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RESEARCH ARTICLE

Real-time characterization of chemical threat agent aerosols for improvement of inhalation studies

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ABSTRACT

Objectives: Deliberate or accidental release of chemical treat agents in the aerosol form can cause an inhalation hazard. Since the relationship between aerosol properties and health hazards is poorly understood, research into the toxicological consequences of exposure to aerosols is needed. The aim of the present study was to improve the characterization of particles for inhalation studies.

Methods: Several aerosol measurement technologies were compared for their potential to physically and chemically characterize particles in the inhalation size range in real-time. For that purpose, we compared the performance of an aerodynamic particle sizer (APS), a scanning mobility particle sizer (SMPS) and an electrical low-pressure impactor (ELPI) in an experimental set-up in which particles were generated by a Collison nebulizer and subsequently delivered into a nose-only inhalation exposure system.

Results: We found that more than 95% of the number of particles, equating to more than 83% of the mass generated by the 6-jet Collison nebulizer, were below 0.5 µm. To characterize the entire size range, the APS as single detector has only limited value, therefore the addition of supplementary instrumentation such as the SMPS or the ELPI is required. After real-time measurements in the size range of 30 nm to 10 µm, ex-situ chromatographic chemical analysis is essential for quantification of the delivered mass concentration.

Conclusions: In summary, the present work demonstrates the utility of the ELPI technology, in combination with off-line analysis, for characterizing aerosols with various size, shape, charge, and composition. This makes the aerosol generation and analysis suite described a promising tool for quantitative inhalation exposure studies.

HIGHLIGHTS

- Multiple analysis techniques were applied for real-time aerosol characterization
- Aerosol size distributions are characterized for inhalation exposure studies.
- Analytical analysis following ELPI measurements is essential for mass quantification.

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Aerosol generation; inhalation exposure; chemical threat agents; particle distribution; ELPI; aerosol characterization

Introduction

Deliberate release of chemical, biological, radiological, or nuclear (CBRN) agents, by either state or non-state actors, cannot be ruled out (Europol, 2020). Conventional chemical threat agents are more or less volatile and are inhaled as vapors. Some recently reported nerve agents are of low volatility (Nepovimova and Kuca, 2018) and therefore need to be dispersed in droplet aerosol form to become an inhalation hazard. Moreover, the dispersion of an opiate-based aerosol in a theater in order to end a hostage situation resulted in many casualties (Riches et al., 2012). Although the probability of a deliberate CBRN incident is perceived as low, these examples point out the extent of the potential impact in the event such an attack occurs. This emphasizes the need to better understand and physically and chemically characterize aerosolized toxic chemicals.

One of the challenges in aerosol sciences is to find relationships between aerosol properties and health risks (Phalen et al., 2021; Sorensen et al., 2019). The COVID-19 pandemic has dramatically emphasized the knowledge gap and lack of consensus regarding airborne transmission and subsequent health risks. Yet, aerosol characteristics, like morphology, particle size and charge, significantly influence the deposition in the respiratory tract, which may result in unexpected health effects. From a traditional toxicological point of view, larger chemical particles have been considered to be more relevant than smaller particles because of the

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larger mass reflected in this fraction. However, the particle deposition and distribution in the body and effective uptake of a compound may depend on size and other characteristics. For certain chemicals with organ-specific toxicological targets, this may result in changes in the toxicological profile (Sorensen et al., 2019). For instance, an increase in deposition in the respiratory system is found for pharmaceutical aerosols carrying a significant electrostatic charge (Vinchurkar et al., 2009; Melandri et al., 1983; Chaurasiya and Zhao, 2020). Moreover, the ability to thoroughly characterize threat agent particles is of utmost importance to understand and improve physical protection against chemical warfare agents (Bergmann et al., 2023). For such purposes, it is necessary to fully characterize an aerosol in terms of particle size distribution.

Despite the need for detailed characterization, it is currently difficult to adequately define the various aerosol characteristics with any single detection method that is applicable to aerosols of any relevant size, shape, and composition. Regulatory acceptance testing of metered dose inhalers requires cascade (Andersen) impactors for aerodynamic particle size distribution measurements (Stein et al., 2002). Yet, this is a labor-intensive method that does not provide real-time information. Time-of-flight detection methods, such as the aerodynamic particle sizer (APS), are widely used in aerosol research for real-time number distribution measurements. However, these methods have significant limitations if employed for particles or droplets smaller than 0.5 µm. In this case, corrections need to be applied for mass-weighted particle distribution measurements, in particular for particles with other than unit density or non-spherical shapes (Pagels et al., 2005; Armendariz and Leith, 2002; Mitchell et al., 2017).

To overcome this shortfall, this study investigates an alternative technique, the electrical low-pressure impactor (ELPI), which combines real-time detection with conventional cascade impaction. The larger particle size bins in the ELPI are similar to the Andersen impactor, which is the traditional system for inhaled drug delivery studies, since the deposition of aerosols in each size bin corresponds well with deposition patterns in various divisions throughout the respiratory tract (Vinchurkar et al., 2009). In addition, the ELPI has the potential to determine the number of elementary charges of different particles, by measuring currents which can be used to determine the aerosol charge (Dekati Ltd, 2016; Marjamäki et al., 2000; Ouf and Sillon, 2009; Glover and Chan, 2004). Aerosols that carry significant negative or positive electrostatic charge will deposit more efficiently in the respiratory system when compared with the behavior of similarly sized particles carrying no significant charge (Melandri et al., 1983).Melandri et al., 1983 showed with CFD simulations that particles that carried an elementary unit charge between +12 and +230 deposit around 10 times more in cascade impactor stages between 0.44 and 0.59 µm, instead of the stages 1.00 to 1.86 µm for neutral particles (Vinchurkar et al., 2009). These results can be used to approximate the effect of charged aerosols on the region of deposition after human exposure. Whereas the use of real chemical warfare agents in this study was considered unnecessarily complicated, some tailor-made, less-toxic simulant agents were used as surrogates. Together with ex-situ analysis of the collection plates by chromatographic analytical methods, the ELPI might be a valuable instrument to quantify the deposition as a function of particle charge.

The aim of the current study was to improve capabilities to characterize aerosols delivered in the course of inhalation studies. This was done by comparing several measurement technologies for their potential to physically characterize particles of interest for inhalation toxicology in real time. For that purpose, we compared the performance of an APS, a SMPS and an ELPI in an experimental set-up in which aerosols were generated using a Collison nebulizer and subsequently transported into a widely used nose-only inhalation exposure system (Lucci et al., 2020), which is a valuable tool for dose-response relationship measurements under various conditions (Mckinney et al., 2013). In addition, ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography with flame ionization detector (GC-FID) were used for supportive ex-situ analysis. Using this approach, various aerosol properties have been successfully characterized, which shows that the combination of measurement instruments employed, provides a promising tool for inhalation toxicity studies.

