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Coronavirus-mimicking nanoparticles (CorNPs) in artificial saliva droplets and nanoaerosols: Influence of shape and environmental factors on particokinetics/particle aerodynamics

Ajay Vikram Singh a,⁎, Aaron Katz a, Romi Singh Maharjana, Ashish K. Gadicherla b, Martin Heinrich Richterb, Jan Heyda c, Pablo del Pino d, Peter Laux a, Andreas Lucha a

a German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Max-Dohrn-Straße 8-10, 10589 Berlin, Germany
b German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Dieseldorfer Weg 1, 12277 Berlin, Germany
c University of Chemistry and Technology (UCT), 166 28 Prague 6, Czech Republic
d Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Departamento de Física de Partículas, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

HIGHLIGHTS

• An experimental model shown with coronavirus like spike NPs-loaded aerosol mimicking speech scenario in a closed environment
• Spatiotemporal particokinetics/aerodynamics of nanoaerosols that reach the air and droplets that deposit are measured
• Method exhibits potential correlation between a particulate pollutant and carrier role it may play in transmission of viruses
• Fluorescent mucin with salts and enzymes in artificial human saliva/sputum was prepared to mimic droplets as CLSM analyzed
• The results implicate for decision making on how many people can be put in a room if the air exchange rate is low

GRAPHICAL ABSTRACT

ABSTRACT

Severe acute respiratory syndrome coronavirus 2, abbreviated as SARS-CoV-2, has been associated with the transmission of infectious COVID-19 disease through breathing and speech droplets emitted by infected carriers including asymptomatic cases. As part of SARS-CoV-2 global pandemic preparedness, we studied the transmission of aerosolized air mimicking the infected person releasing speech aerosol with droplets containing CorNPs using a vibrating mesh nebulizer as human patient simulator. Generally speech produces nanoaerosols with droplets of <5 μm in diameter that can travel distances longer than 1 m after release. It is assumed that speech aerosol droplets are a main element of the current Corona virus pandemic, unlike droplets larger than 5 μm, which settle down within a 1 m radius. There are no systemic studies, which take into account speech-generated aerosol/droplet experimental validation and their aerodynamics/particle kinetics analysis. In this study, we cover these topics and explore role of residual water in aerosol droplet stability by exploring drying dynamics. Furthermore, a candle experiment was designed to determine whether air pollution might influence respiratory virus like nanoparticle transmission and air stability.

⁎ Corresponding author.
E-mail address: Ajay-Vikram.Singh@bfr.bund.de (A.V. Singh).

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1. Introduction

Considering the global pandemic and COVID-19’s potential threat, understanding the transmission of the virus is key to its containment and future prevention. Scientists are determined to elucidate the aerodynamics and particokinetics of the novel corona virus. This would help to understand the aerial transmissions and to evaluate the precautionary recommendations, such as safe distancing in public places to prevent person-to-person spread of the infection. Countermeasures based on this understanding could reduce the possible virus deposition and spread in highly frequented places, such as entrances of public transport, elderly homes, hospitals, churches and schools (Abd et al., 2020). At the beginning of the outbreak in Europe, researchers indicated an impact of air pollution on the severity of COVID-19 due to increased cases in places with poor air quality (Conticini et al., 2020). At first, these findings were interpreted as result of a higher predisposition of a severe COVID-19 infection following any long-term exposure to ambient air pollution (Ali and Islam, 2020). Since so-called spreading events have occurred in indoor spaces (e.g., choir practices), aerosols have increasingly been discussed as potential vectors for transmission of SARS-CoV-2, and as important agents of disease transmission besides droplets (Bazant and Bush, 2021). It was experimentally verified by Van Doremalen et al. (2020) that SARS-CoV-2 is able to remain viable up to 3 h in aerosol. In this context, it is of high interest if and how the indoor air quality might influence the SARS-CoV-2 transmission. For example, does the presence of high particle concentrations stemming from other sources (e.g., smoking, cooking) promote or reduce the transmission? Based on the expectation of a third wave of infections and beyond, most probably coinciding with Christmas, the burning of candles may be an interesting particle source to investigate potential impact on virus transport (Lea Hamner et al., 2020). In addition to vaccines and distancing rules, aerodynamics and particokinetics of virus aerosols will likely also become an important in focus in developing strategies - also in the post-corona era - to diminish the transmission of viral infections and to better prepare for future pandemics. Scientific investigation supported by social and behavioral science will help to reduce the current pandemic’s community transmissions and the emergence of new positive cases as witnessed in current Corona virus (SARS-CoV-2) infection worldwide (Bavel et al., 2020). In terms of understanding time- and space-resolved particle dynamics to support current research and development in this field on the one hand but to avoid laboratory work with viruses on the other hand, we investigated the following key points in this study.

