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Exceeding a resolving power of 50 for virus size determination by differential mobility analysis

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A B S T R A C T

A recently described DMA designed for high resolution viral particle analysis (Perez-DMA; Perez-Lorenzo et al, 2020) is modified to decrease the relative peak full width at half maximum (FWHM) below previously achieved \( \approx 3.3\% \). The electrode radii at the outlet slit \( (R_1 = 1.01 \text{ cm}; R_2 = 2 \text{ cm}) \) and the working length are almost unchanged \( (L = 114.9 \text{ vs. 116 mm}) \). The laminarization trumpet and the radius of the curve merging the trumpet to the working section are both considerably widened to improve gas flow laminarization. DMA evaluation with salt clusters is improved by reducing the flow resistance at the gas outlet, to reach substantially larger sheath gas flow rates \( Q \) near 1700 L/min. Tests with tetraheptylammonium bromide clusters with a center rod diverging at 3° demonstrate FWHM \(< 2.7\%\), without indications of performance loss due to turbulence even at 1700 L/min. Correcting these high flow rate data for diffusive broadening reveals a maximal DMA FWHM in the limit of non-diffusing particles and zero sample flow, \( \text{FWHM}_{\infty} = 1.8\% \). An uncorrected peak width approaching 2\% is independently demonstrated at much lower flow rates of sheath gas with two recently described bee virus particle standards having singularly narrow size distributions at mean diameters of 38 and 17 nm. Correcting raw 38 nm particle peak widths for broadening due to diffusion and aerosol to sheath gas flow rate ratio \( q/Q \) shows an even more ideal response with \( \text{FWHM}_{\infty}<1\% \), where this value includes nonidealities in the DMA as well as possible lack of monodispersity in the viral particles.

1. Introduction

Following the development of electrospray ionization (ESI, Fenn et al., 1989) it became possible to bring to the gas phase large ions such as proteins and viruses for analysis by mobility and mass spectrometry, either separately or in tandem. The approach has been quite mature for many years in the case of proteins. However, it remains in a relatively primitive state for viral particles, in spite of the great interest offered by the possibility to diagnose unknown viral infections based on measuring the mobility or the mass of the virus involved. Mass spectrometry has been severely limited at viral sizes due to the large number of elementary charges \( (z > 100) \) typically carried by electrosprayed viral particles. The combination of this large \( z \) with the slight mass heterogeneity inevitable in viral genomes precludes the identification of isolated mass peaks in full viruses, not just with high resolution mass spectrometers, but even when combining mobility separation and mass spectrometry in tandem. Conventional mass measurements have been possible only on empty viral capsids made up of just proteins, where there is no mass variation between different capsids (Uetrech et al., 2008). The only

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successful mass measurements to date on full viral particles have all been based on the independent measurement of both charge and mass made possible by exquisitely sensitive single-particle charge measurement techniques developed by Jarrold and colleagues (Lutomski et al., 2015). It is important to note that all viral mass spectrometry studies made to date rely on fully charged electrospray ions, because no mass spectrometer exists able to cover the mass range of viral particles carrying only one or a few elementary charges. Mobility analysis has typically considerably less resolving power than mass spectrometry, but its size range is adequate to analyze singly charged viral particles with sizes of 100 nm or above. As a result, a Differential Mobility Analyzer (DMA, Knutson & Whitby, 1975) with a modest resolving power of 10 can easily see individual peaks for singly charged viral particles, being as a result far more effective than a mass spectrometer with a resolving power of $10^4$ for the same fully charged particle. The key to this advantage was the development by Kaufman et al. (1996) of electrospray followed by charge reduction, size analysis with a Differential Mobility Analyzer (DMA) and sensitive detection with a Condensation Particle Counter (CPC). This 24 year old combination, originally named GEMMA, has produced a substantial literature focused on viral particles. The typical resolving power or resolution of GEMMA (defined as the inverse of the Full Width at Half Maximum for a mobility peak normalized by the mean mobility, 1/FWHM) has been 10 (Fernández-García et al., 2019). This is comparable to the resolving power of most commercial DMAs, and is adequate for most aerosol applications, rarely exhibiting very narrow peaks. However, even if viruses from two species were essentially monomobile, while differing from each other in mobility by less than 10%, it would not be possible to distinguish them. Focusing only on respiratory viruses, the various human corona viruses and the influenza have comparable sizes based on electron microscopy. A hypothetical test for covid-19 based on mobility measurement would then have a high probability of false negatives. On the other hand, DMAs have demonstrated in certain cases resolving powers larger than 100 in the size range of a few nm (Amo-González & Pérez, 2018). Extending this performance level to DMAs covering the viral size range would accordingly enable highly sophisticated viral studies. In an effort to approach this ideal, we first surveyed the literature and tested with a diversity of viral particles a number of DMAs available to us (Fernandez-García, 2019). The narrowest published viral peaks we identified had FWHM of 3.9% based on one of Reischl’s DMAs (Kallinger et al., 2013), and 4.7% in an exceptionally careful study based on TSI’s commercial equipment (You et al., 2016). Our own DMAs developed at Yale for size-analysis of particles smaller than most viruses achieved at best FWHM = 3.9%. This suggested the need to develop better DMAs specially designed for the 20–60 nm range. Our first attempt in this direction resulted in a previously described instrument (Perez-Lorenzo et al., 2020), nicknamed the Perez-DMA. Its inner and outer electrode radii $R_1 = 1.01$ cm; $R_2 = 2$ cm and axial length $L = 11.6$ cm were all fairly conventional. We abandoned Reischl’s bullet geometry (supported only on its downstream end) in favor of a center rod (supported both upstream and downstream) in order to achieve good centering in such a long instrument. To avoid an excessively long and heavy device, the inlet laminarization trumpet was small relative to our previous bullet-based DMAs. A key problem in that study was the lack of narrow size standards in the viral size range, needed to evaluate the DMA performance. As a reference, in order to prove unambiguously a resolution of 30 in mobility, the FWHM inherent to the standard itself had to be well below 1/30 in mobility, say 1/50. This implies a FWHM of about 1/100 in diameter. Since such outstanding standards had not yet been developed, we were forced to test that instrument with the only truly monomobile particles then available to us: singly charged cluster ions of a few nm produced by a bipolar source electrospraying alcohol solutions of various relatively large salts. This required a DMA able to deal with both, ~1 nm clusters and viral particles, which had to operate correctly at flow rates from above 1000 L/min down to $\approx$10 L/min. These diverse constraints forced a limit of 30 to the maximum resolution we were able to