Methods

Safety

Experiments with aerosolized toxic chemicals can only be performed in well-validated contained aerosol generation and dispersion chambers. A dedicated system has been designed for experiments with extremely potent chemical warfare agents and other highly toxic compounds, such as synthetic opioids. However, in the present study, low-toxicity simulants were used to evaluate the performance of the system, with a focus on the generation and evaluation of the particle size distributions. Because the system has been designed for the safe use of highly toxic chemicals, precautions were in place to prevent accidental exposure. The aerosol exposure system was placed in a double-walled negative pressure cabinet with the exhaust gas flow filtered by a HEPA/Charcoal filter. The leak tightness of the containment system was determined with fluorescein. After an exposure of 20 min, swabs were taken from 16 locations in the setup. The swabs were extracted with 150 µL water and all samples were assayed with a microplate reader with UV-Vis absorbance (Biotek, Synergy HTX). Subsequently, it was found that the swab contained less than 0.0006% of the generated concentration, based on three generations with 16 swabs each. This was estimated to be sufficiently low for safe use with the earlier-mentioned chemical warfare agents and synthetic opioids.

Experimental setup

A schematic view of the inhalation exposure system is shown in Figure 1. A solution was dispersed by a modified Collison nebulizer (6-jet; BGI by Mesa Labs, Butler, NJ,

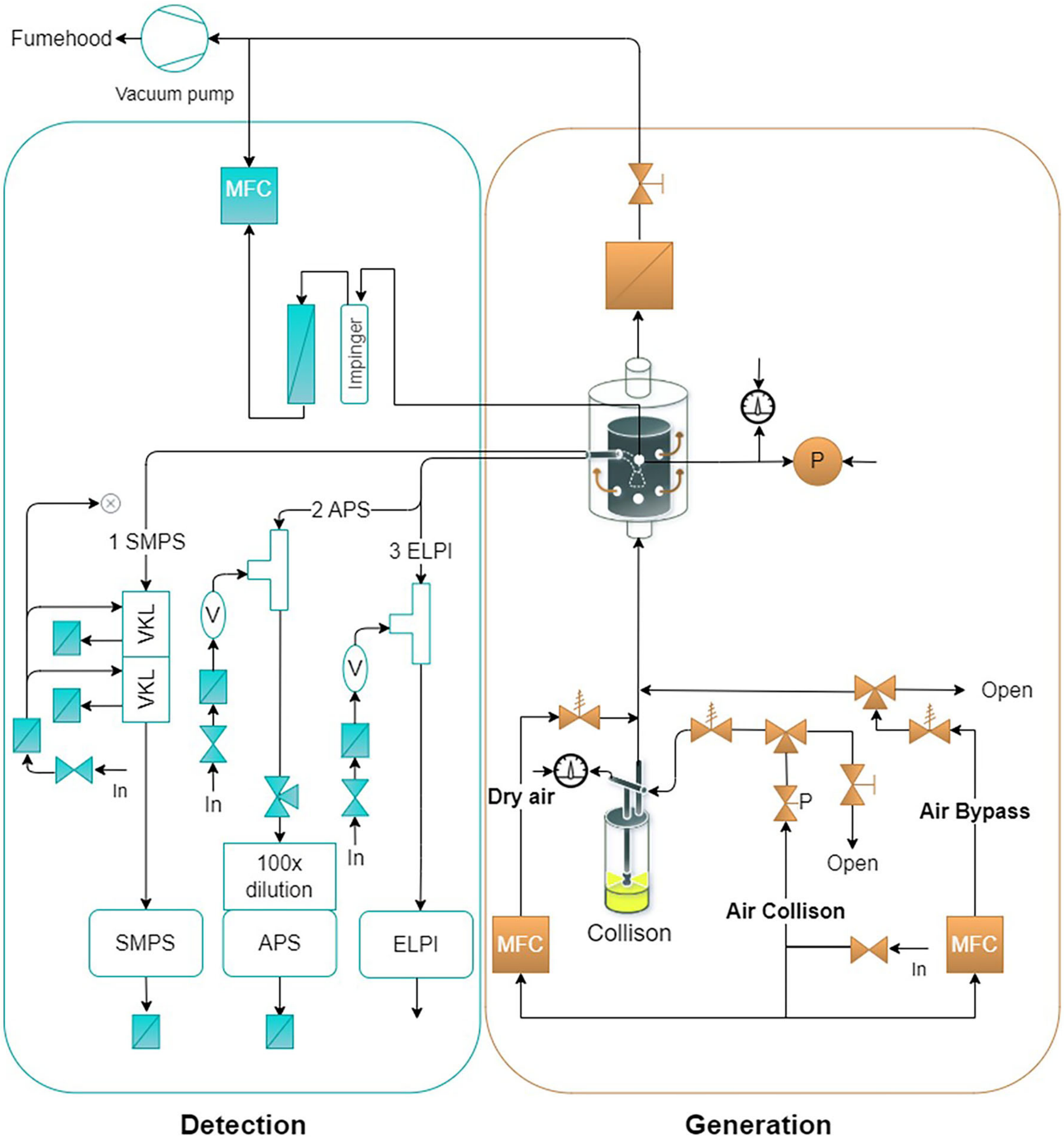

Figure 1. Detailed schematic view of aerosol system with a scanning mobility particle sizer (SMPS), an aerodynamic particle sizer (APS) and an electrical low-pressure impactor (ELPI). an aerosol is generated with the collision nebulizer and directed into the nose-only part of the setup after which the flow is diluted by either VKL diluters, a glass diluter or an aerosol diluter. Impingers were connected to be able to take a sample of the aerosol flow.

USA), where the right-angled top was modified to provide a vertical aerosol outlet (Busker et al., 1999; Jaeger et al., 2006). The aerosol flow was directed into the flow inhalation exposure tower where animals can be exposed noses only. A part of the aerosol flow was sampled through one of the exposure ports for further characterization. The sampled flow was analyzed by a scanning mobility particle sizer, an aerodynamic particle sizer, and an electrical low-pressure impactor (more details are given in Section 2.5). For all collections, isokinetic sampling was used. Corresponding flows and pressures are described in Section 1 of the supplementary material.