- Creating a virus spike like NPs-loaded aerosol mimicking the real life speech situation in a closed environment. Virus shape-mimicking nanoparticles (i.e., sea-urchin like gold nanoparticles) were dispersed in synthetic human sputum and saliva (Ajay Vikram Singh et al., 2022). After characterizing the dispersion, it was aerosolized with a vibrating mesh aerosol generator to create a fine mist of sputum and saliva. One major overview on the study of van Doremalen et al. has outlined the poor simulation of a real cough, especially attributed to the particle size distribution, which we put special emphasis to in this study. The goal was to emulate the real-life scenario of saliva droplets generation by a coughing/speaking person, to acquire and characterize the generated, CorNPs carrying aerosols depending on the environmental factors in saliva aerosols transportation, and to add diagnostic value to this study in light of the current pandemic. To mimic the in vivo situation, saliva/sputum is a viscoelastic soft material, we produced human equivalent artificial biofluids which mimics the virus-loaded human speech droplet transmission (Walker et al., 2015).

- We characterize the aerodynamics and particokinetics post-aerosolization in order to explore how time- and space-dependent particokinetics/aerodynamics are affected by both nanoaerosols reaching the atmosphere, as well as droplets forming on surfaces (Liu et al., 2018; Pan et al., 2019; Smith et al., 2020). Here, condition mimicking speech triggered aerodynamics and particokinetics (long/short duration speech vs infected/uninfected speech) can additionally be investigated in relation with water evaporation in sedimenting droplet (Ajay Vikram Singh et al., 2022). The results can have implications for decision-making on how many people can be put in a room or how to handle teamwork and meetings, especially if the air exchange rate is low. Using a commercially available fluorescent mucus supplemented with salts and enzymes artificial human saliva and sputum was prepared to mimic droplets (Xu et al., 2020). Real-time confocal laser scanning micro analysis (CLSM) was applied to fluorescent droplets containing viral mimicking particles to understand the drying behavior depending on the formulation/particles (Ajay Vikram Singh et al., 2019).

- The method developed herein enabled us to understand the potential correlation between air pollution and carrier role it play in transmission of viruses. The aim is to create background aerosols to mimic the air pollution coming from potential indoor sources (e.g. smoking, candles etc.). By comparing the aerodynamics of the simulated speech event with and without the presence of a background aerosol, information on the interaction of the virus-like nanoparticles with possible indoor air pollution is collected. Based on this data, knowledge on how air pollution may affect the transport and stability of airborne SARS-CoV-2 is strengthened.

2. Methods

2.1. Synthetic saliva with CorNPs formulations to mimic speech droplet generation and chamber design

The experiments were conducted in a custom-made boxed chamber 1 m × 1 m × 1 m (L × W × H) thus the possible maximum dispersion distance is limited by the chamber’s size. Glove box gloves were installed to the transparent chamber door and used for handling the equipment inside as well as starting any experiment. The chamber was equipped with glove. The mesh nebulizer (OMRON MicroAir U100 NE-U100E vibrating mesh nebulizer) was driven by air at a constant flow rate of 5 L/min and the reservoir filled with sterile synthetic saliva mixed with SARS-CoV-2-like gold urchin nanoparticles. Aerosol tubing for assessing the aerosol was connected to CPC and SMPS equipment (Singh et al., 2021b). An exhaled aerosols cloud was revealed as the jet plume and particle count was captured by Condensation Particle Counter (CPC) in the chamber.

2.2. Nebulization experiment for mass-weighted quantitation of aerosol using SMPS and CPC

We nebulized 8 mL of each pure saliva, saliva containing nanourchins and saliva containing nanoparticles samples in the custom-made chamber. Each sample was loaded into the nebulizer and place in the chamber. The experiments were performed at constant relative humidity of 46 % to avoid variations in results due to changes in humidity in chamber (Yao et al., 2007). The chamber was closed. Chamber air was cleaned before every experiment by circulating the air through a connected filter unit until the particle concentration, measured by CPC, was below 10 particles/cm³. Before the actual experiment, two background SMPS measurements were performed. Nebulization lasted for 8 min and approximately 5 to 6 mL of the formulation were left in the reservoir. The 3 Measurements were performed during nebulization. After nebulization, SMPS measurements were continued for around 1 h. To extract the numerical data regarding exponential decay, sedimentation and deposition rate, we performed the quantitative fitting the CPC measurement according to published report on the topic (Crumpl and Seinfeld, 1981).

