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An efficient virus aerosol sampler enabled by adiabatic expansion

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ABSTRACT

Protection of public health against pathogenic viruses transmitted through the airborne route requires effective sampling of airborne viruses for determination of their concentration and distribution. However, sampling viable airborne viruses is challenging as conventional bioaerosol sampling devices operate on inertia-based mechanisms that inherently have low sampling efficiency for virus aerosols in the ultrafine size range (< 100 nm). Herein, a Batch Adiabatic-expansion for Size Intensification by Condensation (BASIC) approach was developed for efficient sampling of virus aerosols. The BASIC utilizes adiabatic expansion in a supersaturated container to activate condensation of water vapor onto virus aerosol particles, thus amplifying the size of the particles by orders of magnitude. Using aerosolized MS2 bacteriophage, the BASIC's performance was evaluated and optimized both from the perspectives of physical size amplification as well as preservation of the viability of the MS2 bacteriophage. Experimental results show that one compression/expansion (C/E) cycle under a compression pressure of 103.5 kPa and water temperature of 25 °C was sufficient to increase the particle diameter from < 100 nm to > 1 µm; further increases in the number of C/E cycles neither increased particle number concentration nor diameter. An increase in compression pressure was associated with physical size amplification and a higher concentration of collected viable MS2. Water temperature of 40 °C was found to be the optimal for size amplification as well as viability preservation. No significant effect on particle size enlargement was observed by changing the dwell time after expansion. The results illustrate the BASIC's capability as a simple, quick and inexpensive tool for rapid sampling of viable airborne viruses.

1. Introduction

Airborne transmission of certain viruses can lead to widespread outbreaks of severe illnesses. Due to their potential to cause pandemics and sometimes fatal diseases, it is very important to understand and control the dynamics of airborne virus transmission. This pathway has been recognized as the most effective route for transmitting episodic respiratory viruses (e.g., Severe Acute Respiratory Syndrome (SARS) Coronavirus, Middle East Respiratory Syndrome (MERS) Coronavirus and H7N9 Influenza virus) and
those more frequent respiratory infectious diseases such as seasonal flu and common colds that can lead to pneumonia (Brankston, Gitterman, Hirji, Lemieux, & Gardam, 2007; Goldmann, 2000; Lapinsky, 2010; Otter et al., 2016; Wang, 2013; Yu et al., 2004). Many virus aerosols are classified by their size under the category of ultrafine particles (UFPs, with aerodynamic diameter smaller than 100 nm) as the size of viruses ranges from 20 to 300 nm, and typically less than 100 nm (Pease, 2012). This magnifies the concern regarding airborne transmission of the pathogenic viruses since the UFPs can travel long distances (Alonso, Raynor, Davies, & Torremorell, 2015) and penetrate deep into the alveoli region of the human respiratory system (Milton, Fabian, Cowlings, Grantham, & McDevitt, 2013). To gain a better understanding of the presence of virus aerosols and better ascertain the inhalation risks they pose, efficient collection of viable virus aerosol is needed.

A variety of samplers for collecting bioaerosol have been developed, but they mostly utilize inertial impaction for particle collection. A significant fraction (42%) of the influenza viruses dispersing into the air after a human cough were detected in airborne particles smaller than 1 µm (Lindsley, Blachere, & Thewlis, 2010). Therefore, the mentioned samplers are inherently ineffective for these ultrafine virus particles. For example, the BioSampler® (SKC Inc., Eighty Four, PA, USA) has been widely used for bacterial and fungal collection, but its collection efficiency is less than 10% for particles smaller than 100 nm (Hogan et al., 2005). In addition to efficient physical collection, preserving viability of the virus aerosol is the other critical factor in assessing the health risk associated with airborne transmission of pathogenic virus.