The sample flow was diluted by two VKL diluters (Palas10, 2401/1508) before it was directed to the SMPS. Theoretically, two VKL diluters have a dilution factor of 100-fold. However, based on monitoring of the pressure, incoming and outgoing flows it was concluded that the dilution factor was 900-fold as demonstrated in Section 2 of the supplementary material. The flow to the APS was diluted 20-fold by a glass diluter and immediately further diluted by an aerosol diluter (TSI, 3302 A) before the APS system, for a total dilution of 2000. The flow of the ELPI was 40-fold diluted by a glass diluter. The aerosol distributions were corrected for a solvent blank. The results are presented using the aerodynamic diameter, unless stated otherwise.

Chemicals and materials

The system was evaluated with various chemicals. The aim was to generate aerosols with various size, shape, and composition. In the first series of experiments, aerosols of sodium chloride, glucose and glycerol were generated representing three categories of chemicals. Sodium chloride (NaCl, ≥99%, Sigma-Aldrich) is a solid inorganic salt with a face-centered cubic crystal structure. The exact aerosol morphology depends on the drying rate. Fast drying results in softer edges and more spherical particles compared to slow drying (Wang et al., 2010). D-(+)-Glucose (≥99.5% BioXtra, Sigma) is a solid organic compound and will restructure into a non-spherical aerosol after fast drying. Glycerol (≥99%, Sigma) is a liquid organic compound with an oily appearance forming spherical particles (Wright et al., 2016). In the second series of experiments acetaminophen (paracetamol, ≥95%, Sigma), a simulant for potent opioids, was used for aerosol generation. MQ water (SimPak® 1) was used as a solvent and water with 50% methanol was used for the analyses. Finally, the liquid compound trioctyl phosphate (TOP, 97%, Aldrich®) was used. This chemical, with a low vapor pressure, was used as a simulant for the chemical warfare agent VX. TOP is not soluble in water, therefore 2-propanol (IPA, >99.8%, Biosolve) was used as a solvent for the analyses. TOP was nebulized in methanol (99.8%, Sigma-Aldrich). Tributyl phosphate (TBP, >99.8%, Aldrich) was used as an internal standard for GC analyses.

Aerosol generation

Aerosol was generated with the 6-jet Collison nebulizer for 20 min and characterized by both SMPS and APS or with the ELPI. The generation was limited by the requirement of using a liquid, therefore solutions of glucose, NaCl, glycerol and acetaminophen in water at 0.01, 0.1, 1 and 10 vol% were nebulized. Also, aerosols were generated from solutions of 0.01, 0.1, 1 and 10 vol% TOP in methanol.

For the charge experiments an aerosol was generated for 30 min with 30-second measurement intervals. The Corona charger of the ELPI was turned off and on again at 5-min intervals. We tested whether the number of particles during switching remained constant and within acceptable detection limits. The total average current per ELPI stage for the intervals when the charger was turned on and off was separately determined. The measurements were carried out with 0.5% NaCl and glucose. For the charge experiments only, no correction for losses was applied to the ELPI collected data.

Aerosol detection

The following sections elaborate on the SMPS, APS and ELPI detection which was schematically presented in Figure 1. Also, the chemical analyses by UPLC-MS/MS and GC-FID are described.

Real-time detection by APS

The aerosol was characterized with an aerodynamic particle sizer (APS, model 3321, TSI Inc.), which measures particles divided over 52 size bins. The APS uses low particle accelerations for aerodynamic size measurements ranging from 0.5 to 20 µm aerodynamic size and 0.3 to 20 µm optical size (TSI Incorporated, 2012). A flow of 0.25 L/min. was sampled from the inhalation chamber, to remain a convenient flow in the chamber for future *in vivo* animal experiments. This was diluted 20-fold resulting in a total flow of 5 L/min. which was sampled by an aerosol diluter and diluted 100-fold after which it was subsequently detected by the APS. For the modeling of the experiments by Matlab and Python 3.9.5, it is assumed that the solvents water or methanol evaporate, resulting in a dry aerosol. The total number of particles detected in the water blank was lower than 0.1% of the particles detected in the experiments and for methanol this was less than 2% (Section 3 of the supplemental material).

Real-time detection by SMPS

To characterize the sub-micron aerosols a scanning mobility particle sizer (SMPS) was used. The SMPS consisted of an electrostatic classifier (TSI model 3082) with a differential mobility analyzer (DMA, TSI model 3081) and a condensation particle counter (TSI model 3775). A flow of 0.25 L/min. was sampled from the inhalation chamber, after which it was diluted to deliver an SMPS sampling flow of 0.3 L/min. The SMPS measures particles in the size range of 16.8 to 572.5 nm in 99 bins. The SMPS makes use of an electrical mobility detection technique and therefore data is provided with the electrical mobility diameter (d_m).

To be able to compare APS and SMPS results, the electrical mobility diameter was converted to aerodynamic diameter using the density and a value for the shape and morphology of the aerosol, calculated with equations explained by (Willeke and Baron, 1993) and (DeCarlo et al., 2004). A more elaborate discussion can be found in Section 4 of the supplementary material. In addition, the combined particle size distribution was corrected for the bin size.

Real-time detection by ELPI

The ELPI (Dekati Ltd. 2014) was used to measure particles in the range 30 nm to 10 µm divided into 12 size bins (Dekati Ltd, 2016). In the ELPI, aerodynamic size classification is based on the inertia of a particle. An inlet airflow of 10 L/min was used. Collection substrates of smooth aluminum foil (Ø 25–26 mm) were used on the impaction plates. Aerosols generated from a liquid solution, with a concentration up to 1 vol% were measured with an electrometer current threshold of 10.000 fA, resulting in an optimized sensitivity. Liquid concentrations above 1 vol% were measured with a current threshold of 100.000 fA. The current value of each channel, connected to an electrometer current amplifier, is proportional to the particle concentration. Subsequently, unless stated otherwise, current and particle concentration distributions were exported with correction for loss mechanisms provided by the ELPI software. The algorithm corrects both diffusion and space charger losses (Dekati Ltd, 2016).

Chemical analysis by UPLC-MS/MS

The collection substrates of aluminum foil were collected from the ELPI and prepared for chemical analysis. Foil with acetaminophen was extracted for 1 h with 5 mL MQ water +

50% methanol and analyzed with UPLC-MS/MS. The experiments were performed on an Acquity UPLC M class system with advanced tandem quadrupole Mass Spectrometry (MS/MS) detection (Waters, Xevo TQ-S). The column temperature was maintained at 21 °C. An Acquity UPLC® HSS T3 column (100 mm x 2.1 mm, 1.8 µm film thickness) was used. The mobile phase consisted of water and acetonitrile both with 0.2% formic acid, using a gradient at a flow rate of 100 µL/min. The following gradient was used: 0 – 10 min. 0 – 80% acetonitrile; 10 – 12 min. 80% acetonitrile; 12 – 12.10 min. 20 – 100% water. The injection volume was 5.00 µL. The UPLC was connected to a triple-quadrupole mass spectrometer equipped with electrospray ionization, for quantification of the analytes in positive ion mode. Section 5 of the supplementary material shows a schematic presentation of the UPLC-MS/MS technique used for the characterization of the aerosol system. For acetaminophen, the monitored transition was set at m/z 152 → 110 with a collision energy of 15 eV. Data acquisition was performed by Masslynx 4.1 software and processed by TargetLynx (Waters Corp., Milford, MA, USA). The analytical methods were validated for selectivity, linearity, accuracy, precision, and matrix effects according to the procedure described in a bioanalytical method validation guidance (Food and Drug Evaluation, 2018).