Fig. 1. Schematic of the experimental setup, including the DMA, the recirculating sheath gas circuit, and the flows associated with the sample entering and leaving the DMA.
Fig. 2. Half cross sections of the new Perez-LT-3° (top) and the earlier Perez DMA (bottom). [0] NW-40 sheath inlet port. [1] Electrical connector. [2] Honeycomb flow straightener (optional). [3] Upper centering structure with perforated top plate (see inset). [4] Upper centering cone. [5] Laminarization meshes. [6] Laminarization trumpet. [7] Annular aerosol chamber. [8] One of the symmetrically placed circularizer holes communicating the annular chamber to the inlet slit chamber. [9] Aerosol inlet(s). [10] Inner electrode. [11] Outer electrode. [12] Insulating and centering carved cone (see inset [12]). [13] Sheath flow throat section. [14] Sheath flow exhaust chamber. [15] NW-40 outlet port for the sheath gas. [16] Antistatic tube. [17] Classified aerosol outlet. [18] Antistatic tube ground contact. [19] Antistatic tube insulator. [A&B] Detail on outlet slit. Three-dimensional views: Perforated inlet plate [3], and Carved insulator centering piece [12].
establish for that instrument. This prevented us from knowing for sure if this maximum demonstrated resolving power was caused by limitations of the DMA, the mobility standard, or both. The present study represents our new attempt to overcome that previous impasse, by seeking substantial improvements in the DMA, the mobility/size standard, and the quality of the electrospay. The effort has been facilitated by two innovations. First a better DMA passing an increased flow rate of sheath gas while still maintaining the flow laminar for evaluation with small clusters. Second, we use two new viral size standards recently described by Fernandez de la Mora et al. (2020).

2. Experimental

The overall structure of the experimental apparatus is shown in Fig. 1.

2.1. Design of the Perez-LT DMA

The LT denomination of the new instrument refers to its Long laminarization Trumpet. Its geometry and main geometrical characteristics are given in Fig. 2 and Table 1 in comparison with those of the earlier Perez DMA. Various known or suspected shortcomings of the earlier model, and their solutions in the LT model are as follows (square brackets refer to the numbering of DMA elements in Figs. 2 and 3):

(i) The flow rate of sheath gas Q was limited to 1200 L/min by the flow resistance at the outlet. An increased Q naturally reduces the diffusive contribution to peak width for a given monomobile cluster. This problem has been addressed by the low-resistance outlet [12] shown in a three-dimensional rendition in Fig. 2. This piece achieves the same goal as its conical ancestor, of centering the rod with respect to the outer electrode. In addition, the cone is now carved with six symmetric rectangular openings occupying 80% of the channel cross section to pass the sheath gas into the exhaust chamber [14]. The flow resistance is also substantially reduced at the four laminarization screens [5] due to their much wider cross section (150 cm$^2$ versus 32 cm$^2$). The former perforated inlet plate [3] centering the upstream end of the center rod offers now far less resistance by using 6 radial bars that connect its outer and inner rings (left inset to Fig. 2) and block just 18% of the laminarization screen section.

(ii) Suspected imperfect flow laminarization and flow quality in the working section, due to an insufficiently wide set of laminarization screens, and to an excessive curvature in the final region of the entry flow (originally forced by the desire of a compact instrument). The quality of the inlet flow has two important effects on resolution. First, directly through the broadening effects of free stream turbulence surviving from the complex flow prevailing upstream the laminarizing screens. Second, the high Reynolds number laminar flow initially established by a suitable inlet geometry still has a tendency to become turbulent, and this transition is accelerated by any surviving free stream turbulence. The maximal flow rate that can be achieved prior to this transition is accordingly increased by a better laminarizer. These circumstances have driven us to provisionally drop the criterion of having a light instrument, in favor of achieving a much better laminarization. The new design therefore incorporates a much wider and longer laminarization trumpet [6], and a smoother transition with a larger curvature radius joining the end of the conical region of the trumpet (arrow [6]) to the point where the sheath gas meets the stream of sample gas entering through the inlet port [9]. The higher curvature of the prior design in this region was seen in flow calculations to accelerate the near-wall fluid upstream the inlet slit beyond the mean speed at that section. This situation subsequently forced a local flow deceleration at the outer edge of the boundary layer, which tends to destabilize the boundary layer.