To address the abovementioned drawbacks, several studies proposed the use of water vapor condensation to amplify the size of virus aerosol particles before collection by inertial impaction (Oh et al., 2010; Pan et al., 2016). When the partial pressure of water vapor exceeds its saturation vapor pressure, supersaturation condition is established and subsequently condensation of water on colder surfaces occurs (Kousaka, Niida, Okuyama, & Tanaka, 1982). There are three common approaches for establishing the supersaturation condition to enlarge airborne particles: 1) mixing a hot saturated vapor with the flow of incoming particles, 2) convectively cooling the airstream of the target particles, and 3) creating an adiabatic-expansion condition in the air volume surrounding the targeted particles (Vanhanen et al., 2011). The first two mechanisms have been implemented for virus aerosol sampling. For instance, Oh et al. (2010) compared a mixing type of bioaerosol amplification unit (mBAU) with a cooling type of bioaerosol amplification unit (cBAU), and observed that the mBAU performed better than the cBAU in collecting viable virus aerosol. The cooling type did not work well when using water vapor as condensation fluid because water’s mass diffusivity is greater than air’s thermal diffusivity (Hering & Stolzenburg, 2005). Thus, condensation mainly occurred on the cold wall surface rather than on aerosol particles. Hering, Spielman, and Lewis (2014) utilized a water-wetted hot wall instead of a cooling tube for the particle growth, and they demonstrated its capability to efficiently amplify nanosized aerosol (down to 6 nm) to sizes larger than 1 µm. High efficiency in collection of viable virus aerosol was further attempted on MS2 and H1N1 influenza virus using the same technique (Jiang et al., 2016; Lednicky et al., 2016; Pan et al., 2016, 2017).

The third approach, i.e., adiabatic expansion, includes an instant volume expansion whereby there is no heat transfer between the contained volume and its surroundings (Bailyn, 1994). For a system with a certain volume, the temperature, volume and pressure of the system before and after the adiabatic-expansion are related as follows:

$$ R_0 V_0^0 = P_0 V_f^0 $$

$$ T_0 V_0^{\gamma -1} = T_f V_f^{\gamma -1} $$

where $P$, $V$, $T$ are pressure, volume and temperature, respectively, subscripts 0 and f refer to before and after adiabatic expansion, and $\gamma$ is heat capacity ratio (i.e., the ratio of specific heat of relevant gas at a constant pressure over that at a constant volume, $\gamma = \frac{c_p}{c_v}$; Strey, Schmeling, & Wagner, 1986). Aitken (1888) implemented this principle to create supersaturation by lowering the temperature of the surrounding air of target dust aerosol. Pollak and O’connor (1955) applied this principle in their photoelectric condensation nucleus counter (CNC), wherein a photoelectric sensor was used to count the number of enlarged mist particles. Pollak and Metnies (1960) investigated the performance of the CNC under different volume expansion ratios (i.e., $\frac{V_f}{V_0}$) and achieved a high saturation ratio of 3.50 under a compression ratio of 1.21, which exceeded the required Kelvin ratio for ultrafine particles (Miller & Bodhaine, 1982b). In their research, the CNC successfully amplified particles as small as 20 nm (Miller & Bodhaine, 1982a). Compared to the mixing and cooling approaches, the adiabatic expansion approach can result in an extremely high supersaturation ratio instantly, which activates the growth of particles in a very short time. This high supersaturation ratio played a key role in activating amplification of ultrafine particles as small as 13 nm (Liu et al., 1984). While there have been a handful of studies on size enlargement of particles using adiabatic expansion as discussed (e.g., the one by Okuyama et al., 1984), there is no study regarding size enlargement of viable virus aerosol by this approach yet.

This study was embarked on to apply the adiabatic expansion principle to engineer a simple but highly efficient size amplification device to address limitations associated with previously mentioned methods. On this ground, a prototype of Batch Adiabatic-expansion for Size Intensification by Condensation (BASIC) sampler was designed and fabricated. Performance of the BASIC apparatus in regards to size amplification was evaluated. Since collection of viable virus aerosols was the main purpose for the new device, experiments were conducted to evaluate its ability in collecting viable viruses. To optimize the BASIC’s operation, sensitivity analyses of key parameters were conducted, including compression pressure, number of compression/expansion cycles (C/E cycles), temperature of the condensing water and dwell time after the expansion.
2. Materials and methods

