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Nanoparticle ζ-Potential Measurements using Tunable Resistive Pulse Sensing with Variable Pressure

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Abstract

Modern resistive pulse sensing techniques can be used to measure nanoparticle electrophoretic mobility, and hence ζ-potential. In contrast to conventional light scattering methods, resistive pulse sensing produces particle-by-particle data. We have used tunable resistive pulse sensing (TRPS) to compare methods for measuring the ζ-potential of carboxylated polystyrene nanoparticles. The five particle sets studied had nominal surface charge den-
sity (σ) between 0 and −0.67 C m⁻², and diameters in the range 160 to 230 nm. Data were collected with pressure in the range ±500 Pa applied across a tunable pore. In each experiment, pressure was varied either continuously or in discrete steps. Calculations of the ζ-potential were obtained by analysing both the rate and the full-width half maximum duration of resistive pulses. Data obtained from duration analyses were more reproducible than rate methods, yielding typical variations smaller than ±5 mV. When σ was greater (less negative) than −0.32 C m⁻², all of the analysis methods studied yielded a monotonic relationship between ζ-potential and σ. Complicated pulse data were observed near the pressure at which the net particle flux is zero, and these observations have been explored by examining competition between electrokinetic and pressure-driven transport. The typical difference between ζ-potentials obtained using TRPS and phase analysis light scattering was 15% (< 5 mV), with an experimental error of ~10% attributable to both techniques.

**Keywords:**
tunable pore, resistive pulse sensing, zeta-potential

1. **Introduction**

Measuring the ζ-potential of nanoparticles in solution is crucial for understanding and predicting the long-term stability of suspensions. Even in a well-characterised solution, it is difficult to accurately predict the ζ-potential from first principles [1]. Existing techniques for ζ-potential measurement draw upon either electrokinetic phenomena, such as electrophoretic light scattering or microelectrophoresis [2], or electroacoustic phenomena [3].
Such measurements are not trivial in nature, especially for relatively non-uniform particle distributions, and they employ experimental procedures and data analysis methods that can affect the \( \zeta \)-potential value. The science of nanoparticle \( \zeta \)-potential measurement can be developed by studying measurement consistency across different experimental conditions, apparatus and analysis methods.

Resistive pulse sensing (RPS) can be used to measure the \( \zeta \)-potential of particles in solution based on their electrophoretic mobility. In RPS, an electric potential is used to drive ionic current through an electrolyte-filled pore within an insulating membrane. If an insulating particle moves through the pore, the resistance across the membrane is increased, producing a transient decrease in measured current from the ‘baseline’ level, known as a resistive pulse (Fig. 1(a)). Subsequent to development of this technique in Coulter counters [4], DeBlois et al. [5] measured the electrophoretic velocity of virus particles passing through polycarbonate pores, perhaps the first indication that RPS could also be used for particle charge measurement. Nanoparticle \( \zeta \)-potentials have since been inferred from individual duration measurements [6, 7]. More recent \( \zeta \)-potential measurements [8–11] have considered resistive pulses in much greater detail, accounting for multiple particle transport mechanisms and conical pore geometry with end effects. Here we present a detailed study and comparison of RPS-based \( \zeta \)-potential measurement methods in which the rate or duration of resistive pulses is measured as a function of applied pressure, with a view to optimising such techniques.

Our experiments employ a variant of RPS known as tunable RPS (TRPS), in which the sensing pore is within an elastomeric membrane, which enables
nanoscale ‘tuning’ of the pore geometry by the symmetric application of macroscopic stretch [11–14]. Tuning may be used, for example, to optimise the signal-to-noise ratio [14], to sterically gate larger particles as the pore diameter is reduced [14, 15], or to employ a single pore to investigate a wide range of nanoparticle sizes [16]. Apart from charge, TRPS can be used to measure nanoparticle size [17] and concentration [18]. Resistive pulse asymmetry [19] and detection of aggregates or clusters [20] have been studied in detail. The range of particles analysed using TRPS now includes dextran particles [21], emulsions [9], liposomes and lyophilisomes [22–24], viruses and bacteria [17, 25, 26], protein aggregates [27], exosomes and membrane vesicles [28–31], expansile nanoparticles [16], magnetic beads [20, 32] and functionalised gold nanoparticles [33]. DNA has been studied as both single molecules [15] and on-bead [14, 21, 34, 35].