2.3. Simulating a background (pollutant) aerosol with candle smoke

The particle number concentration of the background air in the chamber before the nebulization started and the nanoaerosol emission during the nebulization process was measured with a condensation particle counter (CPC, model 3775 from TSI Inc., Minnesota, USA) (Au - Sigloch et al., 2020). The
CPC used in the experiments detects airborne particles down to 4 nm and particle concentrations up to $5 \times 10^4$ particles/cm$^3$ in single counting mode and then up to $10^5$ particles/cm$^3$ in photometric mode. A sampling flow rate of 0.3 L/min was used. A scanning mobility particle sizer (SMPS, model 3938 from TSI Inc., Minnesota, USA) was used to measure the particle size distribution. In the setup, a scan time of 120 s, an aerosol flow of 0.3 L/min and a sheath flow rate of 3 L/min were used. The settings allowed a measurement range of particle sizes between 14.4 and 673.2 nm.

2.4. Inductively coupled plasma mass spectrometry (ICP-MS) analysis of nebulized gold nanourchins

We followed our previous protocols to determine the concentration of corona-like gold nanourchins collected on the deposited surface(Ajay Vikram Singh et al., 2022). We nebulized saliva with or without spherical and nanourchin particle in the chamber setup describe before. Filter papers placed in the chamber were used for passive collection of the aerosols via deposition. The exposed filter papers, pristine papers (as control), as well as the original saliva medium were microwave-digested and the gold content was determined by ICP−MS (ICAP Q, Thermo Fisher Scientific GmbH, Dreieich, Germany).

2.5. Kinetics of sessile drop evaporation dynamic and droplet morphology

For droplet experiments, 0.5 μL of either the pure synthetic saliva with FITC tagged mucin proteins or the synthetic saliva mixed with spherical or corona-like nanourchins containing $\approx 10^{10}$ nanoparticles/mL were pipetted on thoroughly cleaned microscopic glass covers (0.17 mm thickness, VWR scientific Germany). The droplets were evaporated in a microscopic incubation chamber collecting time lapse images (5 frames/second). The change in fluorescence intensity was recorded for the sessile droplets and the final droplet morphology was captured. The drying observations were carried out using an inverted confocal laser scanning microscope (LSM700, Carl Zeiss AG, Oberkochen, Germany) and morphology was recorded over time for up to 340 s with Zen Software with the 488 nm laser line (60 × NA 0.45, zoom 0.6). To minimize the deteriorating effect on the FITC fluorophore the number of cycles of excitation/emission and the laser intensity were optimized.

2.6. Optical microrheology measurements with dynamic light scattering (DLS)

The DLS optical microrheology protocol instructions from the manufacturer were followed Malvern Panalytical GmbH (Herrenberg, Germany) as described in a recent method development report (Maharjan et al., 2022). The experiment was conducted at a temperature of 25 °C and a pH of 7.5. Polystyrene beads of 1.5 mm diameter were used as tracer particles (Polysciences, Inc., Warrington, USA). After checking the tracer compatibility with the sample and the concentration of the tracer, microrheology measurements were carried out. NPs were tested for tracer compatibility by measuring the Zeta potential of 1 mL of the pure formulated synthetic saliva medium. A small volume of NPs was added and the zeta potential was measured again. Significant zeta potential differences would indicate adsorption and aggregation processes between tracer particles and NPs. When both tracer and sample particles are present, the zeta potential should be within the set limit of 5 mV. Subsequently, the tracer concentration was confirmed by following the software instructions of the manufacturer. In a disposable cuvette, 1 mL of the synthetic saliva was first mixed with 5 μL of the polystyrene beads as tracer particles, and then the scatter intensity was measured. With each successive reading, the software recommends how much additional tracer particle should be added so that the relative scattering intensity for tracer particles compared to the NPs in the sample is about 95%.

2.7. Particle physicochemical characterisation using DLS and TEM

The multibranched spike bearing gold nanoparticles as called nanourchins (or nanoflowers alternatively) make a good shape/size mimetic nanodecoy study model (Singh et al., 2022; Ajay Vikram Singh et al., 2022). Considering the size of SARS-CoV-2 virus in the range of 50–100 nm, in our study 90 nm gold nanourchins of were used. We confirmed the shape and size distribution of the gold nanourchins using DLS (Malvern Zetasizer, Malvern Analytical GmbH, Berlin Germany) and TEM imaging (Jeol 1400 Plus, Jeol. GmbH Germany) as shown in supplementary Fig. S1 with size distribution curve calculated by intensity plot (Leibrock et al., 2019). The zeta potential of gold nanourchins was measured – 29.3 mV. The recorded polydispersity Index (Pdi) was 0.24 showing good shape homogeneity of the gold nanourchins.