(iii) Conical inner electrode. The original Perez DMA had a conical inner electrode diverging at an angle of 1$^\circ$. This resulted in a gradual flow acceleration through the working section, which contributes to boundary layer stability (Fernandez de la Mora, 2002; Hoppel, 1968, 1970). Nevertheless, we observed signs of resolution loss in some prototypes at flow rates below the maximum achievable. This we attributed to some incipient form of turbulent transition, caused either by an insufficient convergence angle, or by inadequate laminarization, as discussed in point (ii). To cover for these two possibilities, in addition to the larger laminarization structure of point (ii), we have tested two center rod geometries, with divergence angles 0.5$^\circ$ and 3$^\circ$. The latter center rod [10] is the one shown in Fig. 2.

| Table 1 | Main geometrical parameters for the DMA classification region. |
|---------|---------------------------------------------------------------|
|         | Perez                          | Perez-LT-3$^\circ$ | Perez-LT-0.5$^\circ$ |
| $\alpha_b$ ($^\circ$) | 1                             | 3                  | 0.5                  |
| $L_b$ (mm)      | 116                           | 114.9              | 114.9                |
| $R_1$ (mm)      | 6.07–10.095                   | 4.1–10.095$^c$     | 9.09–10.095$^c$      |
| $R_2$ (mm)      | 20                            | 20                 | 20                   |

$a$ Half angle of inner electrode.

$b$ Axial distance from the upstream edge of the inlet slit (sharp end of trumpet) to the upstream edge of the outlet slit.

$c$ Inner electrode radii at inlet ant outlet slits.

$d$ Outer electrode radius.

$e$ Upper lip radius only.
Other relevant changes are schematically indicated in Table 2. The electric field surrounding the anti-static tube (AST) [16] bringing the monodisperse aerosol from the high voltage of the center rod to ground at the outlet tube provides now a larger effective length of approximately uniform axial field, and therefore a better transmission. The ideal situation to avoid internal radial fields and associated electrophoretic losses is when the AST is immersed in a purely axial external uniform field, which is favored by the two flat electrodes seen in Fig. 2 (top) at the beginning and the end of the tube [16]. Fig. 3 gives more detail comparing the prior (a) and the new (b) outlet geometries. In the past we did not pay adequate attention to the effect of the electric field surrounding laterally the AST, as may be surmised from examining Fig. 3a for the original Perez-DMA. In this case, although the AST is separated by a PEEK insulator from the high voltage electrode around it, the surrounding null external field penetrates through the upper part of the insulator, almost as if the insulator did not exist, and the potential along the AST remains constant down to the point where the metallic piece surrounding the AST ends. Most of the length of the AST is therefore ineffective in bringing the aerosol closer to ground potential. The effective length of the AST naturally depends on its conductivity and its dielectric constant and that of the insulator tube. However, for typical values of these parameters the insulator tube is relatively ineffective in shielding the AST from the external fields. The effective length of the AST in the LT configuration (Fig. 3b) is essentially its whole length.

2.2. Flow rate measurement

The DMA functions such that the product $VZ$ of the classification voltage $V$ and the electrical mobility $Z$ of a particle is strictly proportional to sheath gas flow rate $Q$. Accordingly, accurate values of the DMA constant $VZ/Q$ and $Q$ must be known in order to infer mobility from measured voltage. The constant $VZ/Q$ is fixed by purely electrostatic considerations and, like a capacity, depends only on the geometry (Tammet, 1970). For a strictly cylindrical geometry with inner and outer electrode radii $R_1$, $R_2$ and classification length $L$ this geometric constant is given by Equation (1a) (Knutson & Whitby, 1975). For an axisymmetric shape varying along the axial direction Equation (1b) defines the dimensionless shape factor $K$ based on the radii at the exit slit ($x=L$). For a slowly varying geometry $K$ may be approximated by Equation (1c) (see Appendix of Perez-Lorenzo et al., 2020),

$$
\frac{Q \ln[R_2/R_1]}{2\pi ZVL} = 1; \quad \frac{Q \ln[R_2(L)/R_2(L)]}{2\pi ZVL} = K; \quad K \approx \frac{\log[R_2(L)/R_2(L)]}{L} \int_0^L \frac{dx}{\ln[R_2(x)/R_2(x)]}
$$

where $x$ is the position variable along the DMA, with origin at the center of the inlet slit. For our conical DMAs $L$ and $R_2$ are fixed,

| Table 2 |
|----------|
| Comparison of various characteristics of the new and previous Perez DMA. |
| Perez | Perez LT |
| Separation between Laminarization meshes (mm) | 3.8 | 10 |
| Number laminarization meshes | 3 | 4-5 |
| Trumpet semi angle (°) | 30 | 30 |
| Axial trumpet length (mm) | 29 | 109 |
| Starting trumpet area (cm$^2$) | 32 | 150 |
| Trumpet end bend radius (cm) | 1.2 | 10 |
| Outlet slit lips | Square-square, Fig. 2 top | Square-round (with channel section decrease; Fig. 2 top) |
| Sheath outlet | 18 holes on outer electrode (90° off axis) | 6 rectangular vents (30° off axis) |
| Sheath outlet area at throat (cm$^2$) | 3.5 | 7.4 |
| AST effective length (mm) | ≈8 | ≈47 |

* The outer edge of the LT meshes is bound by a cylindrical instead of a conical surface.
\begin{equation}
R_i(x) = R_i(L) - (L-x)\tan \alpha
\end{equation}

and \(K\) is a function of \(\alpha\) only.

