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Formulations for COVID-19 Early Stage Treatment via Silver Nanoparticles Inhalation Delivery at Home and Hospital

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Abstract

Objectives: For suppressing both viral and bacterial respiratory infections, we investigate the possibility of obtaining real effective minimal inhibitory concentration (MIC) of silver nanoparticles in various respiratory system target locations. Applications include (i) control local outbreaks of COVID-19 via early stage home treatment, and (ii) lower the risk of ventilator associated pneumonia (VAP) in hospital ICU. Our prime objective is to propose a first line intervention measure with the potential to suppress proliferation of the viral infection across the respiratory system, thereby giving more time for proper immune system response and lowering the risk for aggravation and spread of the infection. We further discuss the available credible evidence for human safety consideration, by inhalation delivery, for facilitating immediate clinical trials. In addition, we discuss possible manufacturing and commercial availability of the method elements for near term wide public usage.

Method: Based on previously published experimental data, on the antiviral effectiveness of colloidal silver, we propose a model method and computation for achieving antiviral MIC of silver particles in various respiratory system locations, by: (a) analysing the nanoparticle size dependent required concentration. (b) computing the required aerosol delivery characteristics. In order to compute the require delivery dosage, we take into account deposition fraction losses and also inhalation time fraction of the normal breathing cycle. We evaluate independent targeting of: (i) the trachea-bronchial tree (mucus volume of about 1cc), and (ii) the alveoli (total mucus volume of about 10cc).

Results: The dosage is highly sensitive to the silver nanoparticle size, with 3nm - 7nm being the optimal size. Effective antibacterial MIC 10 μg/ml is estimated, but for more certainty 25 μg/ml is a reasonable target concentration to achieve in the mucus fluid of the respiratory system. In particular, using colloidal silver of 5nm particles, delivering inhalation of standard 5μ diameter droplets aerosol (e.g., using off-the-shelf ultrasonic mesh nebulizers), we assert that sufficient MIC can be achieved with: (i) depositing a total of just 0.25cc of a 100ppm (μg/ml) source concentration in the bronchial tree, and (ii) depositing a total of 1cc of a 250ppm (μg/ml) source concentration in the lungs alveoli. Yet, after accounting for deposition losses and due to the fact that active inhalation time is just about 1/3 of the breathing cycle, we find that that practical effective MIC can be achieved by these aerosolising dosages: (a) for the upper airways and bronchial tree use 2cc of a 100 μg/ml colloidal silver source, while (b) for lungs alveoli delivery use 6cc of a 200 μg/ml colloidal silver source. This would be reduced by a factor 3 if a breath actuated ultrasonic nebulizer is used.

Conclusions: We conclude that effective MIC is achievable, both in the bronchial tree and in the alveoli (though the specific aerosol prescription may differ). Since respiratory infections start most commonly in the upper airways, it would be best to use the presented method early on as a first line treatment to suppress the progression of the infection. The required formulations are presently not available on the market but are easy to mass produce OTC in principle. Using off-the-shelf ultrasonic nebulizers and providable OTC colloidal silver formulations, we posit that our suggested method can be used precautionarily at home by anyone feeling the early signs of a potential infection. In addition, due to the anti-bacterial properties of colloidal silver, our method can serve in hospital intensive care units (ICU) as a new standard of care prophylactic treatment for ventilator acquired pneumonia (VAP).

Introduction

When there are a core scientific and biologically grounded effects, the initial association with fringe unsubstantiated alternative "medicine" should not deter scientists from investigating the potential for success that may come when applying rigorous calculations and analytical reasoning. The antimicrobial properties of silver nanoparticles are well established and is medically FDA approved in field of wound care. A senseless alternative "medicine" practice of ingesting colloidal silver led to widespread disregard by the pharmacological establishment of potential new applications for the antimicrobial properties of silver nanoparticles (beyond wound treatment). In particular, there is no rigorous analysis in the literature concerning the potential of inhalation use for the prevention and/or treatment of respiratory infections.

The goal of this article is to analytically substantiate potential antimicrobial colloidal silver formulations, delivered by inhalation, to minimise the aggravation of respiratory system infections. In the context of the present discussion, we shall distinguish between two scenarios: (A) viral infections (including the recent COVID-19 / SARS-CoV-2); and (B) bacterial infections risk associated with hospital ventilator associated pneumonia (VAP) in intensive care units (ICU) patients on ventilation breathing support.

A common pathogenesis aspect of both is that the infections are commonly initiated mildly in the nasopharynx and/or bronchial tree portions of respiratory system [1,2]. Aggravation of the condition occurs once the pathogens and associated inflammation migrates to lower portions of the respiratory system. Moreover, as the infection spreads, the increased immune response is exacerbated and may cause a greater damage [3]. Therefore, we contend that a desirable effective treatment would be to suppress the proliferation of the pathogens at early stages of the infection, when it is cantered in the upper respiratory system. e.g., (i) when most patients are still at home with mild symptoms, and (ii) from day-one of patient's arrival to the hospital ICU before the signs of any VAP infection. In this article, we will propose potential formulations both for upper respiratory system treatment and for lower respiratory system (alveoli) treatment.