3. Results and discussion

3.1. Characterizing the nebulized aerosols with CPC and SMPS

In this study, as shown in Fig. 1.A-D, a method was developed to simulate speech droplets containing nano- and micro sized droplets with CorNPs (gold nanourchins). An attempt was made to thus simulate the scenario of saliva droplet generation by an infected person. Since saliva is a viscoelastic soft material, artificial biofluids were used to mimic virus/virus−laden human sneeze/cough or speech droplets. Sterile simulated lung fluid (Gamble’s solution, catalog number 1700−0800, Pickingering Laboratories, Berlin, German) used in this study, were commercially purchased.

To experimentally detect the droplet transport, the airborne were analyzed by concentration measurement using a condensation particle counter (CPC) as shown in Fig. 2. A high particle concentration was measured within the first 500 s of the experiments during the active nebulization. The number was three times higher for the aerosol produced from the saliva without CoreNPs. The particle concentration during nebulization of artificial saliva with spherical gold nanoparticles was slightly higher than that of pure saliva. Subsequently, the emission of nebulizing aerosols based on the CoreNPs was compared with the spherical particles. No significant difference (parametric Student t-test $p > 0.21$) was found in the amount of nebulized gold as a function of particle shape when the gold particles were nebulized at the same particle weight concentration in the saliva simulat measured by ICP-MS (Fig. 2.B and supplementary Fig. S2). In relation to the low aerosol concentration of the nebulized saliva with nanourchins, we initially suspected that adding nanourchins might cause changes in viscosity parameters, such as complex viscosity and viscoelastic moduli, which might be responsible for reduced aerosolization of saliva mixed with CoreNPs. We measured microrheology of the saliva mixed with and without spherical and CoreNPs. We observed marginal change in the optical microrheological parameters (e.g., correlation function, complex viscosity, viscoelastic modulus, mean square displacement) of saliva upon mixing with the NPs (supporting info microrheology Figs. S3−S6). This implies that nebulization efficiency is not affected by viscosity, but by the nanoparticle’s shape, meaning that saliva mixed with corona-shaped particles could settle faster upon nebulization compared to pure saliva or spherical NPs mixed with synthetic saliva. One hypothesis is that the shape of the NPs (spherical vs. corona-shaped), i.e., the corona-shaped particles adsorbs more water, which makes it sticky, affects the sedimentation of the aerosol (Malik et al., 2020; Singh et al., 2021d). After nebulization, the exponential decay rates differed significantly between saliva with CorNPs and saliva with or without spherical nanoparticles. Furthermore, the measurements showed that the concentration of nanoaerosols in the air decreased but did not reach zero during the measurement period, which indicates that nanoaerosols are sufficiently stable in the air beyond the measurement period, i.e. 500 s (compare Fig. 2.C-E).

Next, we shortened the nebulization from 8 min time to 1 min and recorded the particle number in the air of saliva or saliva mixed with CoreNPs to investigate short time influence on the generated nanoaerosol stability in air. The stability of aerosols in the air followed the same trend as with longer nebulization, (supplementary Fig. S7). Applying this scenario to a person infected with SARS-CoV-2, one can speculate that a short speech or conversation (1 min or less compared to 10 min duration) from an infected person with a spike-like virus could produce an aerosol cloud that has a
Fig. 1. Motivation, design and application of human patient simulator, speech droplets and aerosol generation. (A-B) Design of SARS-CoV-2 mimicking pseudo nanodecoy with shape mimicking CoNPs (A) and a patient releasing speech droplet, and their transmission in a 1-m periphery and beyond. (C) SARS-CoV-2 infected speech droplet and nanoaerosols mimesis by nebulization of synthetic saliva carrying gold nanourchin fluorescent mucin. (D) Airborne nanoaerosols concentration and size distribution analysis with CPC/SMPS as well as droplet morphology and drying dynamics evaluation with confocal microscopy.