In our past work, the lack of a sensor able to measure \(Q\) with precision led us to infer \(Z\) with the help of a cluster standard of mobility \(Z_{\text{in}}\) by exploiting the inverse relation between mobility and classification voltage: \(ZV_0=ZV\) when operating under fixed flow conditions. Unfortunately this method is problematic when the mobilities of the cluster standard and the particle of interest differ by several orders of magnitude, as is the case with most viruses. The main problem is that, at a flow rate suitable for virus selection, the classification voltage of a \(\approx 1\) nm standard is rather small, resulting in a broad peak and an inaccurate determination of \(V_0\). Accordingly, a reliable mobility inference should rather be based directly on a good measurement of the flow rate. For our non-cylindrical DMA, a proper determination of \(Q\) is also necessary to obtain the geometrical constant \(ZV/Q\), since the expression provided by (1c) is only an approximation.

\(Q\) was measured with a Sensirion Inc. SFM3000 Mass Flow Meter covering up to 200 sL/min (notice that the flow rate standard of this instrument is at 20 °C at 1013 mbar, and is maintained here). The manufacturer claims a maximal error of ±2% of the measured flow rate, with reproducibility of ±0.75%. This suggested the interest of calibrating more accurately the instrument to reduce the ±2% flow measurement ambiguity to ±0.75%. This we have achieved with a Half-Mini DMA (Fernandez de la Mora, 2017) by measuring the classification voltages \(V\) of positively singly charged ion clusters of tetraheptylammonium bromide (Ude et al., 2005) produced by a bipolar ion source (Fernandez de la Mora & Barrios, 2017). We first noticed that the ratio \(VZ/Q\), based on the uncorrected flowmeter reading \(Q\), varied at most by ±2% down at least to 25 sL/min. The Half-Mini DMA has a cylindrical working section (inner and outer radii \(R_1=4\) mm, \(R_2=7\) mm) over 19.03 mm out of its 20 mm length, with a modest change in \(R_2\) (from 7 to 7.43) mm over the first 0.97 mm of its length. Its geometric constant is accordingly expected to be quite close to that of a cylinder. Use of the approximation (1c) shows that this constant differs from that for a cylinder (with \(R_1=4\) mm, \(R_2=7\) mm; \(L=20\) mm) by a factor of 0.9984. This miniscule non-ideality is due to the fact that the departure from a cylindrical shape is initially with zero slope, so the region where \(R_2\) is effectively different from 7 mm is considerably shorter than 0.97 mm. Therefore the error involved in approximating the geometry of this calibrating DMA as strictly cylindrical with \(R_2=7\) mm is well below 0.5%. Accordingly, the ratio \(VZ/Q\) is precisely known without the need of a calibration, and was used to obtain the flow rate \(Q\). By comparing this actual \(Q\) to the value given by the Sensirion flowmeter we have established the calibration relation \(Q = 1.025Q_0 – 0.4509\) L/min, from which we are confident \(Q\) is known within better than 1% when \(Q_0 < 200\) L/min. We have confirmed in subsequent experiments with other DMAs that the ratio \(QV/(ZV)\) based on the calibrated \(Q(V_0)\) relation is considerably more constant than the group \(Q/(ZV)\), supporting the manufacturer’s claim on the relatively high reproducibility of the measure of their sensor. At flow rates above 200 L/min \(Q\) was inferred from the assumption that it is strictly proportional to the voltages at which a given peak appears, with the proportionality constant determined for a given viral particle from a spectrum taken at \(Q < 200\) sL/min.

2.3. DMA circuit

The DMA was operated in a closed circuit of sheath gas with a pump a heat exchanger and a HEPA filter identical to those of Perez-Lorenzo et al. (2020) (Fig. 1). The flow rate \(Q\) of sheath gas was controlled with a feedback loop that fixed the rotation speed of the blower with variations well below 1%. At flow rates below about 100 lit/min the blower speed was fixed at 3200 rpm, and \(Q\) was controlled with a throttle valve inserted in the flow circuit. The experiments with the highest flow rates reported (up to 1700 L/min) could be achieved only by removing the valve and the flowmeter from the circuit, and by using a HEPA filter of high cross section.