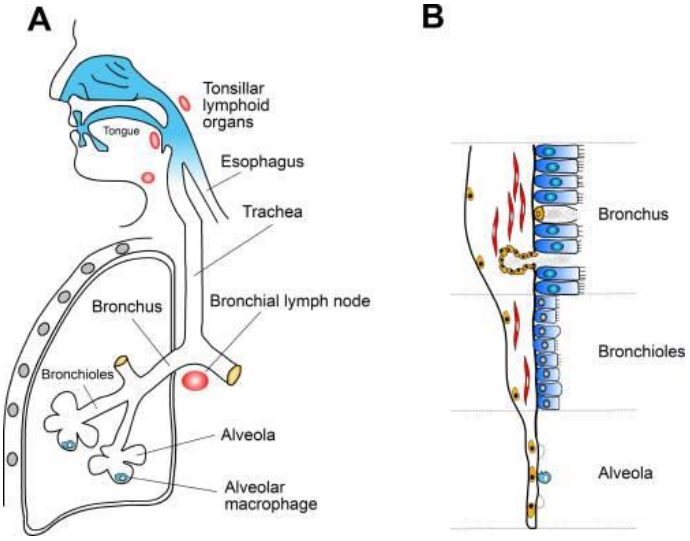

Figure 1: Sites of influenza entry in the respiratory tract. (A) The anatomical and functional structures of the human airways are shown. Influenza first infects the upper airway and the ciliated cells in the bronchus and bronchioli. Resulting clinical syndromes include tracheitis, bronchitis, bronchiolitis, and bronchopneumonia. The adaptive immune response is initiated in lymph nodes along the airways. (B) The respiratory epithelia is especially equipped to defend from incoming pathogens by a layer of mucus (bronchus), ciliated cells (bronchus and bronchioli), and alveolar macrophages (alveoli) [1].

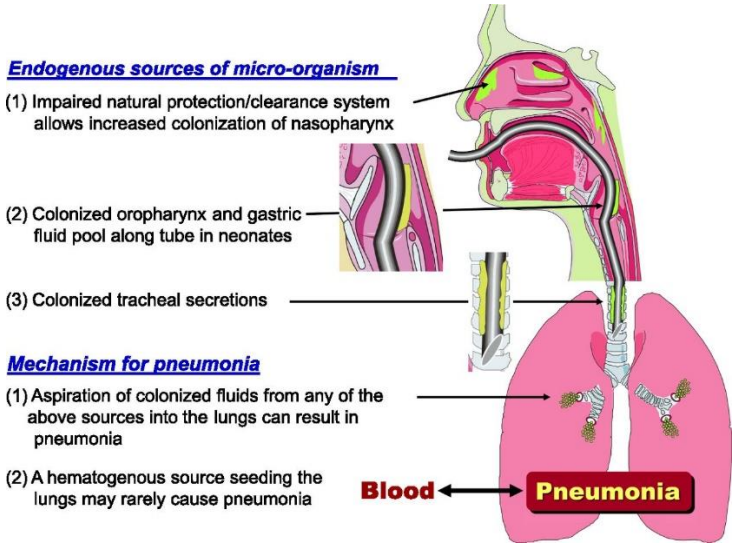

Figure 2: Pathogenesis of hospital bacterial ventilator associated pneumonia (VAP)

Formulations Calculation

To calculate the required delivery dosage, we go through a stepped procedure of analysis, evaluating the effect of each stage between the aerosol production by the aerosolizing device to the final target tissue deposition.

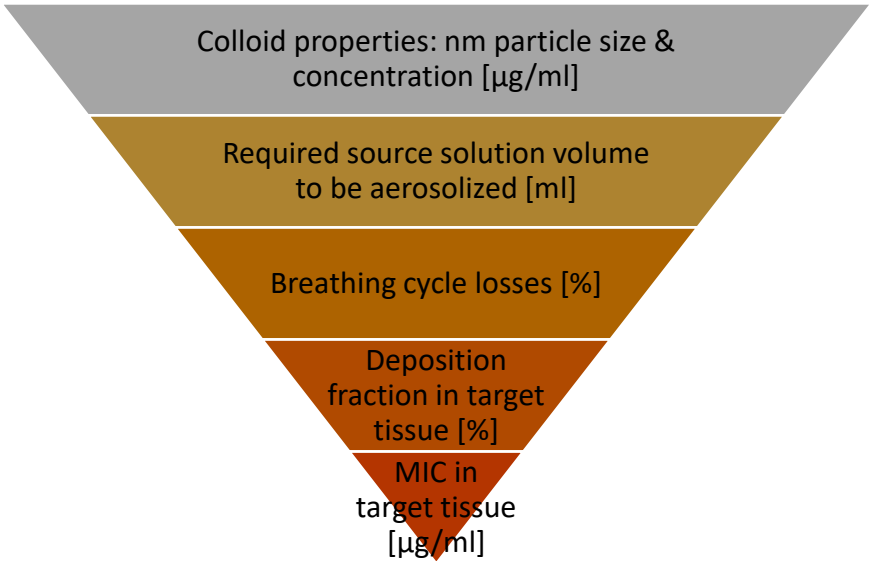

Figure 3: Outline of the dosage calculation logical structure

As elaborate below, we reach the following conclusions:

- **Colloid**: For antiviral applications, nanoparticle size should be in the range of 3nm – 7nm. For antibacterial applications there is less sensitivity to nanoparticle size, since silver ions are the predominant source of antibacterial effect and not the particles.
- **MIC**: For antiviral applications, MIC concentrations is about 10 µg/ml, yet for better confidence, target tissue concentration of 25 µg/ml is recommended. For antibacterial applications, effective concentrations are about half those of the antiviral.
- **Tissue Deposition Fraction**: Under oral breathing of 5µm aerosol droplets, Pharynx 30%, Bronchial tree 30%, Alveoli 25%.
- **Breathing cycle losses**: Inhalation is about 1/3 time of the breathing cycle. Hence, using a continuous aerosol source (most common home medicinal nebulizers) consume 3X the dosage of a breath-actuated nebulizer source.