Fig. 2. Quantitative real time observation of simulated airborne speech droplet, generated by an eight-minute burst of nebulized saliva with and without CoNPs, and spherical particle as control. (A) Chart of particle count versus time (smoothed with a 24-s moving average) representing the aerosol count for saliva (blue), saliva + nanosphere (orange), saliva + nanourchin (grey) CoNPs. The exponential decay curves return to their respective baseline level of ca. 5000 (grey), 15,000 (blue dashed line) and 20,000 (orange dashed line) counts per sec. The 8 min burst of nebulized aerosols started 130 s before time 0. The black arrow (≈ 600 s) marks the start of the exponential fits. (B) ICP-MS analysis to ascertain that a similar nanoparticle concentration (w/v) was nebulized for the particokinetics and particodynamic analysis (S1: Blank_Filterpaper; S2: Saliva_Neb_300ul; S3: Saliva_Drop coated_300ul; S4: Au_nanoUrchin_Neb_Saliva_300ul; S5: Au_nanoUrchin_Drop_Saliva_300ul; S6: Au_Sph_Neb_Saliva_300ul; S7: Au_Sph_Drop_Saliva_300ul; S8: Au_Sph2_Corrected_Neb_Saliva_300ul; S9: Au_Sph2_corrected_Drop_Saliva_300ul). (C) The trend exhibits that when similar volume of saliva without (orange) and with (blue) corona-like particles were nebulized until the whole volume was aerosolized by the nebulizer, instead of only 8 min of nebulization as shown before. (D-E) exhibit count decay of nanoaerosols with time as zoomed in temporal images from 1000 s and 500 s of post nebulization respectively from (C).
high aerosol concentration. However, this aerosol is unstable and settles more rapidly than aerosols from pure saliva. When looking at the particle concentration during the first 500–1000 s of the CPC measurement, slight variations in the particle number concentration were observed. Comparison of saliva mixed with spherical NP mixed or with CorNPs when nebulized until the entire volume was consumed showed a higher aerosol concentration for the spherical NPs. These observations corroborate that the stability of aerosols produced by speech is a complex phenomenon (Heyder et al., 1986; Singh et al., 2021c).

3.2. Effect of pollutants on the stability of aerosol and transport of nebulized saliva with CorNPs

To decipher the role of association between CorNPs interaction with environmental pollutant, we created background aerosols in the experimental chamber to mimic air pollution coming from potential indoor sources (e.g., smoking, candles). Candle smoke exposure in chamber was optimized for the CPC and SMPS measuring range (supplementary Fig. S8). Experiments were performed in precontaminated chamber after candle smoke exposure (~60 s). As shown in Fig. 3A, we compared the size distribution of the particle originated from nebulized pure saliva with the nebulized saliva containing CorNPs using SMPS. We observed broad range of nanoaerosols size distribution from 50 nm to 500 nm in pure saliva or saliva mixed with nanoparticles. The particle concentration, however, was noticed to be highest in pure saliva as shown by the blue line. The differences in airborne stability in presence of candle smoke for the saliva with or without CorNPs can be qualitatively assessed by looking at trends of diameter of the aerosols over time as number- or mass-weighted heatmap plot derived from SMPS measurement. The plots are shown in Fig. 3. B-E for pure saliva nebulization (B-C) or saliva mixed with gold nanoshells (D-E). Increase yellow shaded regions in the number-weighted plot shown in Fig. 3. B compared to Fig. 3. C indicate that nanoaerosols of saliva mixed with CorNPs have a similar particle size distribution as the aerosol from pure saliva and both remain airborne over several minutes. The mass-weighted plot for the two experiments show slightly high size trend on colour scale for the saliva and saliva mixed CorNPs (compare Fig. 3. C to D differential mass of aerosols µg/cm³).

The trend for the particle concentration as function of time with number/mass weighted plots can be seen in line plot in Fig. 3. F-I for saliva and saliva + urchin NPs respectively (see figure captions for details). Looking at the plot, the saliva aerosol concentrations are higher (1.75 e + 04 #/cm³) compared to saliva mixed with CorNPs (1.5 e + 04 #/cm³) which is expected as droplets generated from saliva mixed with CorNPs might have slight higher combined mass, which would lead to faster sedimentation and therefore a faster decrease in concentration. However, the decays in nanoaerosols particle concentration over time from pure saliva and saliva with CorNPs exhibit marginal differences only (Fig. 3. F vs. G and H vs. I). The initial decay during first 40 min show similar drop (5.0 e + 03 #/cm³) in the aerosols of pure saliva and of saliva mixed with CorNPs (compare Fig. 3. H-I below). However, after 40 min saliva mixed with CorNPs takes longer duration to reach base line concentration drops from 1.5 e + 05 to ‘zero’. The mass-weighted plot for the two samples exhibit a similar trend as seen in Fig. 3. H-I.