2.4. Viral particle preparation

The sample was prepared at BVS Inc. by the same procedure previously described (Fernandez-Garcia et al, 2020; Wick, 2015), with more detail on this singularly narrow viral standard given by Fernandez de la Mora et al. (2020). Briefly, 6 g of infected bees collected from the vicinity of a beehive are added to 40 ml of water purified by reverse osmosis, mixing them in a blender for 2 min. The material is then drained through a three layer cheesecloth to remove large fragments such as insect wings and legs. It is subsequently centrifuged at 20,000 relative centrifugal gravities to separate to the bottom of the vial cell fragments and other comparably large objects. The supernatant recovered is mixed with 500 ml of deionized (DI) water and run past a 500 kDa hollow fiber tangential filter that removes particles smaller than about 7 nm and reduces the volume to 2 ml. This sample is then diluted 10-fold into DI water, cleaned with a 0.45 micron mixed cellulose ester filter, and shipped to Yale. These samples are harmless to humans and can be handled at a facility with biosafety level 1 (BSL1). We have no absolute certainty on the nature of the virus in a given sample. A first provisional determination was made at BVS based on the particle size measured with TSI’s commercial system. When studied at BVS, the sample used in this work showed the presence of two main viral peaks attributed to the Sacbrood virus and the Chronic Bee Paralysis Virus (CBPV), both very common in bees (Chen & Siede, 2007). When studied a few days later at Yale, only the CBPV was present. Unlike the Sacbrood virus, this particle was robustly preserved during the shipment from BVS to Yale, and, once refrigerated, maintained high stability in DI water for many weeks. The stability in 90 mM aqueous ammonium acetate is not so good, with low mobility tails developing in a few days, that can be clearly seen with high resolution instrumentation (Fernandez de la Mora et al., 2020, Fig. 4). The aqueous BVS samples were first analyzed as received and gave fairly sharp viral peaks over a slowly varying background. The background could be considerably reduced and the peaks narrowed down by several stages of dialysis in a Thermo Scientific Slide-A-Lyzer mini dialysis device (88,405) with a 20 kDa mass cut-off. We typically exchanged the DI water three times, dialyzing for periods of 2, 2 and 7 h. All the high resolution spectra shown here correspond to the further dialyzed samples, to which ammonium...
acetate was added to a final concentration of 90 mM.

Further confirmation that the sample used in this work is indeed CBPV is given by the fact that the mobility spectra obtained at Yale showed a second sharp peak at about 17 nm, in addition to the CBPV expected at 38 nm. The 17 nm peak is frequently associated with the CBPV, and does not appear alone due to its inability to replicate independently (Overton et al., 1982). It is consequently referred to as the CBPV satellite (CBPVS). In any case, the precise assignment of a viral species to our sample is of no great relevance to our DMA evaluation here, as long as the particles offer a narrow size distribution. We shall accordingly refer to these standards as CBPV and CBPVS, without concern for the correctness of the assignment.

2.5. Aerosol generation

A sample flow of 0.6 L/min was drawn by the detector pump from the ambient (without dehumidifying) through a HEPA filter, and went through a bipolar ES source. The positive capillary sprayed ten parts of the originally purely aqueous viral sample from BVS with one part of 1 M aqueous ammonium acetate buffer to produce a ~90 mM concentration of this salt. The negative capillary facing the positive capillary for charge reduction of the viral particles sprayed deionized (DI) water at a current of \(\approx 140 \text{ nA} \) (Fernandez de la Mora et al., 2020). The gas flow carried the charge-reduced particles from the electrospray chamber into the aerosol inlet of the DMA.

Generation of tetraheptylammonium bromide clusters relied on identical methanol solutions of this salt in both the positive and the negative capillary. In this case we achieve steady cone jets on both capillaries. Work with the Perez-LT DMA and the electrometer detector required flow rates typically of 1.5–2 L/min due to the limited transmission efficiency of this large DMA with 1–2 nm particles.

2.6. Detector

For tests involving virus particles we used Kanomax’s fast CPC (Model 3650) without sheath gas and with a monodisperse aerosol flow rate of 0.6 L/min. Pulses were counted over a certain dwell time \(t\) and are represented as counts/s by dividing the total counts detected by the dwell time. For calibration tests with tetraheptylammonium cluster standards we used a previously described electrometer (Fernandez de la Mora et al., 2017) manufactured by SEADM, having singularly low noise (0.1 fA) and fast response (<0.1 s). Monodisperse aerosol flows were typically 1 L/min in flow calibrations with the Half-Mini DMA, and 2 L/min with the Perez-LT DMA.

2.7. Acquisition of mobility spectra

A home-made computer program stepped through a set of equally spaced voltages (used to drive the high voltage source connected to the inner rod of the DMA), and stored the response of either of the detectors versus the DMA voltage. A raw mobility spectrum is just a representation of one of these variables versus the other. The voltage recorded in the data file is the value \(V_{\text{as}}\) assigned by the computer, not the value \(V\) actually applied to the DMA by the power supply run by the computer. The relation \(V(V_{\text{as}})\) was determined by calibration. No corrections were necessary for the 0–11 kV power supply used for viral work. For flow calibration work with the Half-Mini DMA we used a different 0–5 kV source which required calibration, especially at low flow rates associated to moderate peak voltages. The relation obtained was \(V = 0.98908 V_{\text{as}} + 8.245 V\) (for \(V_{\text{as}} < 1\) kV).

![Fig. 4. Raw mobility spectra for positively charged THABr clusters taken with the Perez-LT-3o DMA, with the blower running at the lowest (3200 rpm, gray) and the highest (9000 rpm, black) settings tested. The aerosol flow was between 1.3 and 1.5 L/min.](image)
3. Results
3.1. DMA evaluation with cluster standards

Fig. 4 shows raw mobility spectra for positively charged THABr clusters at the lowest and the highest settings of the blower used, showing a drastic increase in resolution as the flow rate goes up. The bipolar source used produces dominantly singly charged ions (Fernandez de la Mora & Barrios, 2017), so the ordering of the peaks in the spectrum reveals directly their composition: monomer (bare cation), dimer, trimer, etc, from left to right. The upper spectrum taken at 3200 rpm resolves up to 18 clusters. The lower spectrum taken at 9000 rpm goes only up to the octamer. Note that the noise level is about 0.1 fA, as expected for this electrometer. As a result of the rather low signal, the zero drift of the electrometer is not negligible. It was corrected by assuming a linear variation of the baseline with the voltage, such that the horizontal regions between isolated peaks would be indeed flat. As an example, in the data shown at 9000 rpm (lower continuous black line) the last datum that reads approximately 0 fA when corrected would have read 0.4 fA, and the flat theoretically horizontal regions between peaks 1–6, if not corrected, would have had an unphysical finite upward slope. Uncorrected data are shown also for reference as the dotted line falling slightly above the corrected continuous line for 9000 rpm.