Chemical analysis by GC-FID

The aluminum foil collection substrates were collected from the ELPI and prepared for chemical analysis with gas chromatography. Foil with TOP was extracted with 2 mL IPA and stirred for 1 min with a vortex mixer. The extraction efficiency was determined with methanol, ethanol, IPA, hexane, and ethyl acetate using stirring for one minute with a vortex mixer or rotation for 1 h. The solution was measured with GC-FID. An Agilent CP8822i5 (fused silica) capillary column (30 m x 0.25 mm I.D., 0.25 µm film thickness) was used. The oven program was 80 °C (1 min.) → 60 °C/min. → 320 °C (3 min.). The injection volume was 5 µL.

Results and discussion

Characterization of the aerosol size distribution

Prior to the aerosol generation experiments, a simulation of the aerosol distribution based on a tool provided by the manufacturer of the aerosol generator was performed (MesaLabs, 2022). Section 6 of the supplementary material shows graphs with the aerosol distribution for various concentrations of dissolved chemicals. Although the APS is often used to characterize the aerosol produced by the Collison nebulizer platform (Bowen et al., 2017; Barnewall et al., 2015), our calculations show that the size, of a large fraction of the aerosol and a significant proportion of mass, is expected to be out of the range (< 0.5 µm) of particle sizes the APS can measure. Although mass exponentially decreases with decreasing particle size, the determined distribution is such that the mass represented by those smaller particles should not be neglected from a toxicological point of view. Consequently, the dose to which animals are exposed is in fact higher than would be assumed solely on the basis of APS data. Therefore, an additional or alternative analytical technique is required to enable correct detection and toxicological impact assessment of the entire particle size range.

Characterization from the nanometer range by SMPS combined with APS

To study the hypothesis based on calculations made in Section 3.1 that the APS underestimates the contribution of particles smaller than 0.5 µm, the SMPS was used in combination with the APS to cover the entire particle range between 16.8 nm and 20 µm. Figure 2 shows the aerosol distributions of glucose, glycerol and NaCl at three different concentrations, compared with the theoretical predictions. The SMPS measures the electrical mobility diameter, which was converted to the aerodynamic diameter by using the density. This resulted in slightly different maximum SMPS values for the various chemicals. On the left-hand side (Figure 2(A–C)) the number of particles with respect to the aerodynamic diameter is shown, corrected for the size bin. The figures on the right-hand side (Figure 2(D–F)) show the cumulative concentration distribution, which is the percentage of mass per volume from the total. As hypothesized, the distributions show that the majority of the detected particles and mass result from particles smaller than 0.5 µm. More specifically, 0.1 vol% NaCl yields the lowest mass median diameter (MMD) of 0.27 µm, whereas 10 vol% glycerol yields the highest MMD of 1.7 µm. The most probable cause of higher diameters for glycerol is the more consistent diameter regardless of the angle in the detector. In contrast, the orientation of the non-spherical glucose and NaCl affects the detected particle size, being either smaller or larger than the average diameter.

Furthermore, the figures show that the aerosol distribution measured by the SMPS and APS was comparable to the theoretical distributions predicted for the Collison nebulizer. Only for the lower generated concentrations the measured values with SMPS were higher than the theoretical particle size distributions predicted by the Collison model. A plausible explanation for this is that particles below 0.5 µm are only predicted based on the shape of a log-normal distribution and not experimentally validated (MesaLabs, 2022).

A drawback of using SMPS in conjunction with APS is that the results did not always align. The number of particles in the overlapping range around 0.5 µm measured by APS, were systematically lower than the SMPS results. Only for 0.1 vol% generated solutions of glucose and glycerol, were the SMPS measurements within the error range of the APS (deviation number concentration <40%). An explanation for the discrepancy between the APS and SMPS is the different measurement principle (Das et al., 2022) and consistent underestimation of small particles by the APS. The APS is known to underestimate small particles, due to the recirculation of smaller particles and lower optical sensitivity associated with insufficient light scattering of very small particles (Stein et al., 2002; TSI Inc, 2018; Stein et al., 2003). In order to

