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Modeling Elastic Pore Sensors for Quantitative Single Particle Sizing

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Abstract

An empirically derived model of how the dimensions of an elastic size-tunable pore sensor change with applied membrane stretch is presented. Quantitative modeling of the pore dimensions, in conjugation with a simplified pore resistance model, enabled particle size and translocation velocity profiles to be calculated from the individual particle ‘pulse’ events, at any membrane stretch. Size analysis of a trimodal suspension, composed of monodisperse 220, 330 and 410 nm particles, gave rise to 3 distinguishable particle peaks with coefficient of variances below 8.2% and average size values within 2.5% of single modal dynamic light scattering measurements. Particle translocation velocity profiles, over the approximate 12 μm pore sensing zone, showed that particles entering the small pore were initially accelerated to velocities approaching 5,000 to 6,000 $\mu\text{m/s}$. They then rapidly decelerated due to the pore geometry affects on the forces driving particle translocation, being the electric field strength and fluid flow.

Keywords

nanopore; coulter counter; resistive pulse sensor; elastic pore sensor

Introduction

Resistive pulse sensors enable single particle analysis that is typically more sensitive to sub-populations within a polydisperse sample than ensemble techniques.¹ Recent advancements in nanofabrication and microfluidics have given rise to a plethora of new resistive pulse ‘pore’ sensor devices with the capabilities of detecting the size, shape, charge and concentration of micro to nano-scale objects in solution.^{1–12} Although promising characterization devices, these sensors are currently limited by their static ‘fixed’ pore geometry to the accurate detection of a narrow size range of objects, typically between 2 and ~60% of the pore diameter.^{13–15} Therefore, analysis of polydisperse samples, beyond the pore size limits, typically requires pre-size fractionation of the sample followed by a series of analysis runs through progressively smaller apertures. This is not only time consuming but depending on the size fractionation technique used it can also bias measurements of the ‘true’ size distribution of a suspension.

Supporting Information

Experimentally measured and empirical model values of the ionic current and pore dimensions (Table S1), derivations of the relationship between pore dimensions and membrane stretch (Equation 3 and Equation 4), theoretical resistance profiles through the pore with increasing membrane stretch (Figure 1S), derivation of particle position from cylindrical resistance model approximation of the conical pore (Equation 6), and schematic of pulse signal magnitude due to particle position (Figure 2S) are given in the Supporting Information. This information is available free of charge via the Internet at <http://pubs.acs.org>.

The recent development¹⁶ and commercialization of size tunable elastic pore sensors attempt to address this size range detection limitation by enabling the pore dimensions to be changed in real-time to the sample at hand. Pore size tuning enables improved measurement sensitivity over a larger particle size range. By reducing the applied stretch on an elastic pore sensor Roberts et al.¹⁷ demonstrated a 2 fold increase in the magnitude of the ionic current pulse signal generated by particles. Similarly Vogel et al.¹⁸ recently demonstrated that a 1 mm reduction in applied macroscopic pore stretch gave rise to a 60% increase in particle volume sizing sensitivity. In addition to improving sizing sensitivity reducing the pore size has also been shown to reduce and selectively gate larger particles from analysis.¹⁷

Unfortunately elastic size tuning also presents the unique challenge that the pore dimensions, which must be well characterized for absolute quantitative single particle measurements, are not known. Therefore, quantitative sizing with elastic pore sensors have been achieved to date by calibrating the pore using a series of particles with known size.¹⁸ This calibration relies on the linear relationship between the particle volume and magnitude of the resistive pulse signal.^{15,18} While allowing for particle size distributions to be measured, this method must be completed at every membrane stretch value, which can become time consuming and counterproductive to real-time pore tuning.

Herein we present an empirical model developed from averaged pore geometry measurements that describes the relationship between elastic pore dimensions and the applied membrane stretch. The pore geometry obtained from this model is then used to establish the resistance profile through the pore. This then allows the resistive pulse signals generated by a particle at any membrane stretch to be converted to an absolute particle size and thereby transforming this device from a qualitative to a quantitative single particle analysis instrument.