In this study, the ζ-potential of a particle (ζ\text{particle}) is measured by determining its electrophoretic mobility in the Smoluchowski approximation, in which particle size is much greater than the Debye length. Based on the Nernst-Planck equation, significant contributions to particle flux through a pore \( J \) can be summed as [17]

\[
\frac{J}{C} = \left( \frac{\varepsilon (\zeta_{\text{particle}} - \zeta_{\text{pore}})}{\eta} \right) E + \frac{Q_p}{A}.
\] (1)

Here \( C \) is the volume concentration of particles, \( \varepsilon \) and \( \eta \) are the fluid permittivity and viscosity, and \( \zeta_{\text{pore}} \) is the ζ-potential of the pore wall. \( E \) is the applied electric field, \( A \) is the cross-sectional area of the pore, and \( Q_p \) is the volumetric rate of pressure driven flow through the pore. Pressure-driven flows in resistive pulse sensing have recently been studied elsewhere [36, 37].
Bulk transport via diffusion is typically negligible for TRPS [18], and it is assumed that the gradient of the pore wall is shallow enough (on the length scale of a particle) that the geometry can be considered locally cylindrical.

In experiments, we apply an external pressure across the fluid cell ($P_{\text{applied}}$), and measure the value $P_0$ at which the transport mechanisms are balanced, so that there is no net motion of particles through the pore ($J = 0$), and

$$\zeta_{\text{particle}} = -\frac{\eta Q_p}{\varepsilon E A} - \zeta_{\text{pore}}.$$  \hfill (2)

To calculate $\zeta_{\text{particle}}$, a semi-analytical model has been developed ([8, 9], see Supporting Information) to incorporate specific pore geometry, and therefore to compute $E$ and $Q_p$ in terms of $P_{\text{applied}}$, the additional inherent pressure within the fluid cell ($P_{\text{inherent}}$) and applied voltage ($V$). Independent electro-osmotic flow experiments are used to characterise $\zeta_{\text{pore}}$. Pores used for TRPS exhibit conical geometry, which is modelled as indicated in Figure 1(b). The small opening radius $a$, large opening radius $b$ and length $d$ are model inputs.

Vogel et al. [8] applied this method to a range of carboxylate polystyrene nanoparticles, using TRPS with a custom built manometer to allow precise control of $P_{\text{applied}}$. Somerville et al. [9] used the same technique to measure the $\zeta$-potential of a water-in-oil emulsion, and to explore the possibility of $\zeta$-potential measurements on individual particles. Relevant data have recently been presented by Kozak et al. [11], who used essentially the same model of tunable pores in calculations pertaining to the shape of individual pulses, and by Weatherall et al. [38], who have used a similar but simpler calculation, along with calibration particles of known $\zeta$-potential in place of geometric parameters. Here, we have collected further data for particles of various...
sizes and surface charges. We aim to study the precision and accuracy of
the method used previously [8, 9], as well as three further methods which
identify $P_0$.

2. Materials and Methods

Table 1: Particle sets used in the present study. Diameters and surface charge details are
as specified by the suppliers. Bangs Laboratories calculates charge densities as described
in [39]. ‘CO-psty’ indicates carboxylated polystyrene.

<table>
<thead>
<tr>
<th>Particle Set</th>
<th>Material</th>
<th>Manufacturer</th>
<th>Diameter (nm)</th>
<th>Charge density ($\sigma$) $\text{C m}^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Polystyrene</td>
<td>Polysciences</td>
<td>200</td>
<td>n/a$^i$</td>
</tr>
<tr>
<td>B</td>
<td>CO-psty</td>
<td>Bangs</td>
<td>226</td>
<td>-0.181</td>
</tr>
<tr>
<td>C</td>
<td>CO-psty</td>
<td>Bangs</td>
<td>217</td>
<td>-0.318</td>
</tr>
<tr>
<td>D</td>
<td>CO-psty$^i$</td>
<td>Bangs</td>
<td>194</td>
<td>-0.400</td>
</tr>
<tr>
<td>E</td>
<td>CO-psty$^i$</td>
<td>Bangs</td>
<td>160</td>
<td>-0.666</td>
</tr>
</tbody>
</table>

$^i$ Particles are not carboxylated.

$^i$ Surface groups include both carboxylic and polyacrylic acids.