In order to achieve a desired MIC in the target tissue, assuming the usage of **5nm colloidal silver in water**, the summary equation for calculating the require aerosol for delivery is the following:

(Aerosol dosage) = (Target MIC [µg/ml]) x (Mucus Volume [ml])
/ (Tissue deposition fraction) / (Inhalation time losses)
/ (colloid concentration [µg/ml])

For antiviral applications via oral inhalation of colloidal silver 5nm particles, examples of possible formulations according to our analysis are summarized in table-1. We note that, because of the factor 10X difference in mucus volume, targeting alveoli MIC requires significantly higher concentration of colloidal silver source. Since smaller droplets are more preferentially deposited in the alveoli, it may be recommended to us a 3nm droplets aerosol when targeting the alveoli.

Table 1: Dosage calculation when using a 5µ droplets aerosol

Tissue	MIC [µg/ml]	Mucus Volume [ml]	deposition fraction	Colloid concentration [µg/ml])	Breath activated aerosol [ml]	Continuous activated aerosol [ml]
Bronchial tree	25	1	30%	100	**0.8**	**2.5**
Alveoli	10	10	25%	200	**2**	**6**

Table 2: Dosage calculation when using a 3µ droplets aerosol

Tissue	Tissue concentration [µg/ml]	Mucus Volume [ml]	deposition fraction	Colloid concentration [µg/ml])	Breath activated aerosol [ml]	Continuous activated aerosol [ml]
Bronchial tree	33	1	10%	150	**2.2**	**6.6**
Alveoli	10	10	30%	150	**2.2**	**6.6**

For antibacterial applications, such as for prophylactic treatment of tracheal tube or tracheostomy ventilated patients in hospital ICU, one needs about half the quantity of antiviral.

Distinguishing Ionic vs Colloidal Silver Solutions

It is well established in the scientific literature that silver has both antibacterial and antiviral properties [4,8], including explicitly to influenza viruses. Clinical usage and testing of these properties has thus far focused mostly on wound care. There are is no published clinical research on medicinal inhalation of sliver particles that we are aware of. In this article we intend to guide and lay the grounds for such clinical evaluations to be done.

In the context of inhalations, silver particles suspensions or solution in water is the relevant configuration. It is important to distinguish between *ionic* silver solution and *colloidal* silver. Ionic silver is the case were atomic silver ions are dissolved in water. Colloidal silver is the case where we have nano-size silver chunks of silver matter, commonly in sizes between 1nm – 100nm diameter. When it comes to *antibacterial* properties, both ionic silver and colloidal silver MIC levels are well documented, and it is thought that the silver ions are the most effective agent [23]. In contrast, it has been argued that the *antiviral* properties of the colloidal particles are about 10x more potent than ionic silver [6], as exemplified in table-3 below for HIV. This is very important to realise, since any colloidal silver also contains a significant amount of ionic silver component (as further discuss below). A key implication is that for evaluating the antiviral potency of a colloidal silver, it would be a good approximation to focus analysis on the particle concentration alone.

Table 3: Antiviral effect of silver salts (ionic silver) and nanoparticles against HIV-1 [6]

Silver compound	**IC_{50}***
Silver nanoparticles	0.44 mg/mL (± 0.3)
Silver sulfadiazine (ions)	39.33 µg/mL (± 14.60)

Storage time may have an effect on colloidal silver water suspensions, since silver ions are eluted over time (e.g., during storage) as illustrated in the figure below [7]. It appears that within about 24h of storage in water about 10% of the colloidal particle mass is lost to ions, and within 10 days of storage saturation is reached with about 50% of the colloid mass lost to ions. Yet, we speculate that, intuitively, the colloid particle number density remains the same, only that each colloid silver particle mass is reduce by about 50%.

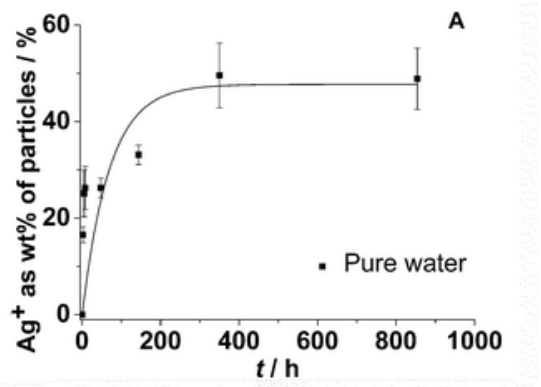

Figure 4: Ageing saturation of colloidal silver in water

The analysis in this article relies phenomenologically on experimental data. While it is not essential for our line of arguments, for the sake of completeness we note that there is growing research on the potential mechanisms of action of the antiviral properties of metallic nanoparticles [5].