Theory

Resistance through a pore

Resistive pulse sensors detect and characterise particles traversing the pore via the *Coulter principle*. In general particles travelling through the pore give rise to a resistance ‘pulse’ in time that is proportional to the excluded volume of the particle in the pore. Therefore knowledge of the pore dimensions and resistance profile enables particle sizes to be calculated from the individual particle ‘pulse’ events.

The resistance through a pore sensor is dependent on the pore geometry, i.e. it is inversely proportional to the cross sectional area of the pore openings and proportional to its length. Previous characterisations, by scanning electron and confocal microscopy imaging, of the elastic pores used in this study have indicated that the pores are approximately conical in shape.^{17,19} Therefore, the total resistance R through the elastic pore can be approximated from the conical model,²⁰

$$R = \frac{4\rho L}{\pi AB} \quad \text{Equation 1}$$

where ρ is the resistivity of the electrolyte media and A , B , and L are the pore dimensions being the small and large pore opening diameters and pore length, respectively. The ionic current through the pore can then be calculated using Ohm’s law ($V = iR$).

A unique characteristic of conical pores is that their geometry gives rise to a resistance gradient through the pore that is highest at the smallest constriction point, i.e. the small pore opening. The profile and resistance at any point within the pore can be calculated from the

conical resistance model by ‘breaking’ the pore into segments. As conical pores are symmetric along the pore axis the resistance at any distance z from the small pore opening, or over any two points (Δz), along this axis can be calculated by,²⁰

$$R = \frac{4\rho(z_b - z_a)}{\pi A_z B_z} \quad \text{Equation 2}$$

where A_z and B_z are the diameters of the smaller and larger pore opening a distance z_a and z_b along the pore axis.

This focusing of the resistance through conical pore makes them sensitive characterization tools but it also presents the challenge that the resistive pulse signal of an object is dependent on its location in the pore. By modelling the change in resistance (ΔR) expected for particles located at increasing distances z from the small pore opening the location and velocity profile of particle translocation can be calculated from the pulse signal.²⁰ This is generally achieved by breaking the pore into Δz segments equal to the particle diameter and multiplying the resistance at this point by the corresponding particle volume fraction. Heins et al.²⁰ improved the accuracy of the excluded volume fraction model by also integrating the resistance gradient, due to the pore geometry, over the length (diameter) of the particle. By modelling the entire pulse signal of a particle in this way it can be used to infer not only information about the particle size but also its position and velocity through the pore.

Materials and Methods

Four elastic pores, donated by IZON Science Ltd. (NZ), were fabricated by puncturing a thermoplastic, Elastollan1160D, polyurethane cruciform membrane (Elastollan1160D, BASF) with a tungsten needle, as described previously.¹⁶ Membranes were mounted on a qNano (IZON Science) and pre-stretched, 5 cycles of stretching the membrane from 42 to 52 mm, to overcome any Mullins stretching effects, prior to imaging and experimental application.¹⁸ The distance between the membrane arms is 42 mm therefore this corresponds to the membrane in a ‘relaxed’ or unstretched state. The pore openings were imaged at 42, 43.5, 45, 47.5, and 50 mm stretches using an IX70 Olympus microscope at 100x and 250x magnification for the large and small opening, respectively. The area of the pore openings were calculated from the image contrast using ImageProPlus software, which was calibrated using a 0.01mm increment calibration slide. The pore membrane thickness was measured at the center of the cruciform using a calliper micrometer. All of the measurements were done in triplicate and the pore was allowed to relax to 42 mm of stretch between measurements.

Polystyrene particles of 220, 330 and 410 nm were purchased from Bangs Labs (USA). All particles were dispersed in 100 mM KCl. 40 μ L of the individual or a mixture of the three particle suspensions were added to the top fluid cell and a minimum of 100 events were recorded for each of the four pore stretches. The current pulse signals were collected and analysed using IZON proprietary software V2.0. The analog digital converter operates at 1 MHz, which is reduced to a sampling rate of 50 kHz through electronic filtering.

Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS. Single modal solutions were prepared by diluting stock solutions of 220, 330, 410 nm polystyrene particles to 0.001 wt. % in 10 mM NaCl. A trimodal suspension was prepared by mixing equal thirds of each single modal solution. Samples were sonicated for >30 min prior to taking 5 measurements, consisting of 10 individual runs, for each sample.

Results and Discussion

As shown in Figure 1, the elastic pore membrane is axially stretched by mounting it between two pairs of opposing stretching jaws. The radial strain is expressed as an applied stretch of distance (X) measured between two opposing jaws, which is 42 mm at rest. Optical microscopy images of 3 separate pore membranes (Figure 1) indicated that the pore openings were roughly circular and increased in size with applied stretch. The large openings displayed polymer folds around their perimeters, i.e. a 'wrinkled' pore opening. Although not directly observed, as they were most likely below optical microscopy resolution ($\pm 0.5 \mu\text{m}$), polymer folds were also expected to be around the small pore opening. These folds are a likely product of stronger crystalline regions in the polymer matrix surviving the puncturing process during pore formation.

The elastic pore dimensions, small and large pore opening diameters (A and B , respectively) as well as the membrane thickness, i.e. pore length L , were measured at 42, 43.5, 45, 47.5 and 50 mm of applied stretch. The membrane thicknesses, measured with a calliper micrometer, decreased from ~ 370 to $315 \mu\text{m}$ with increasing stretch. As they were not perfectly circular the area of the pore openings, including the additional area within the polymer folds, were calculated with Image Pro Plus 4.0 software. The diameters A and B were calculated by approximating the total opening areas as circles. Optical measurements of the 3 membranes indicated that A and B increased from ~ 1.0 to $1.7 \mu\text{m}$ and ~ 34 to $60 \mu\text{m}$, respectively, with applied stretch.

As the measurements of A were close to the microscope resolution we instead used the conical model of resistance through a pore (Equation 1) to calculate A . Previous studies have calculated pore dimensions that are deemed too small for accurate analysis using theoretical resistance models, in a similar fashion as undertaken in this study.^{20,21} Values of A were calculated using the measured ionic currents and experimental measurements of B , and L (Table 1S in the Supporting Information, SI). The resistivity (ρ) of the 100 mM KCl solution was $0.861 \Omega \text{ m}$. As expected the calculated values of A were in most part submicron in size (0.35 to 1.45 nm) and below the resolution of optical microscopy. Therefore, calculated values of A were used in generating the empirical model and subsequently for quantitative single particle sizing.

Stretching the pore: measuring the macroscopic properties

The relative change in the dimensions (L and areas of A and B) of 3 separate pores allowed for the construction of an empirical model to predict elastic pore geometry at any membrane stretch. The generated empirical model was then tested and validated on a 4th 'Test' pore fabricated in the same batch process. An elastic relationship was expected to exist between the increase in pore and membrane areas; axial stretching of the membrane macroscopically should correspond to a similar relative change in the microscopic area of the small and large pore openings. This area change relationship can also be expressed in terms of radial strain and either approach can be used to establish the relationship between the membrane and pore opening dimensions used in this empirical model. For membrane stretches greater than 43.5 mm a linear relationship between the relative change in pore dimensions and membrane area was found, as shown in Figure 2. Stretches below 43.5 mm deviated from this linear trend, which instead displayed pore dimensions more similar to that observed at 43.5 mm of stretch. This is also supported by the higher than expected normalized currents measured through the pores at 42 mm of stretch, shown in Figure 3. The negligible change in pore dimensions at stretches from 42 mm to 43.5 mm is believed to be due to an initial 'lag' before the stretching jaws are able to fully engage the membrane and begin axial stretching. Therefore, 43.5 mm of stretch was taken as a reference point (X_0) for calculating the relative

change in membrane area $((X_i^2 - X_o^2)/X_o^2 \times 100)$ and pore dimensions used to create the empirical model.