2.1. Design of the BASIC platform

The BASIC platform (total height: 21 in., diameter: 4 in.) consists of an expansion bag (volume = 1 L) in a chamber. The bag contains the aerosol sample while the chamber provides space for exertion of compression to and subsequent expansion of the bag (see Fig. 1a, b and c). A certain amount of de-ionized (DI) water is placed inside the expansion bag as the source of volatile vapor for later condensation on the aerosol sample trapped inside the bag. DI water is chosen as the condensation fluid because of its non-toxicity comparing to other common-used condensation media, e.g. butanol, propylene glycol etc. (Agarwal & Sem, 1980; Kousaka, Endo, Muroya, & Fukushima, 1992). The DI water also serves as the medium for collecting the amplified viruses.

2.2. Experimental set-up

A schematic diagram of the experimental set-up is shown in Fig. 1d. First, compressed air was directed to a 6-jet Collison nebulizer (Model CN25; BGI Inc., Waltham, MA, USA) at a controlled flow rate of 6 Lpm to generate the aerosol flow. A diffusion dryer placed at the outlet of the nebulizer removed the water content of the aerosol. The target flow rate was established 15 min prior to each experiment. Before directing the aerosol into the bag, the aerosol inlet/outlet valve was open to let the expansion bag deflate. This process was realized by directing compressed air into the chamber through a compressor air valve, which was immediately closed after the bag was completely deflated. Then, the aerosol inlet/outlet valve was open to allow the sample aerosol flow into the expansion bag from the aerosol inlet port to fill the bag (Fig. 1a). Once the bag was fully filled, aerosol introduction was stopped (i.e. aerosol inlet/outlet valve closed) and compressed air was instead fed to the chamber to increase the chamber pressure. After reaching the targeted compression pressure, the air discharge valve was opened swiftly to quickly drop the chamber's pressure down to the atmospheric pressure level. Thus, the bag expanded instantly, and supersaturation condition was realized inside the expansion bag.

Fig. 1. Schematic diagram of the BASIC, its operation and the experimental system setup.
This process enabled the particles contained inside the bag to be amplified by water vapor condensation as temperature rapidly decreased due to the swift pressure decrease.

2.3. Test aerosol

Lyophilized MS2 (#15597-B1, ATCC®, Manassas, VA, USA) was the source of virus for this work. With an approximate particle size of 28 nm, MS2 is a bacteriophage that only infects male *Escherichia coli* (*E. coli*) bacteria (Davis, Sinheimer, & Strauss, 1961). Since it does not infect humans and due to its robust survivability, MS2 is widely used as a surrogate in research of small airborne viruses (Dawson, Paish, Staffell, Seymour, & Appleton, 2005; Woo, Hsu, Wu, Heimbuch, & Wander, 2010; Zuo et al., 2014). Prior to the aerosol generation process, the lyophilized MS2 bacteriophage was rehydrated in 100 mL DI water to make a stock suspension with a titer of around $10^{11}$ PFU/mL and used soon thereafter. Prior to use, 1 mL MS2 stock suspension was pipetted and diluted into 100 mL DI water to create a titer of $10^9$ PFU/mL.

2.4. Experimental procedure

2.4.1. Physical size amplification

Performance evaluation of the BASIC was split into two phases. In Phase 1, physical size amplification of MS2 virus aerosol was investigated. Amplified aerosol inside the expansion bag was discharged and directed to an Optical Particle Counter (OPC Model 1.108, Grimm® Technologies Inc., Douglasville, GA, USA; size range 0.3–20 µm) for measurements of total number concentration and count median diameter (CMD) of the supermicron particles (i.e., $d_{50} \geq 1$ µm). The CMD was determined using a log-probability plot of the measured size distribution for locating the corresponding diameter of 50% cut-off point of the accumulative number concentration (Hinds, 1999). Most commonly used bioaerosol samplers reach >90% collection efficiency only at supermicron size range. For example, the BioSampler does have 50% efficiency at 0.3 µm, but 90% efficiency is reached only above 0.8 µm. The AGI-30 impinger, which is also widely used for bioaerosol sampling, only has 90% efficiency at 1 µm (Lin et al., 2000). To properly assess the risks of bioaerosol exposure, high efficiency (90% or greater) is needed. This is the main rationale for using information of supermicron particles for further analysis. Control groups were also included by simply introducing the same sample aerosol into the expansion bag without application of any C/E cycle. Source aerosol was measured by introducing the aerosol flow from the outlet of the diffusion dryer into the OPC. One should note that the Collison nebulizer was the only source for generation of the aerosol and our rudimentary tests showed that application of the adiabatic expansion is not a mechanism for generation of the supermicron particles; it only grows submicron particles to supermicron particles by condensation following the cooling process induced by adiabatic expansion.