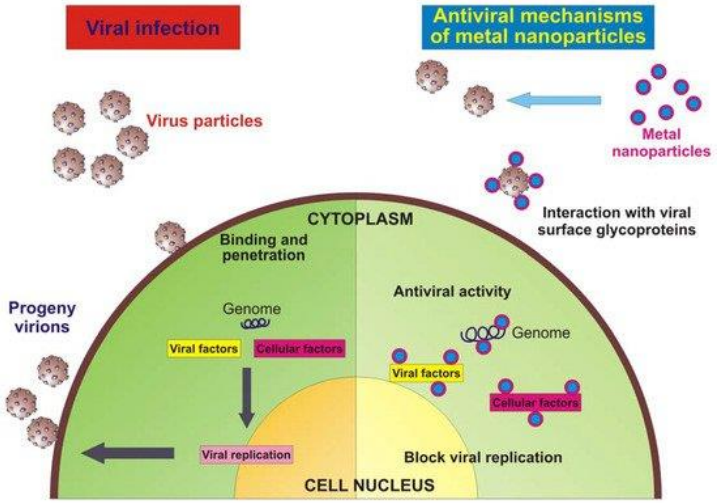

Figure 5: Supposed antiviral mechanisms of action of colloidal silver

Target MIC Determination for Antiviral Applications

The key starting point for calculating any effective dosage is to establish the required target minimum inhibitory concentration (MIC) of the effective agent. In microbiology, MIC is the lowest concentration of a chemical, usually a drug, which prevents visible growth of a pathogen. A major problem in deriving clear numerical conclusions from the published literature is that the MIC – given in weight fraction units (µg/ml) – is very sensitive to the nanoparticle size. For the same weight fraction, smaller

nanoparticles have higher number density than larger nanoparticles (nanoparticle number density is roughly proportional to $1/R^3$, where R is the particle diameter). Since higher density of nanoparticles has higher probability of interacting with pathogens, we would expect that smaller nanoparticles to have higher effectiveness that larger nanoparticles. Therefore, MIC of smaller nanoparticles will be smaller than MIC of larger nanoparticles, which experimentally is indeed the case (see Fig.9). Consequently, since every published research article used different size nanoparticles in its experiments, the resulting MIC values indeed vary significantly between one publication to another.

Moreover, select experimental results, which investigated the size dependence of silver nanoparticle antiviral effectiveness (particularly against HIV), indicate nanoparticles of size less than 10nm have much higher effectiveness than larger nanoparticles (e.g., 25nm or 50nm) [6,9]. This effect is much more significant than what is observed for antibacterial properties. We speculate that this may have to do with the virus size themselves being on the order of 100nm (HIV size is about 120nm, and SARS virus size is also about 100nm). i.e., in order to be effective in interacting with the virus, the silver nanoparticles need to be significantly smaller than the virus, such as less than 10nm. This speculation is given support by direct imaging of nanoparticles binding to viruses [5]. Interestingly, the observed sizes of nanoparticles bound to the virus (see Fig.6) were exclusively within the range of 1–10 nm, with **peak virus attachment effectiveness for nanoparticles size in the range of 3nm – 7nm**. The fact that no nanoparticles greater than 10 nm in diameter were observed to interact with the virus is significant, since the size of ~40% of the overall population in the sample was beyond this range [5]. This provides strong evidence for the size-dependence of interaction.

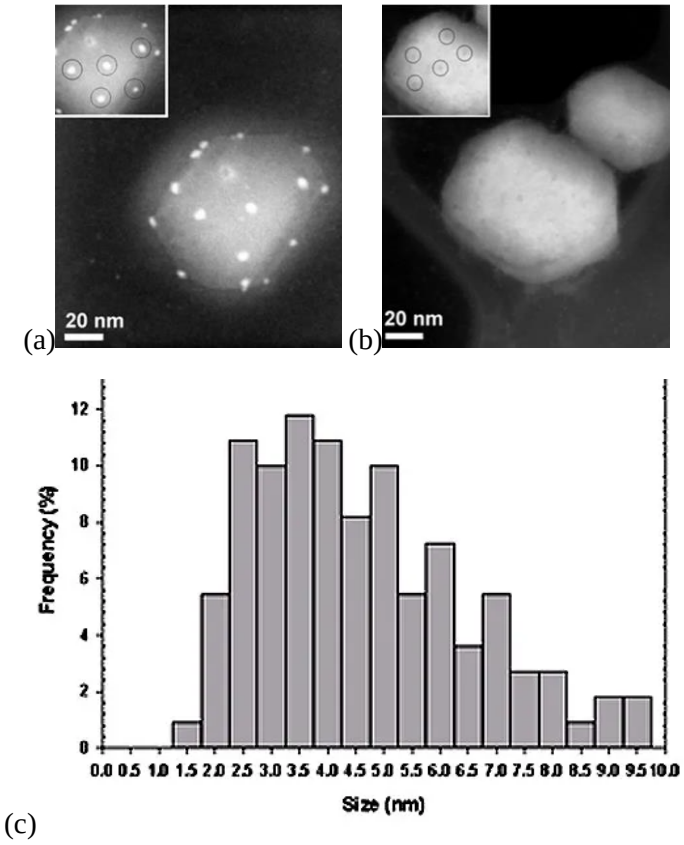

Figure 6: a) image of an HIV-1 virus interaction with silver. b) image of HIV-1 viruses without silver nanoparticle treatment. c) Composite size distribution of silver nanoparticles bound to the HIV-1 virus, derived from all tested preparations, seems to peak at nanoparticle size of about 5nm.