The slope of the relative change in small and large pore opening areas, averaged over the three pores examined, were 11.2 ± 0.4 (R^2 : 0.9944) and 4.0 ± 0.1 (R^2 : 0.9954), respectively. Therefore, it was found that a relative increase in the membrane area of 32% gave on average a 347% and 133% relative increase in the small and large opening areas, respectively. These were much larger than predicted as ideal elastic stretching of a membrane is expected to increase features at a 1:1 ratio. Also the difference in the slopes also indicates that asymmetric stretching is very likely occurring with the relative change in the small opening being greater than the large. These effects are believed to be due to the polymer folds 'wrinkles' observed around the pore openings, which are expected to require a lower energy to open than stretching the polyurethane membrane does. However, further investigation is required. Although surprising the resulting greater dynamic pore size range is in fact beneficial to pore tuning as it allows for the analysis of a larger particle size range compared to ideal elastic pore stretching and even more so over conventional fixed pore devices.

As expected axially stretching the membrane reduced its thickness. The slope of the relative change in membrane thickness and corresponding pore length (L) was -0.38 ± 0.02 (R^2 : 0.9924). This ratio was found to correlate well with the material properties of the polyurethane membrane, i.e. the Poisson's ratio of the Elastollan1160D polyurethane membrane being, 0.4. Changing the Poisson's ratio of the polyurethane membrane polymer by changing the urethane monomer, polymer Mw, or addition of plasticizers is therefore expected to impact the elasticity of the membrane and consequently can be used in the future to tailor the relationship between the changes in pore dimensions with applied stretch.

The experimentally established linear relationship between the relative change in pore dimensions to membrane area was then used to create three empirical models to calculate the pore dimensions A, B, and L as functions of the applied stretch X (derivations provided in SI) by,

$$A(X) = \sqrt{k_i * A_o^2 * \left(\frac{X^2}{X_o^2} - 1 \right) + A_o^2} \quad \text{Equation 3}$$

and

$$L(X) = k_i * L_o * \left(\frac{X^2}{X_o^2} - 1 \right) + L_o \quad \text{Equation 4}$$

where X_o , A_o , and L_o are the values for the membrane stretch, pore diameter and thickness at the reference point used in calculating the relative change (43.5 mm, in this study) and k_i are the pore dimension scaling ratios, i.e. the slopes of the pore dimensions calculated from Figure 2. As A and B have the same geometric relationship to X , B is calculated from Equation 3 by substituting B_o for A_o and using the k_i measured for B (4.0).

At stretches greater than 43.5 mm calculated pore dimensions from the empirical model fit not only the 3 pores the model was generated on but it also fit a 4th 'Test' Pore within experimental error. This 'Test' pore was fabricated in the same batch process as the other 3 pores so although not used in creating the empirical model, i.e. in the calculation of the k_i pore dimension scaling ratios, its pore dimensions were expected to be similar.

Although manufactured using the same puncturing process slight differences in the size of the 4 pores were observed in both optical and ionic current measurements. The ionic currents through Pores 1 and 2 increased from ~9 to 43.5 nA when stretched from 42 to 50 mm whereas Pore 3 and the Test Pore increased from ~15 to 72 nA (Table 1S, SI). Even though the currents through the four pores were different they did give rise to very similar normalized currents with stretch, as shown in Figure 3. This similarity between the pores indicates that the relative or percentage change in pore dimensions due to stretching are independent of the punctured pore size thus supporting the developed universal empirical model to describe these changes. The ionic current through the pore at any stretch, $i(X)$, can therefore be calculated from Ohm's law ($V = iR$) by modifying the model for resistance through a conical pore (Equation 1) with the developed empirical functions (Equation 3 and Equation 4) accordingly,

$$i(X) = \frac{\pi A(X) B(X)}{4 \rho L(X)} V \quad \text{Equation 5}$$

where V is the applied voltage (0.3 V) and ρ is resistivity of the electrolyte medium (~0.861 $\Omega \text{ m}$ for 100 mM KCl at 20 °C).