2.4.2. Viability preservation

The viability of MS2 bacteriophage in the BASIC was studied by shaking the bag to collect the amplified virus aerosol into the water medium in the expansion bag. The collected medium was analyzed using a single-layer virus plaque assay (VPA) following U.S. Environmental Protection Agency standard operating procedures (USEPA, 1984). *E. coli* (#15597, ATCC®, Manassas, VA, USA) was used in VPA as the indicator host cells for MS2 bacteriophage. *E. coli* powder was aseptically inoculated onto a tryptone yeast extract agar (TYA) plate overnight, then a single uniform colony on the plate was aseptically picked and inoculated into sterile tryptone yeast extract broth-1 (TYB-1), and incubated overnight to create an *E. coli* stock suspension. Prior to each experiment, 1 mL *E. coli* stock suspension was cultivated in 30 mL TYB-1 for 6 h to obtain log phase cells of an appropriate concentration. All incubations were held at 37 °C.

For the VPA method, the agar medium consisted of TYA medium. TYA contained 1.0 g tryptone, 0.1 g yeast extract, 0.1 g glucose, 0.8 g sodium chloride (NaCl), and 0.022 g calcium chloride (CaCl2) per 100 mL of medium with 1.0 g additional agar. Tryptone yeast extract broth Type 2 (TYB-2) with all ingredients in TYA except agar was made for dilution of the samples. TYB-1 that contained only tryptone, yeast extract and sodium chloride were also used for cultivating *E. coli*, 6 h prior to each experiment.

Preliminary tests indicated that the undiluted and 1/10 dilutions of the sample material were optimal for VPA counts. Melted TYA was equilibrated to 50 °C in a water bath to maintain its liquid state at a temperature not deleterious to *E. coli* for short-term exposure. Nine mL TYA, 1 mL undiluted or 1/10 dilution of sample and 0.5 mL *E. coli* TYB-1 solution were mixed, vortexed and then poured into a Petri dish and gently shaken for spreading the agar evenly. After they solidified, the Petri dishes were inverted and incubated bottom-up in a 37 °C incubator.

After overnight incubation, plaques showing dead *E. coli* cells lysed by MS2 were visible. Only Petri dishes that contained 10 – 100 plaques were used for counting plaque forming unit (PFU) in order to provide accurate counts (Cormier & Janes, 2014). By multiplying PFU with the dilution factor, the titer of viable MS2 $C_{\text{viable}}$ (PFU/mL) was determined, using Eq. (3).

$$C_{\text{viable}} = \frac{PFU_{\text{plaque}} \times DF}{V}$$

(3)

where DF is the dilution factor and V is the volume of the diluted sample.

2.5. Sensitivity analyses

Compression pressure, number of C/E cycles and water temperature were varied for sensitivity analyses of the physical size
amplification, while dwell time was also included for evaluation of the viability preservation. Sensitivity analyses were carried out by the experimental design shown in Table 1 with the baseline set of variables being 103.5 kPa of compression pressure, one C/E cycle and 25 °C of DI water temperature in physical size amplification tests. The compression pressure was read from the BASIC’s pressure gauge, and the temperature was measured by an Infrared Thermometer (Etekcity® Co. Ltd., Anaheim, CA, USA). No uniform baseline set of values was set in the MS2 viability preservation tests. Instead, each experimental run was paired with a control group (no application of any C/E cycle) and performed on the same day due to the slight variations in atmospheric temperature, humidity and MS2 stock suspension storage time that are known to affect the phage’s viability on different days.