Therefore, we proclaim that **for significant antiviral effectiveness the size of silver nanoparticles needs to be less than 10nm, preferably in the range 3nm-7nm size**.

As seen in Fig.6, multiple silver nanoparticles get attached to a virus. It is thought that a virus function becomes disturbed only when sufficiently covered with nanoparticles [10]. Hence, we speculate that larger viruses would consistently require higher MIC concentrations (we suggest this may be tested in a future experimental analysis or survey of the literature). Indeed, for the large respiratory syncytial virus (RSV) of size ~250nm it appears that concentration of 10 µg/ml is ineffective and a concentration at least 25 µg/ml was needed as MIC (with proper nanoparticle size <10nm) and preferably 50 µg/ml to achieve significant elimination. Yet for the smaller HIV virus concentrations of 10 µg/ml are already effective as MIC, and at concentration of 25 µg/ml effective elimination is obtained [9]. **SARS virus size is similar to HIV. Hence, we speculate that similarly concentrations of 10 µg/ml are already effective as MIC, and at a concentration of 25 µg/ml effective elimination may be obtained**.

Assuming a roughly fixed size of nanoparticles in the colloid, the antiviral potency is expected to increase linearly with concentration (i.e., with mass concentration µg/ml of the silver colloids) which appears to be supported by experimental results [6].

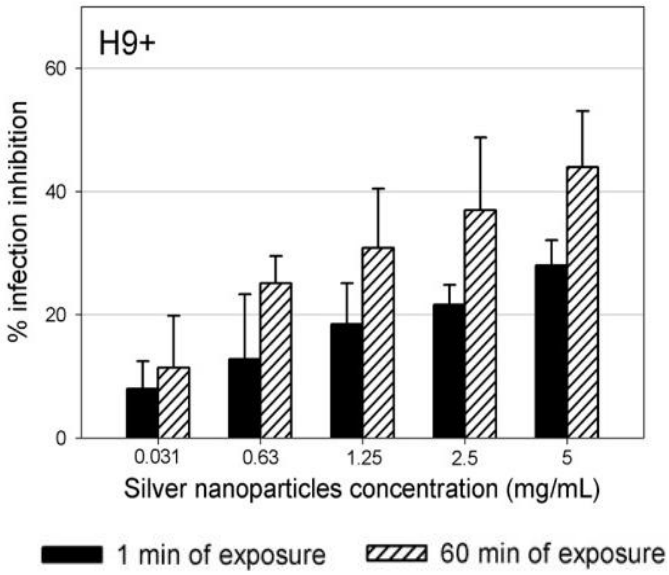

Figure 7: Concentration dependence of HIV-1 infection inhibition by silver nanoparticles [6]. Note that the concentration units in this graph are mg/ml. i.e., the effective concentration of 5 mg/ml = 500 µg/ml. We comment that the above experiment was conducted with nanoparticles of size ~40nm, which may explain the very high concentration need to achieve MIC with this colloidal silver.

Target MIC Determination for Antibacterial Applications

For bacterial infections, the consensus in the literature seems to be that the antibacterial effect is primarily due to the silver ions [15] and not the silver nano particles. Yet, it should be remembered, as we highlighted above, that any silver nanoparticle colloid actually elutes silver ions into the solution. Hence, even when testing colloidal silver source, one cannot attribute the antibacterial effects to the nanoparticle. Therefore, in the context of antibacterial properties, it is better to regard the nano-silver content as a general statement of silver material concentration.

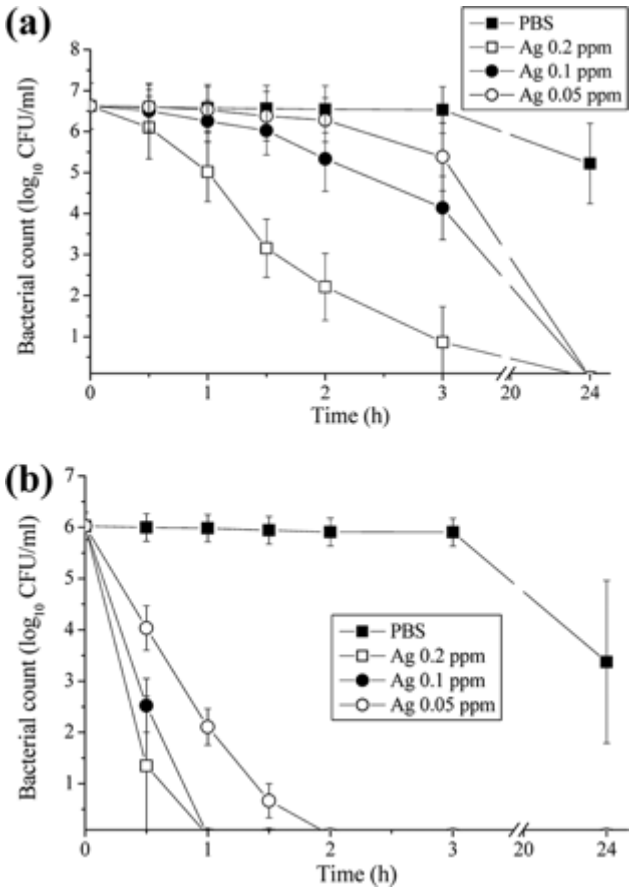

Figure 8: Silver ion solution effect on Staphylococcus aureus (a) and Escherichia coli (b) [15].