The five particle sets studied, summarized in Table 1, consisted of four
sets of carboxylated polystyrene (CO-psty) beads (Bangs Laboratories) and a
set of uncharged NIST traceable standards (Polysciences). All have diameter
close to 200 nm, but the nominal surface charge density ($\sigma$, determined from
titrations during manufacture) varies. Particles were suspended at concen-
trations of $10^9$-$10^{10}$ mL$^{-1}$ in a standard electrolyte buffer (SEB) consisting of
0.1 M KCl, 15 mM 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris), 0.01% v/v Triton X-100 and 3 mM ethylenediaminetetraacetic acid (EDTA), adjusted to pH 8 using HCl. Prior to TRPS measurements, particles were
dispersed by vortexing for 5 s, sonicating at high power for 30 minutes and
passing through a 0.45 μm syringe filter (Minisart, purchased from Sigma-
Aldrich) to remove any remaining aggregates. Values of $\eta = 1.002$ mPa s
and $\varepsilon = 7.1 \times 10^{-10}$ C$^2$ N$^{-1}$ m$^{-1}$ were used in calculations ([40], for water
at 293 K). The Debye length ($\lambda_D$) of SEB is $\sim 1.5$ nm, and all particles and
pores in this study have minimum dimensions in excess of 100 nm, so the
Smoluchowski approximation ($a/\lambda_D \gg 1$) is valid.

TRPS was performed using the qNano system (Izon Science, described
in detail elsewhere [8, 12, 14, 15, 17–19]) which incorporates a fluid cell,
actuation capability for membrane tuning, and customised electronics. Here,
the high-precision customised manometer coupled to the fluid cell uses the
same principle and apparatus as in [8], but with the fluid flow to and from the
reservoir now controlled using a syringe pump (Cole-Parmer model 78961OC,
precise to 0.2 mL) fitted with 2 x 25 mL syringes (Terumo). The pressure
applied by the manometer to the fluid cell ($P_{\text{applied}}$) can be controlled with
precision better than $\pm 5$ Pa (0.5 mm H$_2$O). The net pressure across the
membrane ($P_{\text{net}}$), equivalent to $P_2 - P_1$ (Fig. 1(b)), is the sum of the pressure
applied by the manometer ($P_{\text{applied}}$) and the pressure head within the cell
itself ($P_{\text{inherent}}$). When 40 μL of H$_2$O is loaded into the upper half of the
fluid cell, $P_{\text{inherent}}$ is 46 Pa (4.7 mm H$_2$O) [8].

Pore specimens are produced in thermoplastic polyurethane (TPU) by
mechanically puncturing a membrane using a chemically-etched tungsten needle attached to an actuator [15]. Experiments were performed using a pore specimen designated 'NP200' by the manufacturer (Izon Science), and therefore most suitable for measurement of 200 nm particles. The stretch applied to the membrane was the same in all experiments so that any changes in pore geometry were minimised [12]. To further mitigate possible geometric changes or partial blockages, data were only collected when the baseline current was within 10% of the average observed across all experiments. Based on measurements using SEM, optical microscopy and a micrometer (see Supporting Information), pore opening sizes (Fig. 1(b)) of $a = 184 \pm 20$ nm and $b = 22.5 \pm 0.5$ µm were used in calculations, with a stretched membrane thickness of $d = 179 \pm 7$ µm. Uncertainties primarily arise from measurement resolution, variable application of stretch, and geometric non-idealities. The uncertainty in absolute ζ-potential values due to pore geometry is $\sim 30\%$, comparable to previous work [8]. This uncertainty applies to absolute values of ζ_{particle}, but not to comparative differences between particle sets measured using the same pore. Pulses were identified and analysed using the qNano system’s proprietary software (v 2.2).

To find ζ_{pore}, electro-osmotic flow (EOF) measurements were performed in microchannels that were custom-synthesized in pieces of the TPU used to make pores (BASF Elastollan 1160D) using soft lithography. A laser direct writer (Microtech 405A) produced a master channel (0.022 mm x 0.1 mm x 30 mm) in photoresist (MicroChem SU-8 2015) as described in [41]. Channels were made using thermal embossing rather than using a bonding agent (as used in [8]), which may chemically react with the polymer surface. EOF
measurements were performed using the current monitoring method [8, 42]. Channels were filled with SEB and a potential of 500 V was applied along the channel length using silver electrodes. The value of $\zeta_{pore}$ used in calculations was $-11.4 \text{ mV}$, equal to the mean of 10 repeated measurements with the same microchannel, with a standard deviation of $2.2 \text{ mV}$. After geometry, $\zeta_{pore}$ generates the second greatest uncertainty for $\zeta_{particle}$ measurements. Details of the embossing and results for variable KCl concentration are included in the Supporting Information.