Individual particle sizing theory: relaxing to improve accuracy

Particles traversing a pore sensor give rise to a resistance 'pulse' in time, which is often measured as a change in voltage or ionic current across the pore. Particle size is calculated from the linear relationship between the excluded volume fraction of the particle in the pore and the magnitude of the generated pulse signal. In this way increasing the particle size, or reducing the pore size for the same sized particle, increases the pulse signal magnitude.

Analysis of a trimodal suspension, composed of 220, 330 and 410 nm polystyrene particles, gave rise to ionic current with time traces which contained pulses with three distinct magnitudes, as shown in Figure 4A(insert). As expected larger particles gave rise to a pulse signal with a larger maximum change in ionic current, Δi_m . Histograms of the relative maximum change in pulse magnitude ($\Delta i_m / i_{bg}$, where i_{bg} is the 'background' current through the pore) clearly showed three distinct population signals corresponding to the three particle sizes in the trimodal suspension. Increasing the pore dimensions by stretching the pore reduced the magnitude of the $\Delta i_m / i_{bg}$ populations. This relationship in pulse magnitude with stretch is illustrated by the lower pulse signals observed at the larger 47.5 mm pore stretch than at 43.5 mm of stretch (Figure 4B). The average magnitudes for the three observed pulse populations at all stretches examined are given in Table 1. As expected, increasing the pore size by stretching from 43.5 to 47.5 mm reduced the average magnitude of the 220, 330 and 410 nm particles by 40, 36 and 34 %, respectively. Similar findings of reduced pulse magnitude with increasing pore stretch were previously observed by Roberts et al.¹⁷ Stretching the pore not only decreased the magnitude of the pulse signal populations but it also decreased their coefficient of variances (CV), thus limiting particle size and distribution analysis to qualitative comparisons between pulse signal populations within a sample run.

Using the pore geometry and applying an appropriate resistance model enables the qualitative pulse magnitudes to be used to quantitatively size individual particles. As discussed previously in the theory section these models are based on calculating the increased resistance in the pore due to the excluded volume of a non-conducting particle. Calculating this increased resistance quickly becomes non-trivial for non-spherical particles^{14,22,23} and/or non-cylindrical pores²⁰ due to the inhomogeneous 'blocking' or gradient of the electric field, respectively, in the pore. This can be simplified for conical

pores by approximating the resistance through the pore as a series of cylindrical pores of infinitely small Δz segment lengths. Simplifying in this way enables particle size to be easily calculated from the pulse signal and pore geometry.

In this study individual particle sizes were calculated from the maximum change in the ionic current pulse (Δi_m) using a length independent cylindrical model.²⁴ It was assumed that Δi_m is generated by a particle at the smallest constriction site, i.e. the small pore opening, and that the resistance gradient is negligible over the particle diameter. In this way the diameter d of individual particles at the small pore opening, $A(X)$, of the Test Pore at the 5 pore stretches were calculated by,^{14,15}

$$d = \sqrt[3]{\left(\frac{V}{i_{bg} - \Delta i_m} - \frac{V}{i_{bg}} \right) \frac{\pi [A(X)]^4}{4\rho}} \quad \text{Equation 6}$$

where i_{bg} is the background current through the pore and $V/(i_{bg} - \Delta i_m) - V/i_{bg}$ is equal to the change in resistance (ΔR) due to the particle in the pore.

The calculated diameter of individual particles within the trimodal suspension at the 5 pore stretches examined all gave rise to three distinct particle population histograms corresponding to the 220, 330 and 410 nm particles. In contrast to the decreasing signal in the qualitative relative ionic current values (Figure 4B) the calculated quantitative particle size histograms generated for each stretch overlaid one another, as exemplified in Figure 4C. Over the 5 stretches examined, the variance in the average calculated particle diameters was less than 5%, compared to greater than 50% for the measured average ionic current pulse magnitudes. Furthermore, the average diameter for each particle set, given in Table 1, were within 2.5% of both the manufacture specifications and dynamic light scattering (DLS) measurements of the individual particle sets. The low variance and high accuracy in calculated particle sizes supports the developed empirical pore size model for calculating the pore dimensions and the cylindrical model calculation assumptions.