Spectra were taken at the pump settings and flow rates indicated in Table 3. The peaks for the various clusters in these spectra taken at several pump speeds were fitted to Gaussian curves to obtain the resolving powers shown in Fig. 5. The figure includes the continuous gray line $C \nu^{1/2}$ scaling with peak voltage as theoretically expected from diffusive broadening (Rosell et al., 1996). The coefficient $C$ (1.55 V$^{-1/2}$) used is not obtained by any rigorous theoretical analysis, but empirically as a low-voltage fit (gray line in Fig. 5) to the upper envelope of the data:

$$1 / \text{FWHM}_{\text{diff}} = CV^{1/2}; C = 1.55 \text{Volt}^{-1/2}$$

The value of $C$ analogously obtained for the prior Perez DMA model was 1.35 V$^{-1/2}$, indicating a superior performance of the LT version, perhaps as a result of the better laminarization or the larger convergence angle. One can see at all pump settings that the data for the lower voltages (smaller clusters) approach best the diffusive limit. Yet, past a certain voltage, the resolution shows a drastic reduction. This reduction cannot be due to turbulence, since each series is obtained at fixed flow rate with only the size of the cluster varying. We believe it is primarily an artificial consequence of the fact that the larger clusters are not completely resolved from each other (top spectrum in Fig. 4 above 0.4 kV), so that either the tails from neighbor cluster peaks or ion impurities between main members of the cluster series create a background that is not accounted for by the Gaussian fitting. The resolutions plotted in Fig. 5 for each $Q$ cannot accordingly be trusted past the voltage at which they fall markedly below the diffusion line, which starts at the voltage at which subsequent peaks are no longer baseline separated from each other. This corruption naturally sets in at smaller voltages for smaller pump speeds, so the upper envelope of the various data sets provides a more reliable measure of the real resolution, uncorrupted by peak merging. For instance, the spectrum of Fig. 4 corresponding to a pump speed of 9000 rpm shows isolated peaks for all the clusters included (monomer to octamer), so all the open circles of Fig. 5 ought to give uncorrupted resolutions. The resolutions measured at 9000 rpm for three of the data exceeds 37, well above the best value of about 30 observed with the prior Perez DMA. If the zero drift correction had not been implemented, the maximal resolving power would have been 34, still substantially higher than in our earlier study with the shorter laminarization trumpet.

Concrete mechanisms possibly contributing to $\text{FWHM}_{\infty}$ are:

(i) imperfect axisymmetry of either the instrument or the flow field.
(ii) Even in a macroscopically symmetric device, tiny scratches on the walls may create substantial asymmetries by promoting local boundary layer transition or separation in certain regions of the DMA and not in others.
(iii) Imperfect stretching or localized dirt in the laminarization screens.
(iv) A finite size distribution of the viral standard.
(v) Any unsteadiness in the flow. These may include not just high frequency flow unsteadiness, but could also result from an excessively fast scan of the DMA voltage compared with the particle residence time in the DMA or the detector (this last problem does not arise in this work).

| rpm | 3200 | 4000 | 5000 | 6000 | 7000 | 8000 | 9000 |
|-----|------|------|------|------|------|------|------|
| $V_1$ (V) | 138  | 178  | 229  | 279  | 324  | 371  | 413  |
| $Q$ (L/min) | 565  | 733  | 941  | 1147 | 1332 | 1524 | 1699 |
| $Re=Q/[(R_1(L)+R_2)\nu]$ | 6667 | 8639 | 11,092 | 13,525 | 15,705 | 17,970 | 20,034 |

Table 3
Blower settings, voltage of appearance $V_1$ of the tetraheptylammonium$^+$ ion (monomer) and corresponding flow rates and Reynolds numbers used in ion cluster measurements.
More generally, we consider three separate sources of peak broadening: (i) diffusive, (ii) the contribution due to finite aerosol flow rate (Knutson & Whitby, 1975), and (iii) an additional source collecting the nonidealities intrinsic to the instrument. Their contributions to peak width can be considered to be independent, and thus approximated by the quadratic additive rule

$$\text{FWHM}^2 = \text{FWHM}_{\text{Diff}}^2 + \text{FWHM}_{\infty}^2 + \left(\frac{q}{Q}\right)^2$$

(3b)

The contribution \(\left(\frac{q}{Q}\right)^2\) due to the finite aerosol flow rate \(q\) is negligible for the present cluster measurements, but is important in the analysis of viral particles considered in the following subsection. The theoretical lines used as references for the cluster measurements are accordingly based on the following simplified form of (2-3)

$$\text{FWHM}^2 = \frac{1}{(C V^{1/2})^2} + \text{FWHM}_{\infty}^2,$$

(3c)

with \(C = 1.55 V^{-1/2}\) and \(\text{FWHM}_{\infty}\) determined by a fit to the data. The excellent fit to the 9000 rpm data (excluding the last point) shown as a dashed black line in Fig. 5 is obtained with \(\text{FWHM}_{\infty}=0.018\), implying an intrinsic DMA resolving power \(1/\text{FWHM}_{\infty}=55.6\). This extrapolated diffusion-corrected performance is specific to the conditions of this experiment, with an aerosol flow rate \(q = 1.3\) L/min and a sheath gas flow rate \(Q = 1700\) L/min. Nevertheless, the fact that the cluster data are well fit by a single \(\text{FWHM}_{\infty}\) over a wide range of flow rates supports the interesting conclusion that the quality of the flow does not appear to deteriorate from the smallest to the largest flow rates explored (565–1700 L/min).