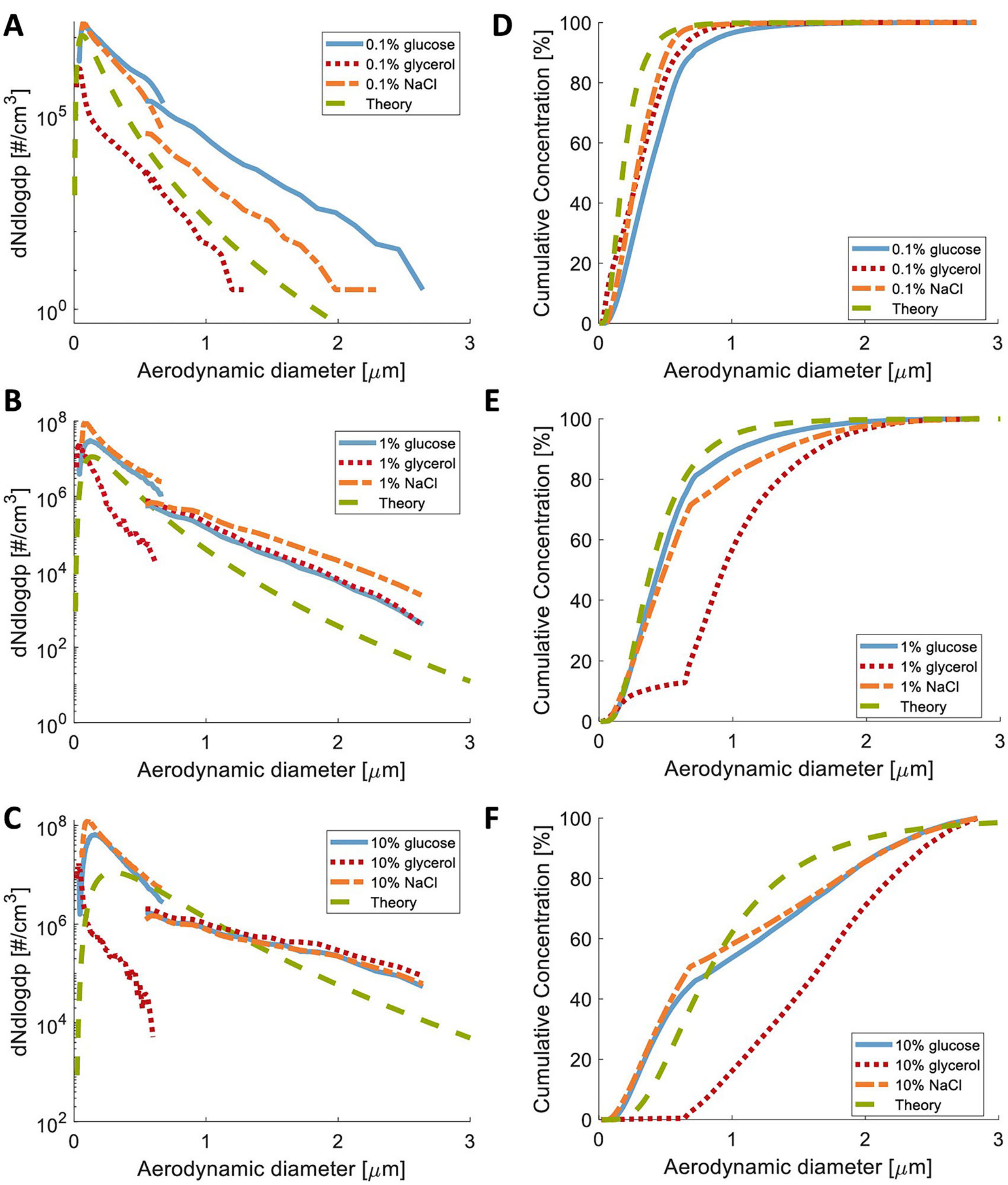

Figure 2. Aerosol distributions of 0.1 (A,D), 1 (B,E) and 10 (C,F) vol% glucose, NaCl and glycerol, measured by a scanning mobility particle sizer (SMPS) up to approximately 0.6 µm and an aerodynamic particle sizer (APS) starting at 0.5 µm. A-C) Number particle size distribution corrected for the bin size. D-F) Cumulative concentration distribution.

simultaneously use the SMPS and APS, a correction should be applied. To avoid this extra data processing step, the next section describes the use of the ELPI that covers the entire size range of the SMPS and APS within a single technique.

3.3. Broad range characterization with ELPI

The experiments with various concentrations of glucose, glycerol and NaCl were repeated and analyzed by the ELPI, which covers the entire particle size range of interest between 30 nm and 10 µm. Advantages of the ELPI are the instrument's potential to combine real-time detection with conventional cascade impaction followed by ex-situ analysis of the collection plates by an analytical chemical technique. However, a possible drawback of the ELPI is the lower size resolution compared to SMPS combined with APS as discussed in Section 3.2. Figure 3 shows the particle size distributions, with on the left the number particle size distribution and on the right the concentration distribution. The ELPI measurements confirm that a major fraction of particles appears to be smaller than 0.5 µm, in line with the theoretical predictions and the APS and SMPS results. A concentration of 0.01 and 0.1 vol% resulted in an aerosol distribution in which 99% of the total number of particles was smaller than 0.5 µm. For the highest concentrations of 10 vol%, 95% of the particles were still smaller than 0.5 µm.

One unanticipated finding was the increase in mass concentration for particles larger than 1.5 µm. A likely explanation for this observation would be losses of small particles in the size bins of large particles which causes the erroneous