The VPA method was conducted on the samples according to the group wherein the sensitivity analysis was conducted. The viable MS2 titer of each sample was then calculated and compared for viability preservation assessment. In order to monitor the stability of MS2 viability in the Collison nebulizer, the titer of viable MS2 in the nebulizer reservoir was also measured for each group. An additional experiment to estimate the rate of aerosol generation was conducted by monitoring the liquid volume remained in the Collison nebulizer at different times; the consumption rate of MS2 suspension was determined using the slope of the linear regression of the data points. The total count of viable MS2 in the expansion bag fed by the nebulizer reservoir was determined using Eq. (4), assuming no loss while transporting and nebulizing.

\[ A_{\text{viable}}(\text{nebulizer}) = C_{\text{viable}}(\text{nebulizer}) \times CR \times t \]  

where \( A_{\text{viable}} \) is the count of viable MS2 consumed in the reservoir (PFU), CR is the consumption rate (mL/min), \( C_{\text{viable}}(\text{nebulizer}) \) is the titer of viable MS2 in the Collison nebulizer reservoir (PFU/mL), and \( t \) is the sampling time (10 s in all experiments).

2.6. Quality control and data analysis

Prior to each experiment, the aerosol generation system was stabilized for 15 min to ensure the variations of the flow rate within ± 0.1 Lpm. Since aerosol size enlargement is realized through water vapor condensation, relative humidity of the incoming aerosol stream should be minimal. Measurement of relative humidity before and after the diffusion dryer showed the relative humidity averagely decreased from ~80% to ~35%. After each experiment, the bag was rinsed by 70% isopropyl alcohol to inactivate residual viable MS2, and then washed thoroughly by DI water. Ten mL of DI water was then poured into the bag as the condensing medium right before the next experiment, and the temperature of the DI water was immediately measured. The expansion bag was then sealed with a lid and held to the chamber to be vacuumed. In viability evaluation experiments, before each experimental run all test tubes and solutions were autoclaved at 120 °C and 1 atm for at least 30 min.

It should be noted that maintaining the water temperature at the highest tested temperature of 60 °C from the time it was poured into the expansion bag to when the adiabatic expansion was applied, was challenging. Based on our measurements right after application of one C/E cycle, temperature of the DI water dropped from 60 °C to 40 °C for the experimental run of 60 °C, and dropped from 40 °C to 35 °C for the experimental run of 40 °C. In other words, due to the temperature decrease of the control volume caused by adiabatic expansion, DI water temperature could not maintain its original value, and the temperature drop was larger at the higher initial temperature. Therefore, the temperatures mentioned in the following paragraphs refer to the initial temperature prior to compression/expansion cycle.

To assess the statistical validity, each experimental condition was triplicated. To analyze the data obtained from the BASIC, a 2-tailed t-test for unequal variance was implemented for comparing the statistical significance between the baseline group and the control group. One-way analysis of variance (ANOVA) was applied for in-group comparison, and a post-hoc test using Bonferroni’s method was applied for comparing two subgroups within a group.

3. Results and discussions

3.1. Physical size amplification

3.1.1. Compression pressure

Size distributions of the aerosol with different compression pressures and without adiabatic expansion are displayed in Fig. 2. As
shown, a fraction of submicron (0.3–1.0 µm) particles picked up some moisture content and were amplified slightly even in the absence of a C/E cycle. In the baseline group, after application of only one C/E cycle at the compression pressure of 103.5 kPa, a significant increase of particles was observed: the largest particle size detected after one C/E cycle was ∼4 µm, and the number concentration of supermicron particles compared to the source aerosol was very high (> 300 #/cm³). An increase in compression pressure resulted in an improved size amplification performance, and the number concentration increased further. When compression pressure increased from 69.0 kPa to 103.5 kPa, the number concentration increased 4–5 times in the submicron size range, and the largest particle size (defined as the size where dN/dlogdp exceeds 10 #/cm³) increased from 2.5 µm to ∼ 4 µm. At compression pressure of 138.0 kPa, the largest size of amplified particles exceeded 5 µm and an obvious increase of number concentration in the supermicron range took place.

