et al. Preparation, characterization and pharmacokinetics of enrofloxacin-loaded solid lipid nanoparticles: Influences of fatty acids. Colloids Surf B Biointerfaces. 2011;83:382–7.

98. Sakellari GI, Zafeiri I, Batchelor H, Spyropoulos F. Formulation design, production and characterisation of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for the encapsulation of a model hydrophobic active. Food Hydrocoll Health. 2021;1:100024.

99. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm. 2000;50:161–77.

100. Joseph S, Rappolt M, Schoenitz M, Huzhalska V, Augustin W, Scholl S, et al. Stability of the Metastable α-Polymorph in

Solid Triglyceride Drug-Carrier Nanoparticles. Langmuir. 2015;31:6663–74.

101. Long C, Zhang L, Qian Y. Preparation and Crystal Modification of Ibuprofen-Loaded Solid Lipid Microparticles1 1Supported by the National Natural Science Foundation of China (No.20536020, No.20476033), the China Distinguished Young Scientist Fund (No.20225620) and Guangdong Province Science Fund (No.04020121). Chin J Chem Eng. 2006;14:518–25.

102. Jenning V, Schäfer-Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Controlled Release. 2000;66:115–26.

103. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Controlled Release. 1997;48:223–36.

104. Vivek K, Reddy H, Murthy RSR. Investigations of the effect of the lipid matrix on drug entrapment, in vitro release, and physical stability of olanzapine-loaded solid lipid nanoparticles. AAPS PharmSciTech. 2007;8:16–24.

105. Abdelbary G, Fahmy RH. Diazepam-Loaded Solid Lipid Nanoparticles: Design and Characterization. AAPS PharmSciTech. 2009;10:211–9.

106. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13:288–303.

107. Chu D, Dong X, Shi X, Zhang C, Wang Z. Neutrophil-Based Drug Delivery Systems. Adv Mater Deerfield Beach Fla. 2018;30:e1706245.

108. Anselmo AC, Gilbert JB, Kumar S, Gupta V, Cohen RE, Rubner MF, et al. Monocyte-mediated delivery of polymeric backpacks to inflamed tissues: a generalized strategy to deliver drugs to treat inflammation. J Controlled Release. 2015;199:29–36.

109. Chu D, Gao J, Wang Z. Neutrophil-Mediated Delivery of Therapeutic Nanoparticles across Blood Vessel Barrier for Treatment of Inflammation and Infection. ACS Nano. 2015;9:11800–11.

110. Wang S, Han K, Ma S, Qi X, Guo L, Li X. Blood cells as supercarrier systems for advanced drug delivery. Med Drug Discov. 2022;13:100119.

111. Brenner JS, Pan DC, Myerson JW, Marcos-Contreras OA, Villa CH, Patel P, et al. Red blood cell-hitchhiking boosts delivery of nanocarriers to chosen organs by orders of magnitude. Nat Commun. 2018;9:2684.

112. Anselmo AC, Kumar S, Gupta V, Pearce AM, Ragusa A, Muzykantov V, et al. Exploiting shape, cellular-hitchhiking and antibodies to target nanoparticles to lung endothelium: Synergy between physical, chemical and biological approaches. Biomaterials. 2015;68:1–8.