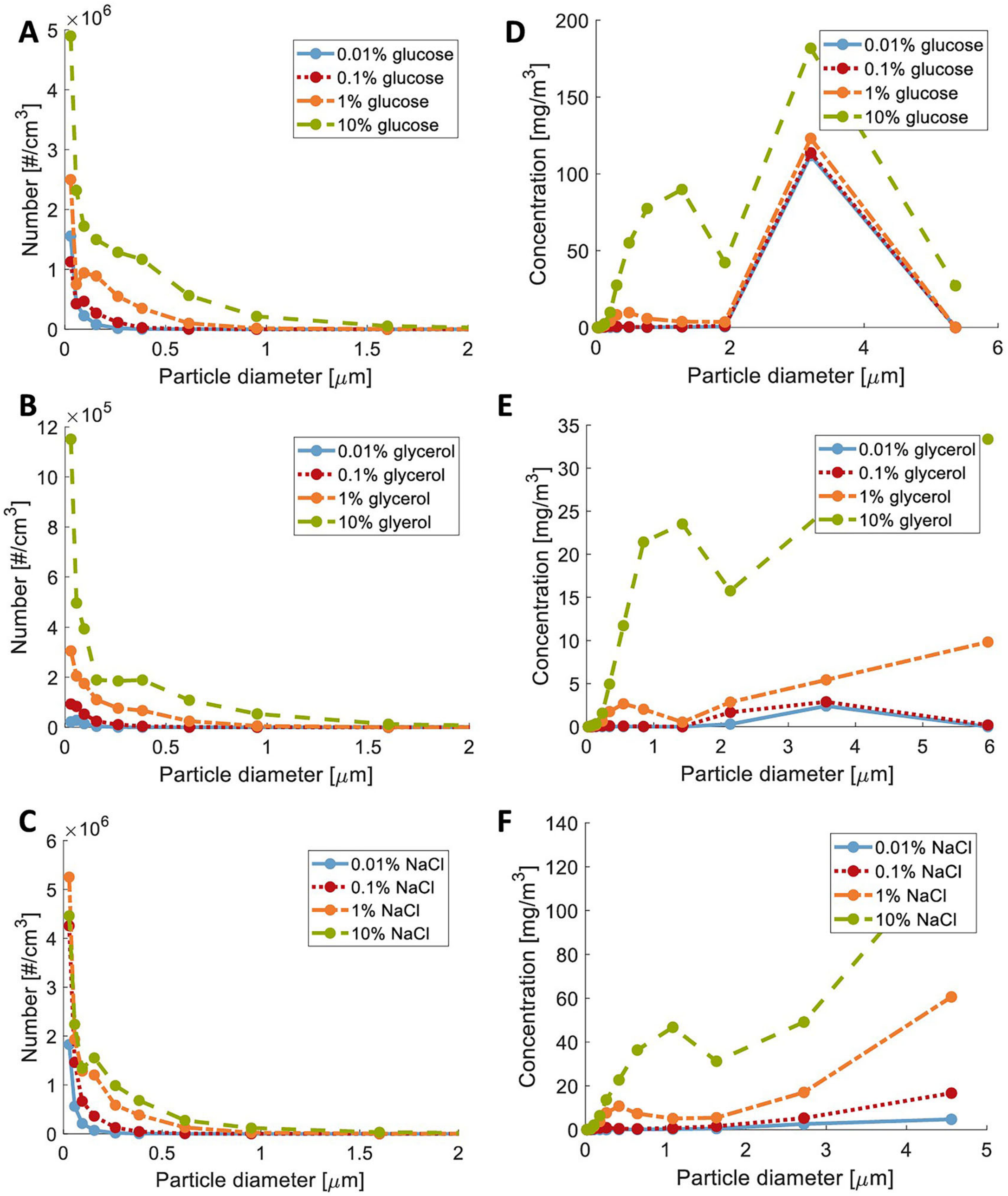

Figure 3. Aerosol distributions of 0.01, 0.1, 1 and 10 vol% glucose (A, D), NaCl (B, E) and glycerol (C, F), measured by an electrical low-pressure impactor (ELPI). A–C) Number particle size distribution D-F) mass concentration distribution.

detection of large particles. This will be discussed in Section 3.4.3. An advantage of the ELPI is the possibility to perform ex-situ analysis of the material on the impactor stages. Therefore, the next section describes the validation of the read-out of the ELPI by comparison with the results from additional analytical techniques.

Quantitative analysis following ELPI measurements

This section first describes the optimization and validation of the quantitative analysis of the ELPI collection substrates by UPLC-MS/MS and GC-FID. Afterwards the analytical methods were applied following real-time detection of acetaminophen and TOP by the ELPI, to verify the mass concentrations.

UPLC-MS/MS optimization and validation

The UPLC-MS/MS method was optimized and validated for acetaminophen. The method was selective for acetaminophen (t_r: 5.76 min.). Linear standard curves were obtained with $R^2 = 0.9985 - 0.9999$. The y-intercept of the calibration curve was 3.2% up to 4.8% of the target value of analyte ($\bar{y}$). The limit of detection (LOD) was 0.1 ng/mL and the limit of quantification (LOQ) 0.5 ng/mL. The mean value of the quality controls (QCs) was within 9% and 1% of the actual value for 1 ng/mL and 100 ng/mL, respectively. Elaborated results can be found in Section 5 of the supplementary material.

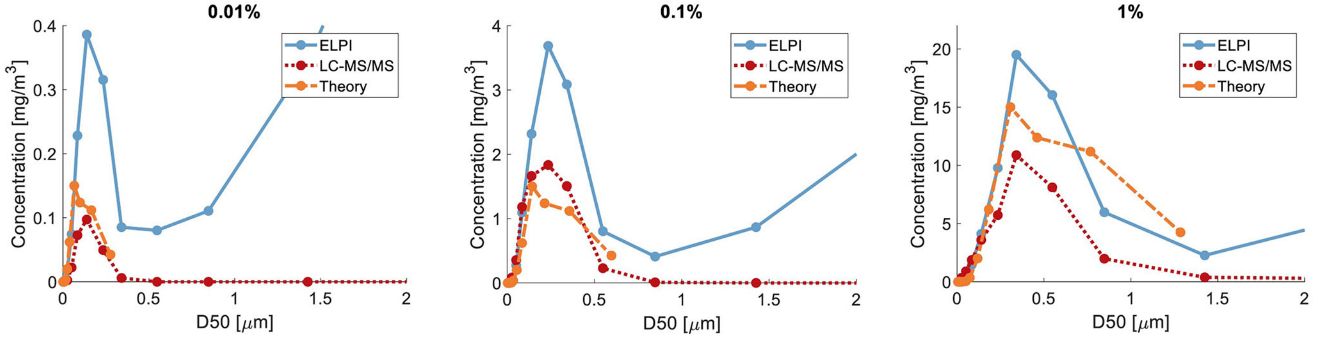

Figure 4. Concentration distribution of 0.01, 0.1 and 1 vol% acetaminophen determined with an electrical low-pressure impactor (ELPI) and ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS).

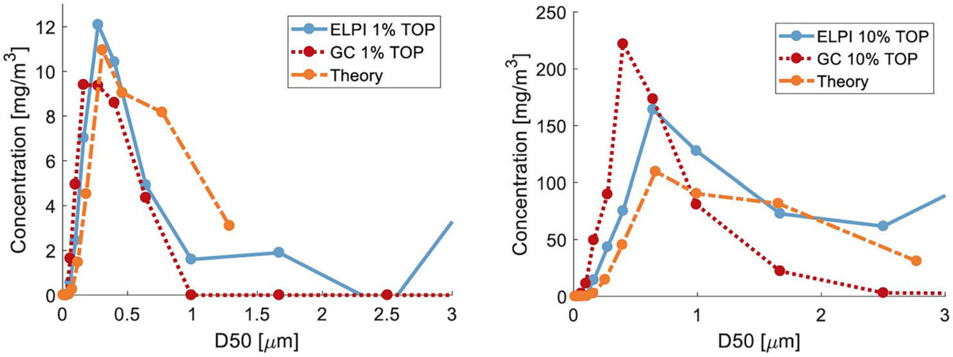

Figure 5. Concentration distribution of 1 and 10 vol% trioctyl phosphate (TOP) determined with an electrical low-pressure impactor (ELPI) and gas chromatography with flame ionization detection (GC-FID).

GC-FID optimization and validation

A GC-FID method was optimized and validated for TOP. The method was selective in the different matrices (t_r: 7.001 − 7.009 min.). Linear standard curves were obtained with $R^2 = 0.998 - 0.9999$. The y-intercept of the calibration curve was −0.02% up to 0.02% of the response for the target value of analyte ($\bar{y}$). The limit of detection (LOD) was 1 µg/mL and the lower limit of quantification (LLOQ) was 2.2 µg/mL. The mean value of the quality control samples of 10 nL/mL ($n = 9$) and 100 ng/mL ($n = 9$) was within 2.6% and 1.4%, respectively, of the actual value. Elaborated results can be found in Section 7 of the supplementary material.

Aerosol distribution of acetaminophen and TOP

After aerosol generation of acetaminophen and TOP, all ELPI-stages in the particle range between 30 nm and 10 µm were analyzed by UPLC-MS/MS. Figure 4 shows the concentration distribution of 0.01, 0.1, and 1 vol% acetaminophen detected by ELPI and UPLC-MS/MS as a function of the aerosol particle size, compared with theoretical distributions. Figure 5 shows the concentration distribution of 1 and 10% TOP detected by ELPI and GC-FID. As expected, the graphs demonstrate that the UPLC-MS/MS and GC-FID do not measure an increase in mass concentration for particles larger than 1.5 µm. This indicates that these large particles likely represent erroneously detected non-chemical artifacts. In accordance with the earlier presented results in this study, 99.9% of the mass is detected in the size bins below 0.5 µm for up to 1 vol% of generated aerosol. Even for 10 vol% generated aerosol concentration, this fraction is still more than 83%.