The t-test results of number concentration of the supermicron particles between groups with (control group) and without adiabatic expansion (baseline group) are displayed in Table 2. The results confirmed that there was a significant difference (P-value < 0.0001) between the two groups. T-test on CMDs of supermicron particles and total particles also proved that adiabatic expansion successfully enlarged particles. Although the fraction of particles larger than 3 µm was negligible, this does not necessarily mean that 3 µm was the upper-limit of the particle enlargement. Due to gravitational settling, larger particles might have settled (on the inner surface of the expansion bag or tubing) before reaching the OPC. In addition, the instant expansion process causes turbulence inside the bag, which is conducive to the deposition of larger particles by impaction (Robertson & Goldreich, 2012). In other words, there are constraints in the current system for accurate measurement of aerosol particles larger than a few microns.

One-way ANOVA results on compression pressures are displayed in Fig. 3. A significant increase in the number concentration of the supermicron and total particles with an increase in compression pressure was concluded. A 10-fold increase in number concentration of supermicron particles was achieved by increasing the compression pressure from 69.0 kPa to 138.0 kPa (Fig. 3a). CMD also increased from ~1.48 µm to ~2.17 µm for supermicron particles (Fig. 3b). A higher compression pressure provides a higher supersaturation ratio, leading to a lower temperature after the C/E cycle. The lower temperature enables more water vapor condensation onto the particles, thus a higher number of the nanosized particles undergoing the size amplification.

### 3.1.2. Number of C/E cycles

One-way ANOVA test results as shown in Fig. 4a and b reveal that neither the difference in number concentration nor CMD within the three groups was significant (p-value ~ 0.21) for supermicron particles. For total particles, the concentration increased while the CMD decreased. After the first C/E cycle, thermodynamic equilibrium inside the bag was reached. While more C/E cycles were originally hypothesized to provide more water vapor for size amplification, the experimental results indicate that when the next cycle of compression was applied, work was done to the aerosol, causing re-evaporation of the water from the amplified aerosol in the bag.

### Table 2

Number concentration and count median diameter of supermicron and submicron particles in the air sample using BASIC, with and without adiabatic expansion.

|                          | Number concentration of submicron particles (#/cm³) | Number concentration of supermicron particles (#/cm³) | Count median diameter of supermicron particles (µm) | Count median diameter of all particles (µm) |
|--------------------------|-----------------------------------------------------|-------------------------------------------------------|----------------------------------------------------|---------------------------------------------|
| Without adiabatic expansion (Baseline) | 18.3 ± 8.3                                           | 0.058 ± 0.021                                          | 1.42 ± 0.05                                         | 0.36 ± 0.00                                 |
| With adiabatic expansion (Control)       | 846 ± 130                                            | 323 ± 109                                              | 1.67 ± 0.04                                         | 0.77 ± 0.12                                 |
| p-value                               | < 0.0001                                             | < 0.0001                                               | 0.0022                                              | < 0.0001                                    |
Thus, a higher number of the C/E cycles simply repeated the first cycle and did no further amplification.

3.1.3. DI water temperature

As shown in Fig. 5a, an increase in the number concentration was observed when the DI water temperature increased from 25 °C to 40 °C. However, the number concentration for supermicron particles decreased when the DI water temperature increased further to 60 °C. The results of One-way ANOVA test also confirm that the experiment run at 40 °C had the greatest size amplification potency for supermicron particles among these three temperatures. However, no obvious difference among the CMDs of these three groups is seen due to the large error bar of the group with 60 °C (Fig. 5b). The concentration of total particles at 60 °C was similar to that at 40 °C, while the CMD decreased. These may suggest that under higher temperature a wider size range of large droplets were produced. A higher temperature was expected to produce more supermicron particles as it would yield a higher moisture content available for condensation. The reason why the number concentration of supermicron particles decreased, when temperature increased to 60 °C, is not yet clear. One related theory is that at this higher temperature, the stability of virus capsid reduced, which might have led to the structure alteration of virion protein (a complete virus particle) (Pinto, Maillard, & Denyer, 2010). Meanwhile, impurities that were likely present in the lyophilized bacteriophage preparation could affect the particle size distribution as temperature varies.