Comparative $\zeta$-potential measurements were made using phase analysis light scattering (PALS) with a Zetasizer Nano (Malvern). Immediately prior to PALS measurements, particles suspended in SEB were sonicated for 5 minutes and passed through a 0.45 $\mu$m syringe filter to remove aggregates. Each measurement was the mean value of 5 consecutive $\zeta$-potential readings. Following each set of 5 readings, the fluid cell was rinsed with deionised water and reloaded with suspended particles. 3 of these measurements (15 readings) were completed for each particle set. To prevent electrode oxidation, each disposable fluid cell was replaced after 5 measurements (25 readings).

2.1. Methods for Finding the $\zeta$-Potential

Four methods were used to analyse TRPS data for particle $\zeta$-potential measurement. In all methods, the strategy is to identify $P_0$ by collecting resistive pulse data while controlling $P_{\text{applied}}$, the pressure applied to the fluid cell. $P_{\text{applied}}$ can be varied continuously or in discrete steps. Exemplar results from each method are presented in Fig. 2. The four methods involve measurement of (a) continuous rate, (b) discrete rate, (c) continuous duration and (d) discrete duration.
The continuous rate method has been described and used previously [8, 9]. $P_{\text{applied}}$ was continuously varied between +500 Pa and -500 Pa, ensuring that resistive pulses were recorded in distinct regimes dominated by pressure-driven flow, and by electrokinetics. The pressure was varied at 1.5 Pa s$^{-1}$, ensuring that a large number of pulses was counted and the chance of a pore blockage during a measurement remained relatively low. $P_0$ is identified as the pressure at which the net flow of particles through the pore is minimised. Figure 2(a) shows the cumulative pulse count with increasing $P_{\text{applied}}$, producing an ‘S’-shaped curve, and $P_0$ is determined by calculating the stationary point of a least-squares cubic fit to this curve. When the polarity of the applied electric field is switched, $P_0$ changes because the direction of net electrokinetic particle transport changes - the sign of $E$ changes in Eq. 2. $P_0$ has been found using both continuously increasing and decreasing $P_{\text{applied}}$.

The discrete rate method (Fig. 2(b)) also identifies $P_0$ as the pressure at which the minimum pulse rate occurs. In this case, the minimum is found by fitting a parabola to discrete rate data. The impact of possible pore blockages and the required measurement time are both reduced in comparison to the continuous rate method. $P_{\text{applied}}$ was varied between +500 Pa and -500 Pa in steps of 49 Pa (5 mm H$_2$O). Over 500 events were recorded at each $P_{\text{applied}}$ over a period of at least 30 s. Each measurement was visually inspected to ensure that the rate was near-constant throughout the collection period, as large deviations typically indicate a pore blockage.

The continuous duration method uses the full width half maximum (FWHM) duration of pulse peaks to indicate the speed at which particles move through the pore. $P_0$ is identified as the pressure at which the average FWHM is max-
imised due to the balance between the electrokinetic and pressure-driven transport. The maximum duration is calculated by least squares fitting a Gaussian function to FWHM data, obtained with pressure varied in the same way as for the continuous rate method (above). A Gaussian function is used because it is simple, symmetric about $P_0$, and accurately represents the single-peak data obtained. Due to outliers (discussed further below), each data point in Fig. 2(c) represents the mean of 5 consecutive FWHM measurements. In the discrete duration method (Fig. 2(d)), data are collected at discrete $P_{\text{applied}}$ values using the same regime as the discrete rate method. As with the continuous duration method, the mean of a Gaussian fitted to the data yields a measurement of $P_0$.