Considering colloidal silver antibacterial properties (see Fig. 9), it appears that typical MIC (~7 µg/ml) for small nanoparticles (~7nm) is roughly half that of the typical antiviral MIC according to our above analysis.

Sample	Minimum inhibition concentration (µg/mL)	
	Bacteria	
	E. coli	*S. aureus*
7-nm silver nanoparticles	6.25	7.5
29-nm silver nanoparticles	13.02	16.67
89-nm silver nanoparticles	11.79	33.71

Figure 9: Antibacterial MIC of colloidal silver [19].

Target Tissue Fluids Volume

For calculating the intake dosage which needs to be deposited in order to achieve MIC, we need to know the volume of mucus liquid into which the delivered droplets of colloidal silver are mixed after deposition. Nasal mucus is about 10–15 µm thick [38] and has two layers: the lower, 6 µm thick liquid layer (also called: periciliary liquid), is covered by the more viscous gel phase.

The total surface area of the bronchial tree is about 1m^2 (10,000 cm^2). But for aerosol droplets of about 5 μm diameter, most of the deposition occurs within the top 15 generations of the bronchial tree. Using the data of the surface areas and mucus thickness from the table in Fig.10, we estimate the combined mucus volume in this top half of the bronchial tree to be about 1cc. The alveoli total surface area is about 100m^2 (i.e., 1,000,000 cm^2) according to some estimations (or 140m^2 according to others) with mucus thickness of about 0.07 μm, resulting in total mucus volume of between 7cc to 10cc.

Generation	$th_{k,PCL}$ *(μm)*	*Airway surface area (cm^2)*	*Mucus production ($\times 10^{-3}$ mm^3/mm^2)*	*Mucus velocity (mm/min)*
0	6.04	70.83	3.67	5.500
1	5.49	32.11	3.84	1.340
2	5.02	40.10	4.01	0.994
3	4.61	50.74	4.18	0.744
4	4.27	63.17	3.91	0.548
5	3.99	63.96	3.58	0.343
6	3.74	81.88	3.26	0.255
7	3.53	121.20	2.94	0.207
8	3.36	171.36	2.61	0.155
9	3.21	228.98	2.29	0.106
10	3.08	289.24	1.97	0.067
11	2.97	353.44	1.64	0.039
12	2.88	422.64	1.32	0.021
13	2.81	520.41	0.99	0.009
14	2.74	687.61	0.67	0.006

Figure 10: Surface area and mucus thickness of various parts of the bronchial tree [21].

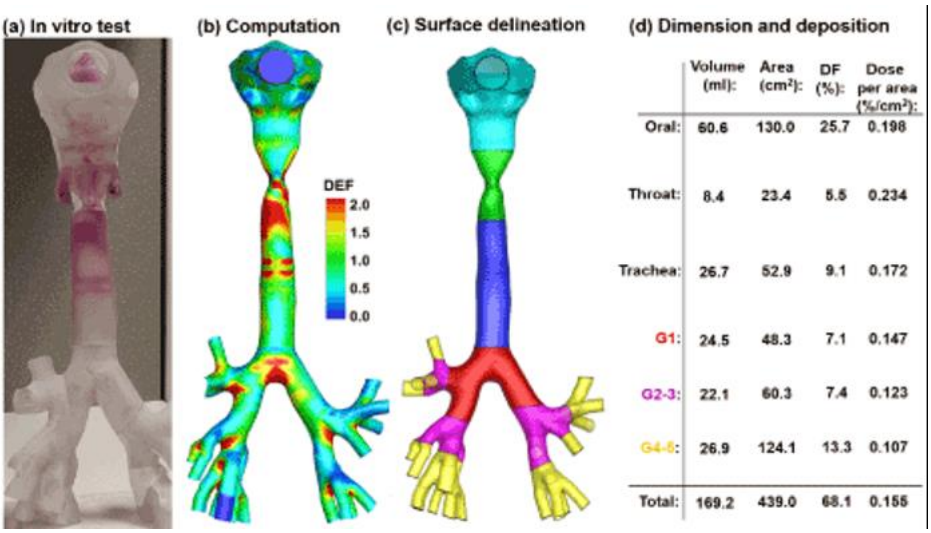

Figure 11: Visualization and quantification of nebulized aerosol deposition in mouth-lung casts under healthy and abnormal breathing conditions [22].

Deposition Fraction in Target Tissue

The deposition fraction factor depends on

i. Target tissue location: extrathoracic / trachea-bronchial (TB) tree / pulmonary (alveoli).
ii. Mode of inhalation (nasal/oral). In early stages, the infection is mainly in the bronchial tree. In particular, the infection and destruction of cilia cells in the trachea-bronchial tree by viral infection is a key aggravation factor in viral pneumonia infections, such as COVID-19 (SARS-CoV-2).
iii. Size of aerosol droplets.

As illustrated in the Fig.12 [14], there is a significant difference in bronchial tree deposition between nasal and oral breathing. Peak nasal breathing TB deposition is only about 10%, while mouth breathing TB deposition is about 30%. Hence, **we recommend oral breathing inhalation**.

The aerosol droplet size dependence of the deposition fraction is also different in each tissue region. In order to achieve optimal utilization, or to facilitate more practical inhalation conditions, it would be preferred to use aerosol of a size corresponding to peak probability of deposition in the target tissue. As illustrated in the Fig.12, (a) peak bronchial tree deposition fraction (of about 30%+) is obtained with aerosol droplets size of about 6 microns, while (b) peak alveoli deposition (of about 30%+) is obtained with aerosol droplets size of about 3 microns.