After the nebulization was stopped and CPC measurement started, the exponential decay in saliva & saliva mixed spherical NPs was >50 % in first 120 s (mean particle concentration 327,97t 1 to 144,51 particle/min for the saliva mixed with spherical NPs vs. 74,54 to 24,71 particle/min). After 1000 s of nebulization stopped till the end of the CPC measurement-time point (Fig. 2A) when there was constant decay observed, the exponential decay was more in saliva mixed with the CorNPs (mean particle concentration 26,467 to 12,321 particles/min) compared to pure saliva/spherical NPs (mixed saliva) exponential decay extracted from the quantitative fit from nanoaerosols concentration. The deposition rate of nanoaerosols extracted from quantitative fit were different for different composition after 1000 s time point and follow trend saliva (22,59 particle/min) > Saliva with CorNPs (14,2 particle/min) > saliva mixed with spherical NPs (9,65 particle/min). The high deposition rate in pure saliva compare to NPs mixed compositions could arise due to the fact that the nanoparticles are heavier than saliva, this additional mass due to added nanoparticles decrease the sedimentation time (Wang et al., 2021). In addition, high nanoaerosols size distribution might play a decisive role (Wang et al., 2022), which we confirmed in next section of our analysis via scanning mobility particle sizer (SMPS) count. Further, in this context, the candle-experimental optimization phase number- and mass-weighted plots of the diameter and particle concentration as function of time are demonstrated in supplementary Fig. S9.

3.3. Confocal Laser Scanning Microscopy (CLSM) for fluorescent droplet analysis

Droplet sedimentation time, the duration droplets spend in air before settling to the ground, is thought to be a primary determinant of airborne transmission and exhibit linear proportionality to droplet stability (Netz, 2020). Airborne transmission is believed to be one of the most important means of transmitting infectious diseases, such as SARS-CoV-2 (Wang et al., 2021). Reduced droplet mass caused by evaporation lengthens the sedimentation time. Especially small droplets that were almost completely evaporated can stay airborne for a long time as droplet nuclei depending on their solute content. Since viruses can stay viable in aerosols for hours, the formation of droplet nuclei which stay airborne long can substantially increase the viral load in the air (Fig. 4. A). Consequently, in this section we investigated the droplet morphological factors that are associated with droplet evaporation and sedimentation times.

A theoretical study has recently demonstrated that the dominant solute osmotic-pressure effect of water correlates with time-dependent evaporation and droplet sedimentation in speech droplets (Netz and Eaton, 2020). However, no experimental verification exist currently. Water mediated evaporation phenomenon may prolong the viral stay in air by reducing the sedimentation behaviors of the speech droplet. At a given time in a mid-size room, a mask less infected person may produce 10 K virions in air at a steady state air load while speaking, which makes it prone to inhale the people present in that room an average 2.5 virion/min (Eiche and Kuster, 2020). Low relative humidity, as result of seasonal changes or indoor air conditioning (e.g., in laboratories, hospitals, airplanes), may exacerbate the airborne atmospheric load of virions.

To mimic the SARS-CoV-2 generation via speech droplets released from infected person, we performed fluorescent droplet (FTTC-tagged mucin protein) analysis released from a vibrating mesh nebulizer. The droplets were collected on thin microscopy slides placed at different distances as shown schematically in Fig. 4. B. The droplets were imaged in situ using CLSM and analyzed using ImageJ to quantify different size and shape descriptors as shown in Fig. 4.C-H and supplementary Fig. S10. Using the Laplacian of the Gaussian method and binary function in ImageJ helped the fluorescent mucin distribution in the droplets could precisely be determined and allowed a fine morphology assessment (supplementary Fig. S11). As shown qualitatively in the supplementary Fig. S10 and quantitative analysis in Fig. 4.F-H, the CLSM micrograph showed that the biggest number of estimated droplets could be found closest to the emission. However, the area of the individual droplets is highest at a middle distance and mid-landing nanodroplets. Interestingly, we found a greater number of droplets on sample collectors placed closest to the nebulizer, mid and lower-mid height and distance show almost similar features but larger droplets. The droplets released in air while travelling longer distance might coalesce forming bigger droplets. We also collected the droplets at different heights and, as seen qualitatively in supporting Fig. S12, the results are similar for samples S1-S4 placed at variable distances except the images landing from highest level (H4) which exhibit a more distorted morphology. Typical fractal-like drying effect can be seen for the dried droplets collected at mid and mid-distance collected droplets. Contrary, the droplets that travelled the longest distance or height showed the most irregular shapes and least in number as quantified using the Ferret’s diameter, which is a measure of an object size along a specified direction (Walton, 1948). For the identification/selection of dried droplets. Ferret’s diameter (# of a nonround
concentration during nebulization of 300 µL of either pure saliva (B) or saliva mixed with the CorNPs (C) after 10 s candle burning. (D-E) Heatmap of the number-weighted total particle aerosol concentration during nebulization of 300 µL of either pure saliva (D) or saliva mixed with the CorNPs (E) after 10 s candle burning. (F-G) Number-weighted total particle aerosol concentration during nebulization of 300 µL of either pure saliva (F) or saliva mixed with the CorNPs (G) after 10 s of candle burning. (H-I) Mass-weighted total particle aerosol concentration during nebulization of 300 µL of either pure saliva (H) or saliva mixed with the nanourchins (I) after 10 s of candle burning.