3. Results and Discussion

3.1. Pulse Rate Methods

$P_0$ data obtained using the continuous and discrete rate methods are shown in Figure 3(a). Measurements were performed at both $V_0 = +0.5$ and $-0.5$ V, and corresponding values of $P_0$ are separated by the horizontal line corresponding to $-P_{\text{inherent}}$, equivalent to $P_{\text{net}} = 0$. For typical values of $\zeta_{\text{particle}}$ and $\zeta_{\text{pore}}$ in these experiments, the electro-osmotic and electrophoretic transport mechanisms drive particles in opposite directions, but electrophoresis is larger. With positive applied voltage, particles are electrophoretically driven towards the lower half of the fluid cell (Fig. 1(b)), so the opposing pressure required for $\mathbf{J} = 0$ is negative. In the continuous case, each data point represents two experiments, in which $P_{\text{applied}}$ was either increased or decreased over time. The typical variation in $P_0$ between these cases was less
than 5%, and systematic variation is removed by plotting the average of the
two values.

All data sets show a monotonic trend with respect to nominal surface
charge for the three data sets at $\sigma \geq -0.32 \text{ C m}^{-2}$ (i.e. less negative than
$-0.32 \text{ C m}^{-2}$). The trend extends more weakly to particle set D ($\sigma =
-0.40 \text{ C m}^{-2}$). Particle set E ($\sigma = -0.67 \text{ C m}^{-2}$) is exceptional, gener-
ating widely varied $P_0$ measurements and resulting $\zeta$-potential values. These
observations, further discussed in Section 3.4, can be partly attributed to
the use of polyacrylic acid (in addition to carboxylate groups) to function-
alise set E. Comparing continuous and discrete rate measurements, the trend
with respect to surface charge is identical for $\sigma \geq -0.40 \text{ C m}^{-2}$. However, ab-
solute values of $P_0 - P_{inherent}$ are consistently smaller for the discrete rate
measurements.

Figure 3(b) plots $\zeta$-potentials calculated from $P_0$ measurements in Fig. 3(a)
using Eq. 2 and experimental inputs from Section 2. The calculation accounts
for the polarity of $V_0$, so measurements at $\pm 0.5 \text{ V}$ are treated as repeats. The
variability in these measurements is greatest for particle set E, giving unreli-
able data, and smallest for sets A and B. Consistent with Fig. 3(a), discrete
measurements give lower absolute values of $\zeta_{\text{particle}}$.

Data obtained using PALS (Fig. 3(b)) agree with the TRPS data. Ig-
noring particle set E, the average PALS value is close to the discrete and
continuous results, and consistently lies between them. The continuous rate
data are within experimental uncertainty of the PALS data, with maximum
differences of 4.6 mV (absolute) and 24% (fractional) across sets A-D. The
equivalent maximum differences for the discrete rate data are 8.3 mV and
It is notable that $|\zeta_{\text{particle}}|$ is lower for particle set D than for set C in three of the four data sets plotted. The exception to this trend [8] used the same particle sets (A-D) in experiments.

The two key advantages of the rate methods are, firstly, that considerable data obtained over a wide range of conditions are brought to bear on the task of finding $P_0$, and secondly, that pulses do not need to be further analysed once they have been identified. The primary difficulty with rate methods is that pulses recorded within $\sim 50$ Pa of $P_0$ are often non-ideal (further discussed in Section 3.4), and identification of $P_0$ is strongly dependent on these pulses. Previously [8] these issues have been partially mitigated by discarding events within 50 Pa of $P_0$ prior to fitting.

In general, the continuous rate method offers more precision than the discrete rate method. The latter method involves a trade-off between precision and time per measurement, which is dependent upon the discrete step size. The discrete raw data (Fig. 2) are smoother near $P_0$, but the parabolic fit has uncertainty on a similar scale to the step size. The discrete method also has advantages, namely that it is less vulnerable to spurious pulses near $P_0$, it is not terminally interrupted when a pore blockage occurs, and it does not require pressure changes in chronological sequence. For the continuous case, the latter requirement can be mitigated by checking and averaging results for increasing and decreasing pressures.

3.2. Duration Methods

Measurements of $P_0$ using duration methods are summarized in Fig. 4(a). There are broad similarities to data obtained using the rate methods, such as the division of $P_0$ values for different polarity of $V_0$ about the horizontal
line equivalent to \( P_{\text{net}} = 0 \). Again, \( P_0 \) monotonically increases with \( \sigma \) at low absolute values, including particle set D \((\sigma = -0.4 \ \text{C m}^{-2}\)) , although data for set E is again inconsistent. In contrast with Fig. 3(a), there is no clear systematic difference (and indeed very good agreement) between discrete and continuous data. Overall, \(|P_0 - P_{\text{inherent}}|\) data are smaller than those produced by the rate methods, and it is notable that these values are greater at positive rather than negative values of \( V_0 \). These trends are further explored in Section 3.4.