Standard mass-produced devices presently on the market have droplets of size 5 micron on average, which would work reasonably fine. **For 5 µm droplets, bronchial tree deposition fraction is about 30% and alveoli deposition fraction is about 25%**.

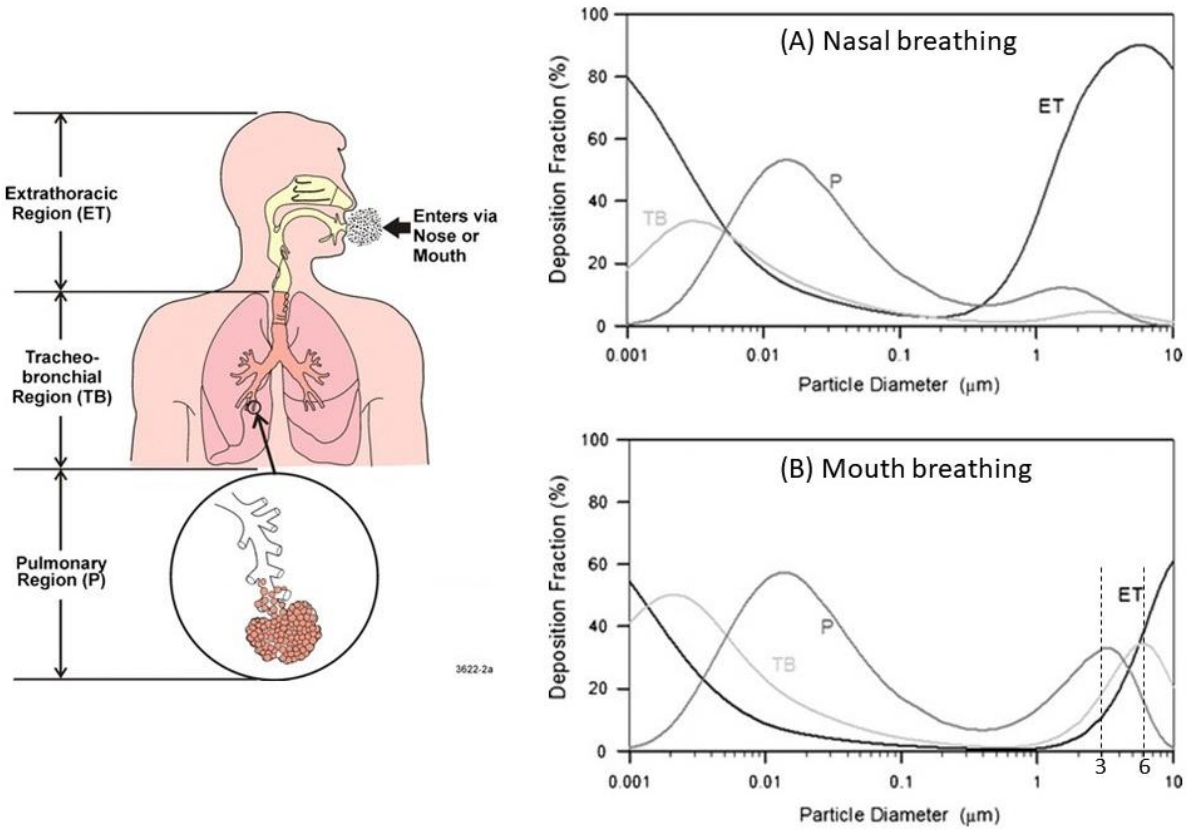

Figure 12: Primary structures of the respiratory system and associated nanoparticle deposition fractions at rest breathing [14].

Inhalation Timing Losses

Inhalation duration is only about 1/3 of the full breathing cycle. Therefore, if one is using an aerosol source which is continuously active, only about 1/3 of the aerosolized dosage is really counted for effective tissue delivery. This would be the common situation for home users when using standard commercial medicament aerosol which are available for purchase in pharmacies. Of course, having a breath actuated nebulizer (also available commercially) would correspondingly save on wasted silver colloid solution.

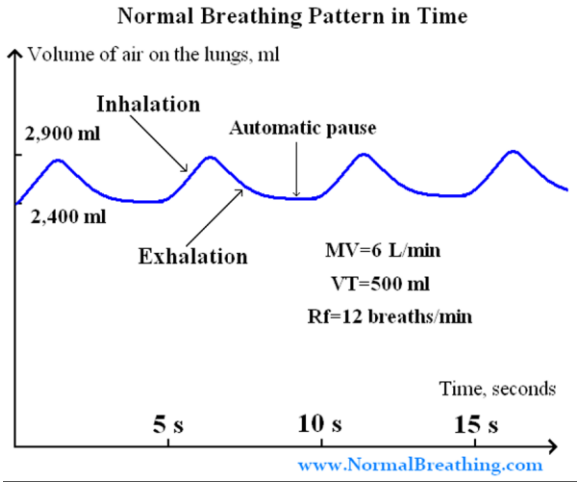

Figure 13

Clinical Safety

We are normally and daily exposed to silver intake via food and water. The human body has inherent normal mechanisms for disposal of silver. Most foods contain traces of silver in the 10–100 µg/kg range. The median daily intake of silver in a research from 84 self-selected diets, including drinking-water, was 7.1 µg. Higher figures have been reported in the past, ranging from 20 to 80 µg of silver per day [16]. The biological half-life in humans (liver) ranges from several days up to 50 days. Most of absorbed silver is being excreted with the bile in the faeces [16]. Published scientific experiments on colloidal silver antiviral and antibacterial properties, which also contain examination of cell culture toxicity, consistently show non-toxicity at the relevant MIC silver concentrations.