Absolute single particle sizing, as achieved through this study, also enables quantitative analysis of the size distribution of a particle population. Characterising particle size distribution is important in applications such as photonic crystals and drug delivery where even small variances or outlying populations can impact the material properties and performance. The average CVs measured for the 220, 330 and 410 nm particle sets over the 5 pore stretches examined were found to be 8.2, 3.1 and 3.8%, respectively. These narrow distributions correspond to the manufactures specifications that all three particle sets were monodisperse, i.e. have a CV < 10%. In contrast CV's measured by DLS (Figure 4D) were much larger, being 21, 22 and 23% for the 220, 330 and 410 nm particle sets. Furthermore, DLS was not able to discriminate between the three particle sets when measured as a mixed trimodal suspension. Instead DLS gave rise to a single peak at 420 nm corresponding to the largest particle set in the trimodal suspension. This is because the detection signal of DLS is biased toward larger particles within a suspension.²⁵

Modeling particle location and velocity profile

In addition to particle sizing, a few studies have explored modelling the particle resistance pulse profile to enable particle surface charge (ζ -potential)^{26,27} and shape measurements.^{14,22,23} As detailed previously (Equation 2) the geometry of conical pores gives rise to a resistance gradient, which extends into the pore from the small pore opening. Increasing the angle of the pore will increase the 'focusing' of the resistance gradient at the small pore opening. Particles within this focused region give rise to an excluded electrolyte

volume that will result in a change in resistance (ΔR) above the background noise, i.e. a detectable pulse signal. The distance into the pore where objects are detectable is often referred to as the sensing zone. A common approximation of the maximum 'potential' sensing zone length is the distance into the pore where the resistance profile reaches 85% of the total resistance through the pore. However it is important to note that this approximation of the maximum potential sensing based on the resistance gradient due to pore geometry and that background noise will be greater than 15% of the total pore resistance. The actual sensing zone is dependent on the pore geometry, amplitude of the background noise, and the particle size with larger particles giving rise to larger and therefore detectable signals over longer distance than smaller particles.²⁰

Resistance profiles for the test pore at the 5 stretches examined were found to all be very similar (Supplementary Information). Stretching the pore slightly increased the maximum potential sensing zone from 27 to 32 μm . This indicates that the sensing zone in these pores is expected to always be less than 10% the total pore length at any stretch. The slight change in maximum sensing zone length (27 to 32 μm) with stretch is due to the small change in pore angle being, 2.7 to 5.6 degrees. As the detection signal is also dependent on the particle size a more accurate approximation of the sensing zone and therefore also the pulse signal with particle location in the pore can be made by theoretically modelling the change in resistance for a specific sized particle at increasing distances into the pore.^{20,28,29}

Theoretical modelling of ΔR , calculated here as Δi , expected for a 220, 330 and 410 nm particle at increasing distance in the pore using the developed cylinder approximation, exclude volume fraction, and Heins model are shown in Figure 5 at a membrane stretch of 45 mm. It is important to note that as outlined in this study these models only predict the resistance change expected for particles within the pore. The models predict either the ascending or, as is this case for this study, the descending half of the pulse curve depending on the direction of particle translocation, i.e. from big opening to small or vice versa, respectively. Additional modelling of the resistance gradient outside the small pore opening, beyond the scope of this study, can be employed to predict the entire pulse curve.

All of the models showed similar curves with the magnitude of the expected signal increasing with particle size and proximity to the small pore opening. The volume fraction and model developed by Heins et al²⁰ produced similar Δi vs distance curves. This is expected as they are based on similar geometric parameters, with Heins's model also accounting for the resistance gradient over the particle diameter. The cylindrical approximation gave rise to very similar shaped curves but higher Δi values than the other models. The sensing zone lengths predicated from the cylindrical model for the 220, 330 and 410 nm particles, based on a measured instrumental noise of 0.01nA, were ~6, 10, and 12 μm , respectively. As expected these sensing zone lengths are all smaller than the maximum potential sensing zone due to pore shape and indicate that particle detection only occurs within the first 4% of the pore. In addition to calculating the sensing zone, theoretically modelling Δi vs particle position also enables the velocity profile of a particle through the pore to be calculated.