In addition to determining the resolution of the LT-3° DMA, cluster standards have been used to obtain the calibration constants collected in Table 4 for the LT-3° and the LT-0.5° Perez DMAs. The measured values are included in convenient units in the second column, while the third column re-expresses this measured value as the dimensionless shape factor \(K\) defined in Equation (1 b). The fourth column is the approximate value computed for the shape factor \(K\) via Equation (1c), which is fairly close to the measurement for the two angles studied. Fig. 6 compares these two measured data (including the purely theoretical unity datum at \(\alpha = 0\)) with the approximation (1c) for the dimensionless shape factor \(K(\alpha)\).

The resolving power of a different DMA prototype using the LP-0.5° inner rod was also briefly investigated with ion clusters, but was not as high as that found in the Perez-LP-3° prototype due to some flow or geometric imperfection that remains to be identified. We do not think the problem with this prototype is related to the 0.5° angle.

Note finally that the largest \(Q\) achieved corresponds to a maximal gas velocity of only 30 m/s, so compressibility effects are negligible under all conditions investigated.

### Table 4

| DMA      | \(ZV/Q\) (\(\text{cm}^2/\text{s}\)/L/min) | \(Q/R_3/(L/R_1(L))\) | \(2\pi ZVL\) | \(K(\alpha)\) |
|----------|----------------------------------------|-----------------------|--------------|--------------|
| LT-0.5°  | 0.175                                  | 0.933                 | 0.9316       |              |
| LT-3°    | 0.236                                  | 0.678                 | 0.6770       |              |

3.1.1. DMA evaluation with viral particles

Mobility spectra obtained with the LT-3° DMA for the 3 viral particles produced by our sample have been previously presented and analyzed with the goal of characterizing the particles themselves (Fernandez de la Mora et al., 2020). Our present analysis of the same data is aimed at evaluating the DMA, especially at the modest flow rates required for virus analysis. The prior study by Perez-Lorenzo...
et al. (2020) did explore this key low flow rate region (down to $Q = 47$ L/min) with protein particles having relatively broad size distributions ($FWHM > 6\%$). Their conclusion that the DMA response was close to that ideally expected up to $q/Q$ ratios of 5–10% was based on spectra showing peak widths between 6% and 8%, and provided no direct assurance of the DMA’s ability to reach resolving powers of even 20 with particles larger than 20 nm. The situation is now more favorable thanks to the new viral standards.

Fig. 7a–b shows mobility spectra for the CBPV and CBPVS viruses, where each viral particle is measured several times (and appears at several voltages) during a single voltage scan involving different $Q$ values. The smaller peak to the left of the CBPV peak is too weak to yield a precise width, and will accordingly not be used here as a mobility standard. In experiments carried out several months after those shown in Fig. 7a we have observed that the dominant peak and its shoulder decrease and increase in height with time. This evolution has enabled a more accurate determination of the width of the slightly more mobile peak, which was seen to be comparable to that studied here for the slightly less mobile peak. Data on inverse peak width are reported in Fig. 8 for the two main particles. Note first that both figures show resolving powers even larger than the cluster experiments of Fig. 5. The width reported has been inferred following several criteria. Gaussian all refers to a fit of the whole peak to a Gaussian curve. This fit reflects best the real $FWHM$ of the measured peak, including its right tail (attributed to involatile impurities in the solvent incorporated as contaminant residues into some viral particles). Gaussian Left implies a fitting of the left and top of the peak to a Gaussian, ignoring the right side of the peak potentially contaminated with involatile residues from the solution. This value reflects better the intrinsic width of the particles and the DMA by removing contamination from ES imperfections. Linear Left and Linear All similarly refer to a fit to either the whole or the left half of the peaks to a triangular shape. That the peaks are approximately triangular, as predicted theoretically in the absence of diffusion (Knutson & Whitby, 1975), can be seen in Fig. 7b for the satellite (shown in more detail in Fig. 6 of Fernandez de la Mora 2020). The real peaks are missing the three sharp edges of the triangle at its top and its two bottom regions, as a result of either diffusion, or of a finite width of the real particle size distribution (see Fig. 2b of Fernandez de la Mora, 2017). Accordingly, the height of the theoretical triangle exceeds slightly that of the experimental peak. This leads to a smaller $FWHM$ for the triangular shape than for the actual peak, explaining why the Gaussian fit data in Fig. 7 lie below the linear (triangular) fit data.

At the modest values of $Q$ used for the data shown in Fig. 7, the $(q/Q)^2$ contribution from the sample flow rate variable ignored in our prior analysis of the cluster data is no longer irrelevant.