Calculated \( \zeta \)-potentials with measurements at \( \pm 0.5 \ \text{V} \) treated as repeats (Fig. 4(b)) yield a monotonic relationship between \( \sigma \) and \( \zeta_{\text{particle}} \) other than for particle set E. The relatively large uncertainty in \( \zeta \)-potentials for particle sets C and D relative to sets A and B is a feature of both Figs. 3(b) and 4(b). For these particle sets, \( \zeta_{\text{particle}} \) values may have high dispersity, or random measurement uncertainty may be relatively large for the specific measurement parameters (including \( \sigma \)) used here.

The maximum difference between duration data and the corresponding PALS data is 7.0 mV (absolute) or 25% (fractional) for sets A-D. Across these four particle sets and all four methods (i.e. 16 measurements), the average difference between TRPS and PALS \( \zeta_{\text{particle}} \) values was 3.4 mV (absolute) or 15% (fractional). The average uncertainty attributed to repeated measurement was 10% for the TRPS methods and 11% for PALS. PALS and rate measurements (Fig. 3(b)) indicated a higher absolute \( \zeta \)-potential for particle set C than for set D, suggesting that values of \( \sigma \) (manufacturer-specified) and \( \zeta_{\text{particle}} \) may not be monotonically related. This trend was not observed in Fig. 4(b), although as with the rate data, PALS results are consistent with
duration data for all particle sets A-D.

Overall, duration measurements of $\zeta_{\text{particle}}$ are more reproducible than measurements using pulse rates. Figures 3(b) and 4(b) each contain data for six individual measurements using each particle set (four measurements for set B). When each set of six is treated as repeats, all five particle sets have a lower coefficient of variation (equivalent to standard deviation as a fraction of the mean) in the case of duration measurements.

As with the rate methods, the discrete duration method usually requires less measurement time than the continuous method, but affords less precision depending on the discretization. Although pulses close to $P_0$ are again problematic (naively, the FWHM tends to $\infty$ at $P_0$), each pulse is analysed more closely than for the rate methods, resulting in less uncertainty. Nevertheless, the Gaussian fit can be significantly affected by individual events, and indeed it is prudent to partially discretise the continuous data by averaging five consecutive individual events for each data point (Fig. 2). FWHM pulse durations do not change greatly with pulse magnitude, the latter being proportional to particle volume [17]. For example, for a sample of set C particles the pulse FWHM varied by $< 14\%$ between the largest (1.3 nA) and smallest (0.5 nA) pulses, corresponding to $< 30\%$ variation in particle diameter. The range of mean particle diameters for the sets used here was $\sim 40\%$ (Table 1). This could partly account for the low $P_0$ value for particle set E.

3.3. Comparison of Methods

Figure 5 summarizes $\zeta$-potential measurements using TRPS, plotting the difference between the value for each method and the mean value over all
methods. Duration measurements, both continuous and discrete, are always within ±∼5 mV of the mean, further indicating that duration measurements produce more precise (self consistent) results than event rate methods. Occasional variations closer to 10 mV are obtained using the rate methods, especially for more highly charged particles. For particles of unknown charge, there is clear advantage in taking measurements using multiple TRPS techniques to check for self consistency, and to avoid measurements such as those found to be characteristic of highly charged particles here.

Some of the trends observed in Figs. 3 and 4 are further evident in Fig. 5. The continuous and discrete rate data lie either side of the mean in all cases, due to the consistently lower absolute values of $\zeta_{\text{particle}}$ derived from discrete measurements. The continuous rate data point for particle set C appears here to be an outlier with low reproducibility. This demonstrates how the inconsistent trend observed for sets C and D in Fig. 3, discussed above and supported by PALS data, could originate from measurement uncertainty rather than from characteristics of the actual particle distribution. The possibility of measurement error in the nominal charge densities (Table 1) should also be noted.

