FUNDAMENTALS OF TUNABLE RESISTIVE PULSE SENSING (TRPS)

THEORY MANUAL

IZON

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1 / FUNDAMENTALS OF TRPS

Tunable Resistive Pulse Sensing (TRPS) enables the physical characterisation of nanoparticles suspended in electrolyte; specifically, TRPS is capable of measuring particle size, concentration, and zeta potential. It is the only technology that can deliver the concentration of particles in fluid as a number per volume across a specified detectable size range, as well as calculate effective surface charge of individual nanoparticles.

TRPS technology uses the Coulter principle on the nanoscale. Voltage is applied across a fluid cell via silver/silver chloride (Ag/AgCl) electrodes, creating a baseline current due to the flow of electrolytic ions (**Figure 1**). A temporary decrease in current is detected as particles pass through the nanopore, which allows for the analysis of particles suspended in electrolyte.
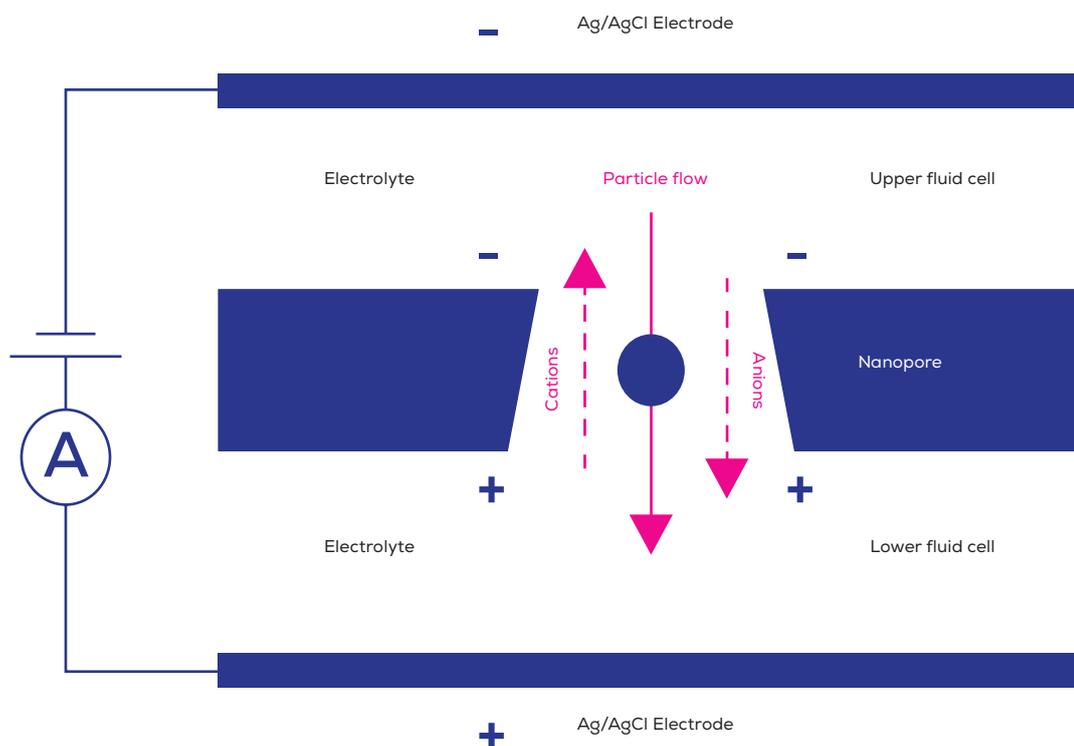

Figure 1: A schematic representation of Tunable Resistive Pulse Sensing (TRPS), showing the relative position of the nanopore, electrodes and fluid cells, and the direction of ion and particle flow.

2 / CALIBRATING DATA

2.1 Size and Concentration Measurements

Sample particles are driven through the nanopore by applying a combination of voltage and pressure, and each particle translocation event causes a resistive pulse or "blockade" signal that is detected and measured by the application software as displayed in **Figure 2**. By comparing sample particles to calibration standards with a known size and concentration, accurate size and concentration data can be obtained.