Fig. 4. Fluorescent speech droplet morphology analysis. (A) Context of respiratory and speech emissions from infected person releasing the nanoaerosols (long range transmission) and droplet (short range sedimentation to within a one meter periphery). (B) Experimental nebulization setup to mimic speech droplets by use of fluorescent imaging after collection on high resolution imaging-compatible microscopic slides. (C-E) Fluorescent droplets from pure saliva, with spherical NPs and saliva mixed CorNPs respectively (samples were collected on microscopy slides in distance S2 and imaged with 63× CLSM objective). (F-H) Particle shape and size descriptors quantified from spatially resolved fluorescent droplets normalized intensity with ImageJ representing Feret’s diameter (F), droplet volume (G), and droplet count (H) collected in different s distances (Bln: blank or control; CorNPs: corona-like NPs or spike NPs; sal: saliva; Sph: spherical; Cor: corona-like nanoparticle).

Fig. 3. Candle experiment to investigate the role of airborne contaminants affecting the stability of nebulized CorNPs. (A) Exemplary SMPS analysis and particle size distribution during nebulization of the highest investigated concentration (50 µg/mL for each) for pure saliva (blue), saliva mixed with Nano spheres (orange) and saliva mixed with CorNPs (grey) after 10 s of candle burning. (B-C) Heatmap of the number-weighted particle size during nebulization of 300 µL of either pure saliva (B) or saliva mixed with the CorNPs (C) after 10 s candle burning as imitating precontamination conditions. (D-E) Heatmap of the mass-weighted particle size during nebulization of 300 µL of either pure saliva (D) or saliva mixed with the CorNPs (E) after 10 s candle burning. (F-G) Number-weighted total particle aerosol concentration during nebulization of 300 µL of either pure saliva (F) or saliva mixed with the CorNPs (G) after 10 s of candle burning. (H-I) Mass-weighted total particle aerosol concentration during nebulization of 300 µL of either pure saliva (H) or saliva mixed with the nanourchins (I) after 10 s of candle burning.

3.4. Evaporative drying dynamics of microscopic morphology analyses of fluorescent droplets

The drying dynamics of saliva with or without NPs were also investigated by analyzing the drying patterns and droplet shapes of sessile microdroplets by fluorescent intensity as function of time with high resolution confocal microscopy to understand if the presence of spherical nanoparticles or CorNPs may affect drying. A concentrated 0.5 µL droplet of saliva with FITC-tagged mucin (as control) was compared to a droplet of saliva with FITC-tagged mucin mixed with CorNPs and to spherical NPs.

In the optimized imaging environment in the microscopic incubation chamber with controlled temperature (37 °C), humidity (95 %) and gaseous exchange (5 % CO2), the water-dependent fluorophore mobility and diffusion can be recorded by microscope photo multiplier tube. Analyzing over all viscosity of the condensate phase of saliva droplets, one can analyse qualitatively the drying dynamics of droplets (Wüstner et al., 2012). As shown above in Fig. 5.A-C, the starting fluorescence recorded in saliva mixed urchins was approximately 10 fold higher compare to pure saliva or mixed with spherical NPs. This could be due to nanoparticle shape and size mediated fluorescence enhancement in aqueous environment (Ayala-Orozco et al., 2014). Further drying dynamic was similar in spherical NPs mixed saliva and pure saliva, where intensity drop was more linear and uniform over time in pure saliva (~200 s). However, in spherical NPs mixed saliva, trend observed was biphasic with rapid drop in fluorescence intensity (1st 0~90 s), then 2nd phase from 90 to 220 s. Unlike spherical NPs mixed saliva, in spike bearing gold urchin mixed saliva, the linear drop was recorded ~166 s. The fluorescent images as shown in Fig. 5. B and F qualitatively exhibit the top view of the completely dried droplets. The pure saliva droplets show smooth periphery with asymmetric shrinkage of droplet probably arising due to off-centered viscoelastic changes in biomolecular composition in the droplet (Fig. 5.B). Saliva mixed with spherical NPs show splitting of the biomolecule concentration into doughnut like a pattern with quasi-smooth periphery. Saliva mixed with CorNPs a periodic zigzag peripheral pattern is noted with symmetric distribution of bimolecular concentration in the droplet (Fig. 5.F). The differences in morphology of saliva mixed with NPs compared to pure saliva might arise due to the fact that NPs make homogeneity in saliva composition, and did not allow peripheral movement of biomolecules. Another important aspect shown by saliva droplets as green-colored aggregated FITC-tagged mucin lumps. After careful analysis of these patterns, we anticipated that in the saliva mixed NPs, tiny water droplets remained homogeneously distributed due to NPs while drying with uniform pattern formation.
Spike NPs show 105 particles/cm³, which we speculate due to shape medi-102
ated pure saliva and saliva mixed with spherical NPs. However, saliva mixed
shared at this time as the data also forms part of an ongoing study.