A probable explanation for the unexpected large particles detected by ELPI are space-charge and diffusion losses (Pagels et al., 2005; Marjamäki et al., 2005; Virtanen et al., 2001). Space charge losses occur when particles collide with the walls of the detector tubing due to electrostatic force. This leads to a significant loss when the number concentrations are high but can be neglected for lower number concentrations. In the current study, large losses were encountered for low-number concentrations as well. Therefore, the results cannot be wholly explained by space-charge losses. Also, diffusion losses can be a probable explanation for the erroneous detection of large particles. In impactors, diffusion losses cause particles that are smaller than the cutoff diameter of an impactor stage to be collected to some extent. Virtanen et al. calculated the diffusion particle loss to be below 2% particle concentration for particles between 30 nm and 10 µm (Virtanen et al., 2001). For number distribution measurements the effect of particle loss is relatively low. However, when the results are converted to mass concentrations, the effect is very large. The results of the current study showed a 1000 times larger mass of TOP and acetaminophen in the higher-size bin compared to the mass measured by analytical chemical analysis. Therefore, it can be concluded that the unexpected large particles are artifacts associated with the loss of small particles that are erroneously detected as large particles.

For reliable future mass concentration measurements, it is advised to not use the results of the highest size bins of

Table 1. Mass median diameters (MMD) of acetaminophen measured by an electrical low-pressure impactor (ELPI) and afterwards determined with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for 0.01, 0.1, 1 and 10 vol% aerosolized liquid concentrations.

Concentration (vol%)	MMD measured by ELPI (μm)[a]	MMD analyzed by LC-MS/MS (μm)	Theoretical MMD (μm)
0.01	0.14	0.13	0.10
0.1	0.23	0.21	0.21
1	0.34	0.31	0.46
10	0.55	0.31	0.99

[a] For this calculation the higher ELPI size bins are not included. A normal distribution is assumed, where the ascending concentrations after the second minimum are neglected.

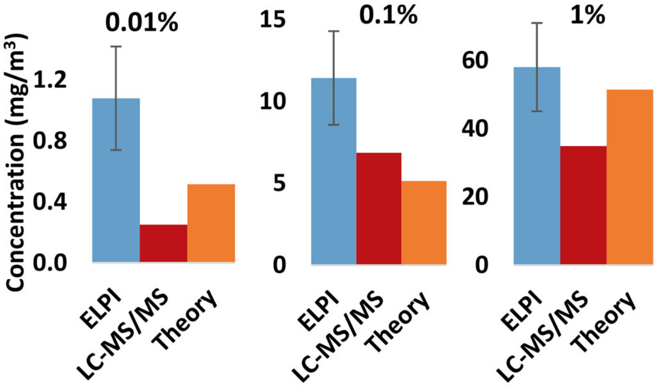

Figure 6. Total concentrations of acetaminophen measured by an electrical low-pressure impactor (ELPI) and afterwards determined with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for 0.01, 0.1 and 1 vol% generated volume percentage. Bins from 0.03 to 0.4 μm, % bins up to 1 μm or bins up to 1.5 μm were considered for respectively 0.01, 0.1 and 1 vol%. the error bars present the within-variation (standard deviation, $n = 120$).

the ELPI if most particles are below 0.5 μm (as shown previously in Figure 5). When these size bins are not included, the total concentrations and MMD up to 1 vol% are consistent with theory and LC-MS/MS results. Table 1 compares the MMDs detected by the two techniques and predicted by theory. The MMDs are similar for lower concentrations and somewhat lower for higher concentrations compared to theory. The latter effect might be due to gravitational losses or the falling apart of aerosols in smaller particles in the sampling line of the ELPI. When the MMDs are compared to results for TOP, higher values for acetaminophen were observed. This difference was expected, since a solvent with increased vapor pressure often causes the generation of smaller particles compared to the same concentration in water (Shum et al., 1993; Kahen et al., 2004). Figure s 6 and 7 present the total mass concentrations measured by ELPI, LC-MS/MS or GC-FID compared to theoretical values. The concentrations of the ex-situ analyses were comparable with theory and did not deviate more than a factor two.

Differentiating between particle distributions

The potential use of the system for differentiating between two particle distributions was examined. This is relevant for studies to get more insight into the effect of particle size on the deposition in the body and a possible relation to toxicological consequences. According to ICRP66 (ICRP, 1994) the particle deposition for aerosols between 0.01 and 10 μm in the respiratory system varies for different sizes (Section 8 in the supplementary material). Figure 8 illustrates two normalized particle concentration distributions for acetaminophen and TOP, created by varying the dissolved concentration. The overlapping area for two concentrations acetaminophen and TOP is respectively 38 and 57 w%. The unique fraction of the distribution is expected to be administered in a different part of the respiratory system, resulting in different toxicological effects. Therefore, with the current nebulizer, it is possible to generate notable different size distributions, although it depends on the application whether this difference in distribution is significant enough. In future applications another nebulizer with a larger primary droplet size can be used that generates doses with even less overlapping particle size distributions, resulting in particle deposition in separate parts of the body with different uptake regimes.

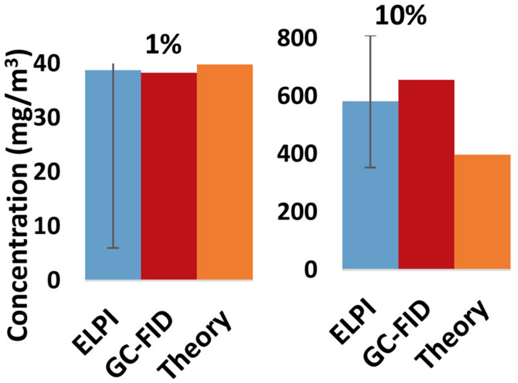

Figure 7. Concentrations of trioctyl phoshate measured by an electrical low-pressure impactor (ELPI) and afterwards determined with gas chromatography with flame ionization detection (GC-FID) for 1 and 10 vol% generated concentration. Bins up to 1 μm or 2.5 μm were considered for respectively 1 and 10 vol%. the error bars present the within-variation (stdv, $n = 120$).