The position (Figure 6A) and subsequent velocity (Figure 6B) profiles for individual particles traversing the pore were calculated from the descending half of the experimental pulse peaks, shown in Figure 4A, using the simplified cylindrical model presented in Equation 6. Particle position was determined by calculating the pore diameter A_z required to generate Δi pulse signals along the signal curve at times greater than the Δi_m . From these A_z values the distance from the small pore opening were geometrically determined from the pore dimensions (derivations provided in SI). The resulting particle position vs time graphs for the 220, 330 and 410 nm particles traversing the Test Pore at 45 mm of stretch produced

complex curves over the ~ 0.01 sec detection times. As expected all three particle sizes gave rise to similar position profile curves through the pore with estimated sensing zone distances of approximately 5, 9, and 11 μm s, increasing with particle size, supporting the theoretical predictions of the sensing zone lengths. Although the curves did not reach a plateau when approaching the detection limit, the data did undergo a significant spread due to background noise fluctuations in the current signal dominating the calculations. Thus the sensing zones were calculated from the average distance measurement when significant data spreading was observed, i.e. at times greater than 0.005 sec.

The velocity profile of the 220, 330 and 410 nm particles (Figure 6B) were calculated from the slope of the position vs time curves using a 5 data point moving average to reduce the error from noise fluctuations in the pulse signal. As expected the velocity profiles were complex yet similar for all three particle sizes. Particle velocity increased rapidly over the first micron of the pore reaching a maximum of between 5,000 and 6,000 $\mu\text{m/s}$. Particles then rapidly decelerated over the next few microns reaching negligible velocities at distances greater than 5 microns into the pore. This acceleration and then deceleration of particles through the pore is believed to be a product of the pore geometry. Initial particle acceleration near the small pore opening is expected to be due to the higher resistance gradient, increasing electrophoretic force on the particle, and the establishment of fluid flow into the pore due to inherent pressure forces in the apparatus set-up. Particles then undergo deceleration as they travel further into the pore due to the reduction in the electrophoretic forces and fluid flow³⁰ acting on the particle as a result of the increasing pore diameter. As demonstrated from the resistance profile modelling, moving greater distances, z , into the pore increases the pore diameter A_z . This increase in cross-sectional pore area reduces the resistance gradient by $1/A_z^2$. Similarly the average fluid velocity through the pore also reduces approximately as a function of $1/A_z^2$.^{30,31} Therefore, as particle velocity is determined by one or a combination of both of these forces particles will decelerate as they move into the pore, as illustrated in Figure 6B. Characterising the contribution that each of these forces plays on particle translocation can potentially enable single particle electrophoretic mobility measurements.²⁴

Conclusions

The dimensions of a conical elastic size tunable pore were empirically modelled by relating the pore geometry to the applied macroscopic membrane stretch. Knowledge of the pore dimensions and applying a simplified length independent cylindrical model of the resistance through the pore enabled quantitative single particle analysis at any pore stretch. Size analysis of a trimodal suspension, composed of three monodisperse polystyrene particle sets were easily discernable from each other at all stretches and the calculated diameters were within 2.5% of both the manufacture specifications and dynamic light scattering (DLS) measurements. Theoretical modelling of the resistance gradient through the conical pore and the subsequent resistance pulse generated by a particle within the pore indicated that particles were only detectable when they were within $\sim 12\mu\text{m}$ of the small pore opening. This very short 'sensing' zone length, which was less than 4% of the overall pore length, negligibly changed with applied stretch due to the small change in pore angle with stretch. Modelling the resistance pulse with time of particle translocation was also used to calculate the particle velocity profile through the sensing zone. Pore geometry affects on the main particle driving forces, being the electric field strength and pressure driven fluid velocity, correlate with the complex velocity profile being an initial particle acceleration followed by deceleration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Elastic polyurethane cruciform membrane mounted into the qNano stretching jaws. Schematic of a conical pore with large B and small A opening diameters and pore length L . Optical microscopy images of the large and small openings as well as confocal cross sectional images of the pore thickness at 42, 45 and 45 mm of applied stretch.