Most importantly, in the context of inhalation delivery, it has been shown that even after 90 days of continuous exposure to high dosage of silver nanoparticles inhalation (total of 1,143µg silver per day), cumulated tissue levels return to normal within about 12 weeks of recovery. It is a very strong indication of short-term inhalation exposure safety, in the sense of having no permanent cumulation in body tissue.

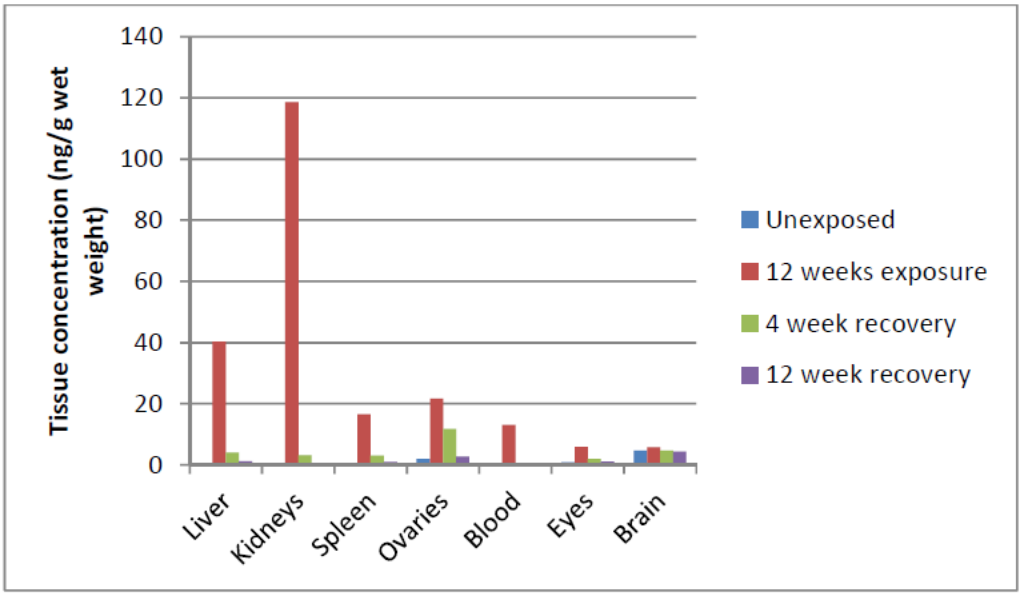

Figure 14: Tissue silver concentrations in female rats exposed to the high AgNP dose (381 µg/m3, silver nanoparticles ~15nm diameter, for 6 h/day, amounts to an inhalation dosage 1,143 µg silver per day) in a 90 day inhalation study, followed by a 12 week recovery period [17,18].

There are several occupational guidelines and exposure limits in the United States for airborne silver. All are defined on a mass basis. 13,74 OSHA71 has adopted the threshold limit value on a time-weighted average (TLV-TWA) for a 40-hr/week exposure from the American Conference of Governmental Industrial Hygienists (ACGIH) of 0.1 mg/m^3 for metallic silver. Under normal breathing (500L/h air breathing), this amounts to work environment (8 hours per day) inhalation of about 400µg daily of silver nanoparticles.

Commercial Availability

The scientific testing in the published literature were provided by speciality manufacturers at a high price of about $3,000 for 30ml bottle of 1,000 µg/ml in water. At this price, a single treatment of 2ml dosage at 100 µg/ml concentration would cost about $20. That's quite a lot, though not prohibitive (considering the pneumonia risk). Moreover, since the above pricing is presently for specialty laboratory usage, we assume the price can be significantly reduced for commercial consumer market volume sales. The online commercial sources, which we could locate, all seem unreliable and mostly do not even provide the information with which we can assess the suitability of the product. Yet, in the literature there are descriptions of protocols for cheap manufacturing [22] of colloidal silver with exactly the recommended properties.

Discussion

Though marred by charlatan claims of unprofessional commercial products, there is well-established scientific research on the antibacterial and antiviral properties of colloidal silver. Yet, its potential application for the treatment of respiratory infections was never properly explored, to the best of our knowledge. The surveyed literature indicates that colloidal sliver of particle sizes between 3nm – 7nm can be highly effective to suppress viral mechanisms of infection. We further conclude that MIC concentrations of such colloids is about 10 µg/ml, yet for better effectiveness confidence, target tissue concentration of 25 µg/ml is recommended. Yet, these values we obtained by making somewhat indirect inferences and therefore more focused research is called for. We estimate that these formulations can be effective for prevention and treatment of respiratory viral infections at early stages, including COVID-19 / SARS-CoV-2.

For bacterial infections, particularly in the context of ICU prevention of hospital ventilator associated pneumonia (VAP), the same formulations can be applicable, only at dosages of about half those of antibacterial. An additional risk reduction benefit of colloidal silver inhalation treatment for ventilated patients is the possibility of suppression of biofilm formation inside the endotracheal or tracheostomy tube.

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