Although the rate methods depend on pulses near $P_0$ (Section 3.1), the analysis required for these methods is relatively facile, requiring only accurate identification of each event. In comparison, duration measurements employ a detailed analysis of each individual event. This provides advantages, such as the ability to discard individual events if they are considered to be outliers, and the possibility to extract more information from each individual event. Indeed, $\zeta_{\text{potential}}$ can in principle be calculated from a single event, without
variable pressure [11]. However, the model is designed for an ideal particle travelling smoothly along the central pore axis, and so for individual particle charge measurements factors such as off-axis trajectory, steric interactions and polydispersity should be considered. The comparison between continuous and discrete methods can be summarized by noting that the precision of discrete data is limited by the discrete step size, but that discretization allows flexibility over the step size as well as timing and quality control of measurements.

3.4. Highly Charged Particles

In Section 3.1, the high variability of $P_0$ data and derived $\zeta$-potentials for particle set E (Figs. 3 and 4) was partly attributed to differences in functional groups. In addition, set E has the highest nominal charge of the particle sets used (Table 1), which may give rise to complications due to competing flow effects near the pore constriction, at the smaller opening (Fig. 1(b)). Figure 6 employs the semi-analytic model used above for $\zeta$-potential calculations to plot the relative contributions to particle transport for a specific set of experimental parameters. Due to the differences between electrokinetic and pressure driven flows, the dominant transport mechanism can switch within (say) a few tens of nanometers of the geometric discontinuity at the pore opening, causing the particle flux to change sign. Additionally, particle transport varies across the width of the pore [32]. The pressure-driven flow profile is approximately parabolic (as in Poiseuille flow), while electro-osmosis is nearly a plug flow, and electrophoresis depends on the electric field geometry. This consideration of transport details reveals that complexities in particle transport will not be captured by Eqs. 1 and 2.
Figure 6 shows that particle transport can be asymmetric about the pore constriction. In this example, any on-axis particle near the small pore opening (on either side) will be transported away from the pore, potentially creating a region of depleted particle concentration around the pore opening. Particles approaching the constriction from above the membrane move differently to those moving from within the pore. This asymmetry could explain differences between \( P_0 \) magnitudes (relative to \( P_{\text{inherent}} \)) for positive and negative applied voltages, which are especially evident for duration method data.

Perhaps more importantly, competing mechanisms produce a higher likelihood of abnormal resistive pulses near \( P_0 \) due to steric or Brownian mechanisms. Abnormal pulses (see Supporting Information for an example) can be generally characterized as those caused by particle lingering near the pore, perhaps passing through multiple times, rather than cleanly passing through. In our experiments, competition between pressure-driven and electrokinetic transport is increased when particles have high charge. There is also greater range of \( P_{\text{applied}} \) at which abnormal pulses were observed, although this could also be caused by relatively high polydispersity. As demonstrated in Figure 7, fitting of a cubic to the continuous rate method can be uncertain under these conditions, with multiple possible inflection points observed. The accuracy of \( P_0 \) measurement is similarly uncertain for particle set E.

Possible differences between \( P_0 \) for the cases of \( J = 0 \) and maximised duration could explain why \( \zeta \)-potentials are consistently smaller when obtained by the duration methods, in comparison with the rate methods. As identified in rate experiments, \( P_0 \) is the pressure at which the number of particles passing through the constriction is minimised. This may differ from
the pressure at which the average particle flux is zero, because it is possible for particles to be moving through the pore in both directions, promoted by transport variation across the pore width. As for maximised duration, the dominant transport mechanism acting on a particle can vary as it moves along the z-axis on length scales comparable to the size of the particle.

This Section reveals clear directions for future improvement of TRPS-based particle charge measurement methods. A significant step would be to establish a process to identify (and discard) abnormal pulses, and draw upon event asymmetry [19] to establish the direction of particle motion through the pore. Research into the importance of precise pore geometry and competition between transport mechanisms will be ongoing. The convergence of the electric field at the pore constriction may give rise to significant DC dielectrophoresis. Further work is required to establish the working range of particle charge measurements for pores of different sizes and in different electrolytes.