3.2. Viability preservation

The MS2 titer in the Collison nebulizer reservoir was 1.5 (± 0.32) × 10⁹ PFU/mL, thereby implying the system supplied a stable
size distribution of the aerosol source for different experiments. The consumption rate of MS2 suspension in the Collison nebulizer was about 0.3 mL/min, and the bag filling time was set at 10 s for each experiment. Consequently, the consumed volume of the nebulizer liquid was about 0.05 mL, and accordingly the expected MS2 titer in the sampling air was estimated to be ~7.5 × 10^7 PFU/L of air, assuming no loss due to transport or inactivation by the nebulization process. Detailed results for each system parameter investigated are reported in the following sub-sections.

3.2.1. Compression pressure

Results of the statistical analyses are displayed in Fig. 6a. The figure shows an increasing trend of collected viable MS2 with an increase in the compression pressure up to 103.5 kPa. Shaking of amplified aerosol trapped inside the expansion bag increased the probability of amplified virus aerosol depositing into the DI water. The results indicate the viability was preserved in the investigated range of compression pressures (69.0 – 138.0 kPa). Past studies showed that application of a high pressure on a virus-containing suspension can inactivate noroviruses (Aertsen, Meersman, Hendrickx, Vogel, & Michiels, 2009). However, this concern applies to an extremely high pressure (> 60,000 psi) wherein virus capsid protein or lipid envelope may be damaged (Tang et al., 2010). By using MS2 as a surrogate at a high pressure (40,000 psi), Pan (2015) observed that MS2 did not suffer significant viability loss under that pressure held for 3 min. Our statistical analysis results infer that the increased collection of viable MS2 after adiabatic expansion was significant compared to the case without adiabatic expansion; the collected amount increased as compression pressure increased up to 103.5 kPa, although there was no significant difference between cases with compression pressures of 103.5 kPa and of 138.0 kPa.

Compared to the increase in the number concentration of supermicron particles when using 138.0 kPa vs. 69.0 kPa (Fig. 3a), the level of increase in collected viable MS2 virus was not as prominent. While the size amplification did increase the probability of particle collection, how MS2 was distributed in the amplified particles was unknown. In other words, the increase in the measured number concentration of the supermicron particles did not necessarily lead to a proportional increase of collected MS2 aerosol. Meanwhile, the difference might also imply some deactivation when using high compression pressure. Thus, caution should be taken at the higher compression pressure, and the distribution of viable MS2 in the amplified particles should be a subject of future investigation.

3.2.2. Number of C/E cycles

Comparative results of collected viable MS2 under different numbers of C/E cycles are presented in Fig. 6b. In contrast to the physical size amplification, a higher number of cycles is inversely related to the viability preservation of MS2. As seen in Fig. 6b, the highest viable virus titer (4620 ± 930 PFU/L air) was achieved at one C/E cycle, and the difference from other groups (3 cycles, 5 cycles, and no adiabatic expansion) was statistically significant. Pollard (1960) reported that gradual exertion of pressure on viruses leads to an increase of bond stability in virion protein, thus leading to the better stabilization of virus. However, exertion of an instant pressure loss (as in adiabatic expansion) may cause the breakage of the viruses. In other words, when the number of C/E cycles increases, viruses suffer from frequent and instant pressure changes, causing virions to expand and break apart. The other possible reason is the change in particle size distribution. However, Fig. 4a shows that the concentration and CMD of supermicron particles remained the same for different numbers of C/E cycles; thus, the low resistance to frequent pressure change is the more likely reason for the reduced viable MS2 collection. In summary, when using the BASIC for collection of viable MS2, one C/E cycle gives the optimal outcome.