Data availability

A.V.S., and R.S.M., A.K., A.G.; funding acquisition, A.L.

A.V.S.; supervision, A.V.S., M.R., P.L. and A.L.; project administration,

A.V.S., R.S.M., J.H., A.G. and P.D.P.; graphic design and visualization,

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4. Conclusion

To conclude, in the present study, we developed a simplistic method to
simulate the speech droplet/nanoaerosols production mimicking infected
(saliva + nanourchins) and healthy (saliva) person speech. Nebulized aerosol
emissions and particle size distributions were measured from pure sa-
liva, saliva mixed spherical NPs and saliva mixed CorNPs. Particle
number concentrations were found in a range of 10⁶ particles/cm³ for
pure saliva and saliva mixed with spherical NPs. However, saliva mixed
spike NPs show 10⁶ particles/cm³, which we speculate due to shape medi-
ated faster agglomeration of particles in the air. Fluorescent droplets, which
are bigger in size and sediment closer to nebulizer sources, were analyzed
after collection at different heights and distances. The results showed vary-
ing patterns of droplet shapes, sizes and nonround features for saliva com-
pared to saliva mixed with NPs (spherical vs. CorNPs). Further drying
pattern and final morphology of the sessile drops corroborated the similar
trend for CorNPs mixed saliva compare to pure nebulized saliva. Such find-
ings shed light on how aerodynamics and particokinetics of nanoaerosols
generated during speaking in closed environment differ in context with in-
fected vs. healthy person speech. We further demonstrated via a candle ex-
periment that contaminating particles in such environment have minimal
role in airborne nanoaerosols stability. The simulation and characterization
of the saliva aerosol and environmental aerosol as well as their possible in-
teraction adds value to this study in terms of exposure and risk assessment
given the current pandemic.

CRediT authorship contribution statement

Conceptualization, A.V.S.; data curation, A.V.S., R.S.M., A.K. and A.G.;
writing—original draft preparation, A.V.S.; writing—review and editing,
A.V.S., R.S.M., J.H., A.G. and P.D.P.; graphic design and visualization,
A.V.S.; supervision, A.V.S., M.R., P.L. and A.L.; project administration,
A.V.S., and R.S.M., A.K., A.G.; funding acquisition, A.L. & P.L. All authors
have read and agreed to the published version of the manuscript.

Data availability

The raw/processed data required to reproduce these findings cannot be
shared at this time as the data also forms part of an ongoing study.

Declaration of competing interest

The authors declare that they have no known competing financial inter-
ests or personal relationships that could have appeared to influence the
work reported in this paper.

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Appendix A. Supplementary data

A list of additional supporting information with the size distribution of
gold nanourchins by DLS, measurements of viscoelasticity, aerodynamics/
particokinetics repetition verification, aerodynamics of candle experiment,
microscopy of droplet morphology, ImageJ segmentation and binary anal-
ysis and change in droplet morphology collected from different height
after nebulization is supplied. Supplementary data to this article can be
found online at doi: https://doi.org/10.1016/j.scitotenv.2022.160503.

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Fig. 5. Time-resolved microscopic drying dynamics by fluorescence intensity (top, A, C, E) and final morphology patterns as top view of dried droplets (bottom, B, D, F) of sessile droplets of (A-B) saliva with FITC-tagged mucin, (C-D) saliva with FITC-tagged mucin mixed with spherical NPs and (E-F) saliva with FITC-tagged mucin mixed with CorNPs.
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