Prediction (3b) is shown in Fig. 8a for the same value $C = 1.55$ previously inferred from cluster studies, and for two values of $FWHM_{\infty}$. This quantity was associated in the prior cluster data with any possible non-ideal broadening mechanism from geometrical or flow imperfections in the DMA. This was then justified because the cluster standards are believed to be strictly monomobile. The same has never been established for any viral standard, so that now $FWHM_{\infty}$ combines nonidealities in the response of the instrument with the perhaps finite width of the mobility distribution of the viral particles. It is noteworthy that, once the diffusive and $q/Q$ corrections...
have been included in the theory, it fits the data with a value of $FWHM_{\infty}$ approximately half-way between 0% and 1%. In other words, even if the DMA had a perfectly ideal response, we can state that the intrinsic width of the size distribution of this virus is about 0.5%. Alternatively, even if the virus was perfectly monodisperse, the resolving power of the DMA for non-diffusing particles at $q/Q \to 0$ would be about 200! These inferences are based on the linear-left criterion to determine the resolution. The choice is justified because our goal is to gauge the imperfections on either the viral size distribution or the DMA response, rather than the evident imperfections in the electrospraying process responsible for the asymmetry of the peaks. We conclude from the analysis of the data for the CBPV particle that both the DMA and the particle standard are closer to the ideal than could have been anticipated.

The fact that the values of $FWHM_{\infty}$ inferred are independent of the flow regime ($Q$) both for the CBPV ($FWHM_{\infty} \approx 0.5\%$) and the cluster data ($FWHM_{\infty} \approx 2\%$), yet differ so much among themselves is puzzling. This paradox is illuminated by the resolution data for the CBPV Satellite (Fig. 8b), which span flow rates intermediate between those used for the CBPV and the cluster studies. In this case no Gaussian analysis is used as the peaks look manifestly triangular. The theoretical lines shown in Fig. 8b represent Equation (3b) with $C = 1.45$ and $FWHM_{\infty}$ of 0% and 2%, as indicated in the legend. The choice of $C = 1.45$ used here differs slightly from the $C = 1.55$ obtained in the prior cluster measurements and the $C = 1.35$ reported by Perez-Lorenzo et al., 2020, perhaps because the effective diffusivity is enhanced by free stream turbulence, and therefore depends on the quality of the laminarizer and the Reynolds number. In spite of the scatter of the data, one can clearly recognize a region of high resolution to the right, and another of lesser resolution to the left, with a jump (marked with a vertical line) from one to the other taking place somewhere in the interval $159 < Q/(L/min) < 177$ ($q = 0.6\ L/min$). The corresponding critical DMA Reynolds numbers defined in Table 4 is in the range $1874 < Re^* < 2087$. These are values typical for turbulent transition in a tube in the absence of laminarization, and it is most unusual for an instability to set in in a DMA so close to this critical condition. The general situation is also unusual for a variety of reasons. First, the discontinuity seen is rather mild, as $FWHM_{\infty}$ changes from about 1.8-2% at $Q > 170\ L/min$, to about 0.5% below it. This small effect was undetectable in our previous Perez DMA design. All other prior work with supercritical DMAs running laminarily at Reynolds numbers larger than 2000 has involved considerably shorter working lengths $L$, which decreases the time available for the growth of perturbations. These prior studies have also tended to work always at $Re^* \approx 2000$, whence, if a regime change took place at $Re = 2000$ it would have been missed. In perspective of the unusual situation, we have looked for other possible explanations for the jump in Fig. 8b. One possibility is that the quality of the electrospay deteriorated slightly at one point as the series scanning over $Q$ was carried out. This hypothesis is not unlikely, since achieving the rather high resolving powers recorded in Fig. 8 requires that all components of the system (virus, ES, charge reduction, DMA) be close to their best. The data of Fig. 8a do not allow resolution of the “turbulence versus ES quality” ambiguity because the maximal flow rate achieved in work with CBPV is substantially below the $\approx 160\ L/min$ at which the transition was observed in the CBPVS study.

4. Conclusions

DMAs designed to run at high Reynolds numbers often exhibit nonideal behavior as a result of the natural tendency of some level of upstream turbulence to survive through the laminarization elements, or of some form of new turbulence to develop in the working section. Nevertheless, we have shown here that it is possible to achieve flow conditions where the DMA operates almost ideally. This regime is not easy to attain, but has been obtained here by using a wide laminarization trumpet with a particularly smooth transition into the working section. This ideal regime was not easy either to identify with prior evaluation methods relying on cluster standards useful mainly at fairly high flow rates. Fortunately, the availability of two recently identified viral standards with diameters of 17 and 38 nm now allows high resolution probing of DMAs at modest flow rates compatible with this ideal behavior. Exceptionally low uncorrected $FWHM$ values have as a result been measured, approaching 2%. Once corrections are made for peak broadening by diffusion and the finite aerosol flow rate, we estimate a $FWHM_{\infty}$ of 0.5%, including both DMA nonidealities and the finite width of the viral particles. This implies that the two viral particles tested are monomobile for all practical purposes, and also indicates that the
DMA operates close to ideally. By future improvements in signal/noise we hope to obtain better values of the Q dependence of the uncorrected FWHM, and better bounds for the corrected DMA and virus FWHM∞.

A confirmation of the difficulties involved in achieving near-ideal DMA performance is given by the fact that only one of the two Perez-LT DMA prototypes tested performed as exceptionally as reported here. We are confident that the performance of the inferior prototype was unrelated to the different inner rod angle, being rather due to one of the many usual causes for non-ideal DMA response.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Following Yale rules JFM declares an interest in the company NanoEngineering commercializing the DMA here described.

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