4. Conclusion

We have studied variable pressure methods for ζ-potential measurement using TRPS. Measurements are comparable to PALS data between zero surface charge and -0.4 C m⁻², both in terms of absolute values and repeatability. Use of tunable pores enables multiple measurement and analysis methods, an advantage over light scattering. Key advances in this paper include introduction and comparison of four relatively simple analysis techniques, improved experimental control, and more rigorous determination of ζ_pore (a key parameter for ζ_particle calculation). Our results are more reproducible.
when calculations are based on FWHM duration data than when rate data
are used. Collection of data over a range of experimental conditions has
ensured that uncertainties are clear. Uncertainty increased for more highly
charged particles due to competing electrokinetics and pressure about the
pore opening. Future work should focus on measurement uncertainties due
to pore geometry and surface charge parameters used in calculations. TRPS
charge measurements could also be extended to a wider range of particles.
Transport of larger particles is readily dominated by pressure-driven flows,
so low-conductivity electrolyte and modified electronics should be used to
allow the application of larger voltages necessary to measure electrokinetic
transport.

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Figure 1: (a) shows typical tunable resistive pulse events. Each downwards pulse in current from the baseline level indicates that a 200 nm carboxylated polystyrene sphere (from set C, Table 1) has passed through the pore constriction. Inset, an expansion of the red outlined region. Events are asymmetric because pores are near-conical in shape. (b) is a schematic section of a tunable pore, showing the truncated conical pore geometry with small and large pore openings $a$ and $b$ respectively, the membrane thickness $d$, and the cylindrical polar co-ordinates $(r, z)$. The net pressure across the membrane is $P_{\text{net}} = P_2 - P_1$. 
Figure 2: Indicative results for particle set C (Table 1), demonstrating analysis methods. Vertical dotted lines indicate derived values of $P_0$. (a) and (b) show data obtained from continuous and discrete particle rate measurements respectively. Cumulative data in (a) are normalized by the total pulse count in each run. (c) and (d) show data from continuous and discrete measurements of full width half maximum (FWHM) duration respectively. Each data point for ‘discrete’ cases is the average of at least 500 pulses. Error bars in (d) indicate the interquartile range around the median FWHM.
Figure 3: Data obtained using rate methods for particle sets A-E (Table 1). Particle set labels appear directly above the relevant data in (a), which plots applied pressure at minimum pulse rate ($P_0$) as a function of nominal surface charge density ($\sigma$). The horizontal black dotted line indicates $-P_{\text{inherent}}$. Error bars for continuous experiments indicate the range spanned in cases of increasing and decreasing pressure. (b) $\zeta$-potentials calculated from the rate data. For continuous experiments, error bars indicate the standard deviation of four contributing data points; for discrete experiments, error bars indicate the range spanned by the two values at +0.5 V and -0.5 V (see Fig. 3(a)). Data from Vogel et al. [8] (green squares) were obtained using the continuous rate method. Mean values obtained using PALS are plotted in (b), with error bars ($\pm 5$ mV maximum) omitted for clarity. In both (a) and (b), error bars smaller than symbol size are omitted.
Figure 4: Data obtained using duration methods for particle sets A-E (Table 1). (a) Applied pressure at maximum pulse duration ($P_0$) as a function of nominal surface charge density ($\sigma$). (b) Corresponding $\zeta$-potential data. Error bars and the horizontal black dotted line are as described for Fig. 3, with error bars for PALS ($\pm 5$ mV maximum) and those smaller than the symbol size omitted for clarity.

Figure 5: Summary of all $\zeta$-potential data, plotted as a deviation from the mean over all methods for each particle set. Error bars correspond to those plotted in Figs. 3(b) and 4(b), and are omitted if smaller than the symbol size.
Figure 6: Example of simulated particle velocity components in which the direction of transport reverses at the pore constriction. The simulation is based on Eq. 1 using the following parameters: $P_{net} = -30$ Pa, $V_0 = 0.5$ V, particle radius 100 nm, $a = 450$ nm, $b = 46$ μm, $\zeta_{pore} = -12$ mV, $\zeta_{particle} = -30$ mV.

Figure 7: Example results using particle set E (Table 1). Vertical dotted lines indicate $P_0$, determined as described in Section 2.
References


Graphical Abstract

Balanced

V wins

P wins

Pulse Count

ΔP
Highlights:

- 3 new ways to find zeta potentials using tunable resistive pulse sensing (TRPS).
- Comparative measurements using 5 particle sets, 4 TRPS methods and light scattering.
- Values and reproducibility are comparable to the standard light scattering method.
- Significantly developed assessment of uncertainty relative to previous TPRS work.