Figure 2.

Relative change in pore dimensions, being the area of the small (□, ■) and large (○, ●) pore openings as well as the membrane thickness L (◇, ◆) with relative change in membrane area due to applied axial stretch. Open symbols are the average and standard deviation of pores 1–3 and closed symbols are the measured values for the test pore. Best fit lines through average of dimension measurements of pores 1–3 and the origin are subsequently used for the empirical model.

Figure 3.

The ionic current measured through the four pores (○) normalized to the highest current signal measured at 50 mm of stretch. Values and errors are the average and standard deviation of four measurements.

Figure 4.

Analysis of a trimodal suspension composed of 220, 330 and 410 nm polystyrene particles.

A) Overlay of single ionic current pulse-traces recorded for 220 nm, 330 nm, and 410 nm particle. Insert is the ionic current raw data of the trimodal suspension clearly displaying three different pulse magnitudes which correspond to the three particle sizes. B) Histogram of the relative maximum change in ionic current signal and corresponding C) calculated particle diameter histograms at 43.5 mm and 47.5 mm of applied stretch. D) Dynamic light scattering number distribution of 220 nm, 330 nm, 410 nm, and Trimodal particle suspensions.

Figure 5.

Theoretical change in ionic current for 220 nm, 330 nm, and 410 nm particles 'placed' at increasing distance from the small pore opening. Cylindrical model predictions (solid lines) were similar but greater than theoretical models based on excluded particle volume (dots) and Heins's model (dash), which also takes the inhomogeneous electric field over the particle diameter. All of the models demonstrate the signal dependence on particle size and that particles must be within 10 μm of the small pore opening to give rise to a detectable change in signal.

Figure 6.

Particle translocation position A) and velocity B) profiles. Distance calculated from the cylindrical model for 220 nm ($\circ$), 330 nm (Δ), and 410 nm ($\square$) particles to generate the measured change in resistance at increasing times during translocation of a single particle – i.e. the pulse-trace generated by a particle travelling through the pore.

Table 1

Ionic current signal of a trimodal particle suspension and corresponding calculated particle sizes using a cylindrical and the empirical model values for A.

Applied Stretch [mm]	Relative Blockade Magnitude [$\Delta I/I$] $\times 1000$				Cylindrical Model		
	220	330	410		220	330	410
42	6.3 $\pm$ 0.8	24 $\pm$ 1	46 $\pm$ 6		87 $\pm$ 4	138 $\pm$ 3	172 $\pm$ 8
					202 $\pm$ 9 *	319 $\pm$ 6 *	399 $\pm$ 18 *
43.5	5.2 $\pm$ 1.1	19 $\pm$ 2	38 $\pm$ 3		208 $\pm$ 14	323 $\pm$ 12	409 $\pm$ 11
45	3.6 $\pm$ 0.7	13 $\pm$ 1	25 $\pm$ 2		216 $\pm$ 12	335 $\pm$ 9	413 $\pm$ 9
47.5	2.1 $\pm$ 0.5	6.8 $\pm$ 0.9	13 $\pm$ 2		220 $\pm$ 16	330 $\pm$ 14	412 $\pm$ 16
50	1.9 $\pm$ 0.9	4.4 $\pm$ 0.4	8 $\pm$ 1		230 $\pm$ 39	314 $\pm$ 10	377 $\pm$ 22
Dynamic Light Scattering				216.1 ** $\pm$ 1.12 ($\pm$ 41.2) ***	346.1 $\pm$ 2.646 ($\pm$ 67.5) ****	412.5 $\pm$ 7.760 ($\pm$ 78.9)	420.6 ***** $\pm$ 7.649 ($\pm$ 67.3)

* Particle size calculated using the small pore diameter calculated directly from the ionic current.

** Single modal particle suspension. Error is Standard Deviation over 5 measurements.

*** Standard deviation of the number distribution.

**** Trimodal particle suspension.