Charge measurements with the ELPI

An additional feature of the aerosol generation system with ELPI characterization was the possibility of measuring the charge of the particles. The equations used to convert the currents to charges are explained in more detail in Section 9 of the supplementary material. Equation 1 shows the final equation that was used for the calculations. The average number of elementary charges per particle for uncharged particles ($n_{uncharged}$) is equal to the current for uncharged particles ($I_{uncharged}$) times the number of elementary charges per particle for charged particles ($n_{charged}$), divided by the current for charged particles ($I_{charged}$). Because the particles are not charged by the Corona charger a charger efficiency (P) of 1 is expected.

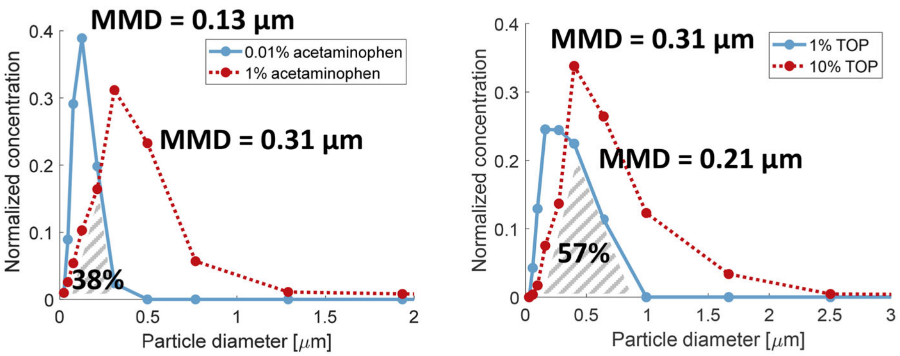

Figure 8. Comparison of two normalized concentration particle size distributions. A) 0.01 and 1 vol% acetaminophen measured with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). B) 1 and 10 vol% trioctyl phosphate measured with gas chromatography with flame ionization detection (GC-FID).

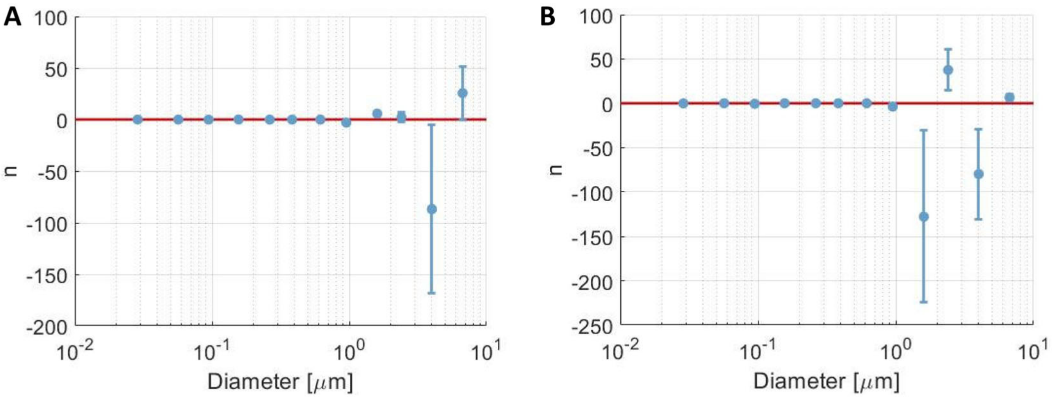

Figure 9. Number of elementary charges for different particle diameters for A) glucose, B) NaCl (>1000 particles/cm^3). the error bars represent two times the standard error of mean (±SEM, $N = 3$-4 repetitions).

$$Pn_{uncharged} = \frac{I_{uncharged} * Pn_{charged}}{I_{charged}} \qquad \text{Equation 1}$$

Figure 9 shows the results where the number of elementary charges for glucose or NaCl is shown for each particle size bin. The number of particles in each size bin was at least 1000 particles/cm^3. For both the glucose and NaCl experiments, no particles below 1 µm were significantly positively charged. For glucose more particles were significantly negatively charged than for NaCl. Particles above 1 µm carried higher negative and positive charges. Because negatively charged particles are seen as smaller particles compared to neutral particles (Vinchurkar et al., 2009), it is expected that these aerosols may deposit in deeper stages of the lungs than neutral particles (Chaurasiya and Zhao, 2020). In case more potent chemicals than NaCl and glucose are examined, the influence of the charge may influence the health risk. Current research demonstrates the possibility of examining a small range of aerosol charges. Future research could focus on applying an additional charger to the current setup to explore the toxicological effect of a wider range of aerosol charges.

Conclusions

In the present study, the ability to correctly characterize aerosol exposure in toxicological studies by three real-time aerosol detectors (SMPS, APS, and ELPI) was examined. By using a combination of these detectors together with a nebulizer and an inhalation exposure system, it proved the possibility of connecting various measurement techniques, enabling the qualitative and quantitative evaluation of aerosols with a broad range of characteristics. The APS was used in conjunction with the SMPS to cover the entire particle size range generated by the Collison nebulizer.

This study demonstrated that the measurements based solely on the APS underestimate the contribution of particles below 0.5 µm. This is a major drawback, as such small particles can contribute significantly to the inhalation hazard. This study employed an experimental set-up that generated aerosols of which more than 95% of the number of particles were smaller than 0.5 µm (solute concentrations up to 10 vol%). This corresponds with more than 83% of the generated mass, emphasizing the limitations of the use of the APS instrument alone. Combination of the APS with the SMPS provides only a partial resolution of the measurement issue because the two instruments show a discontinuity in the overlapping range of 500 to approximately 600 nm. An iterative determined correction factor provided by other researchers might correct for this discontinuity, which is caused by the morphology of the particles.

In contrast to the combination of SMPS and APS in the same experimental set-up, the ELPI appeared to successfully monitor, in real-time, the aerosol concentration and charge in a particle range between 30 nm and 10 µm. This technology offers the capability to follow concentration in real-time and permits verification of the measurement results by offline analysis of the deposition onto the several stages. However, particle losses resulted in artifacts, due to losses of small aerosols that were erroneously detected as large particles. It was confirmed by UPLC-MS/MS and GC-FID that no particles occurred in the size bins above 1.5 µm. It is important in toxicological studies to accurately measure this particle size range because it corresponds with relevant pharmaceutical aerosol administration sizes. If the highest size bins were not considered, a relatively reliable mass concentration result was obtained by the ELPI, however, the number of affected bins varied because it was dependent on the particle size distribution. Therefore, it is more reproducible to use an analytical chemical measurement technique as the gold standard, with real-time measurements used only for monitoring purposes during the experiments.

To conclude, the aerosol monitoring system described enables the generation and characterization of toxic aerosols in real-time during inhalation toxicity studies, followed by offline analytical chemical confirmation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The raw data that support the findings of this study are available from the corresponding author, Mirjam de Bruin-Hoegée, upon reasonable request.

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