3.2.3. DI water temperature

Collected viable MS2 as a function of the temperature of pre-injected water is plotted in Fig. 6c. In operating the BASIC, DI water was used as a medium for both condensation and collection. When applying DI water of 40 °C, an increase of viable MS2 collected in
water was observed in comparison to the case with DI water of 25 °C. However, when the water temperature increased further to 60 °C, the titer of viable MS2 collected in water decreased greatly. Previous studies found that temperature can play a vital role in the viability of many viruses (Ausar, Foubert, Hudson, Vedvick, & Middaugh, 2006). Pinto et al. (2010) investigated the effect of temperature on MS2, and reported that high temperatures cause structural change of virion capsid and therefore virus inactivation.

One-way ANOVA results confirm that by applying water temperature of 40 °C, the titer of collected viable MS2 was significantly higher than those at 25 °C and 60 °C. Different from other key parameters, higher water temperature exerts positive effect on physical size amplification but negative effect on viability preservation. As MS2 shows vulnerability at 60 °C, therefore 40 °C is the optimal DI water temperature, considering both physical collection and viability preservation.

3.2.4. Dwell time

There was no benefit for increasing the dwell time since the viable MS2 titer did not vary much among the four dwell times studied (see Fig. 6d). This is confirmed by the 1-Way ANOVA test proving no statistically significant difference among the four dwell time groups. Wu and Biswas (1998) reported that the growth of an aerosol particle in the free molecular regime due to condensation was independent of its original size, and it could achieve 50% of its final size at ~3 characteristic times. When the number concentration of ultrafine particles was above 10^4 particles/cm^3, the characteristic time was less than 0.3 s. In other words, in a short period of time (less than 1 s), ultrafine particles could grow to 50% of its final size (calculated as ~10 µm). Hence, a long dwell time for particle growth is not essential (neither for particle amplification nor for more virus collection in this given system).

4. Conclusions

This study focused on the performance assessment of the BASIC in enabling ultrafine virus aerosol sampling as well as the ability in preserving the viability of airborne virus. MS2 phage was used as the test agent in assessing both amplification effectiveness and viability preservation. Results for physical size amplification tests showed that increasing compression pressure in the range of 69.0 – 138.0 kPa had a positive effect on the CMD enlargement and increase of aerosol number concentration. This was attributed to the higher saturation ratio at higher compression pressure. The application of C/E cycles yielded physical size amplification, and one
cycle was the optimal condition. Increasing water temperature had a double-edged effect on collecting MS2 aerosol. Increasing initial water temperature from 25 °C to 40 °C resulted in a positive effect, but it exhibited a negative effect as temperature increased from 40 °C to 60 °C.

In evaluating the performance of viability preservation, the results showed that increasing compression pressure also produced an improvement in the total amount of collected viable MS2 in DI water, although the increase was not as prominent as the increase in the number concentration of supermicron particles. Regarding the number of C/E cycles, applying one single cycle was the optimal for collecting viable virus aerosol in the expansion bag as multiple C/E cycles caused the viability decrease of collected viruses. Low resistance to frequent pressure swings may be an attributing factor to the reduced viable MS2 collection. Similar to the results in the physical size amplification, 40 °C initial water temperature increased the amount of collected viable MS2 virus, while lower quantities of viable virus was collected at 60 °C. The results are in agreement with previous research wherein high temperature above a threshold value could lead to inactivation of viruses. Increasing dwell time from 0 s to 120 s yielded no obvious difference in the titer of viable MS2 because particles reach their equilibrium size in less than 1 s in this system.

In conclusion, the BASIC system showed its potential in highly efficient sampling of ultrafine virus aerosols. If the BASIC is combined with a rapid airborne virus detection and analysis approaches, the resulting system could be a significantly improved virus aerosol detection and identification system for infection control, agriculture and biodefense applications. In this study, only MS2 was used for evaluation of the BASIC. Since MS2 has been reported to be highly robust, i.e. resistant to high pressure and high humidity, operational pressure and temperature should be considered in order to achieve good performance when applying this technique to other viruses. Further research could be launched to increase the compression pressure to a higher extent, or to choose other health-related airborne viruses as the challenging virus aerosol.

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