- Blockade magnitude is directly proportional to the particle diameter.
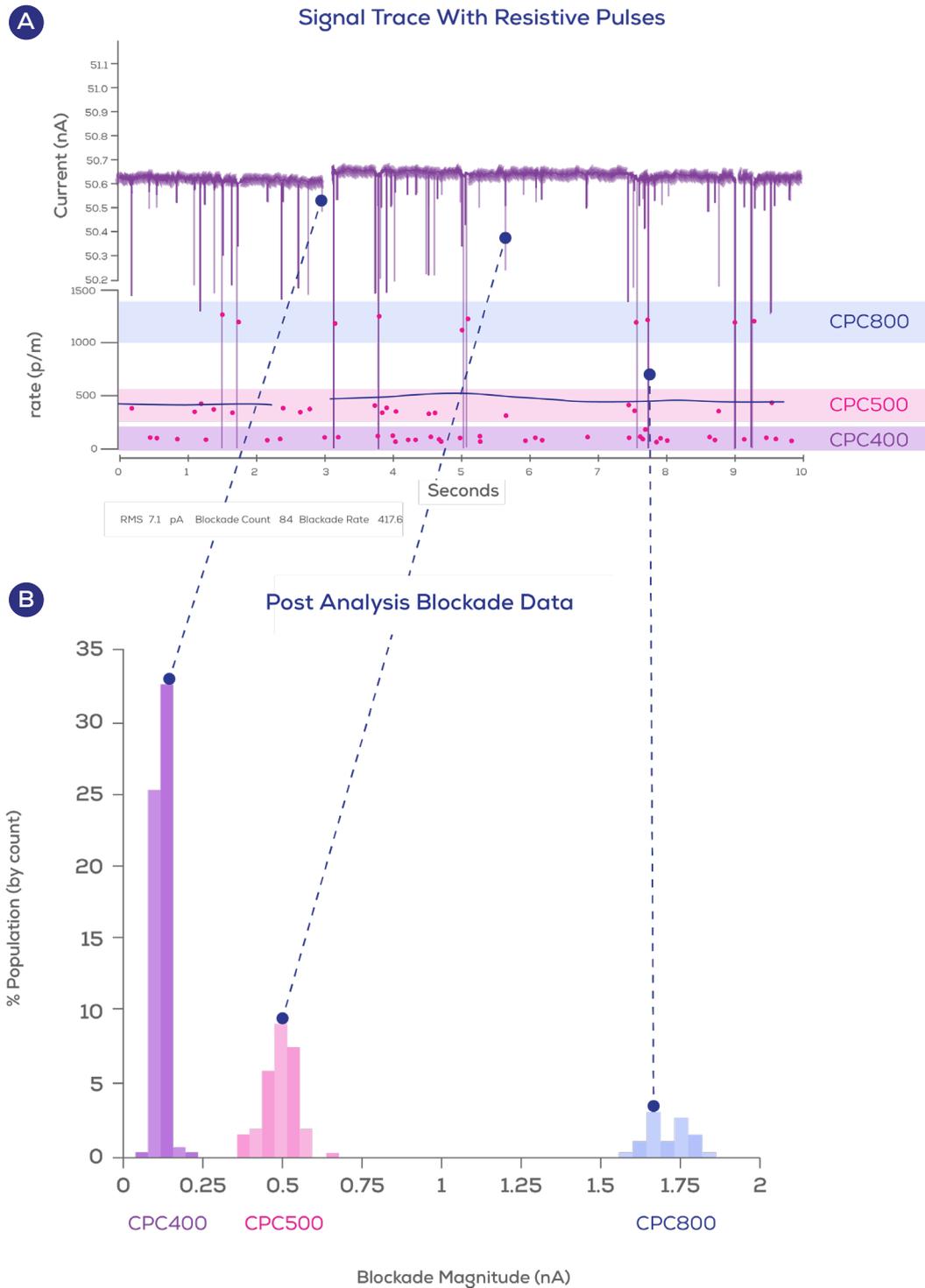- Blockade frequency is used to determine particle concentration.

Figure 2: Magnitude and frequency measurements are converted into particle size and concentration by calibrating with particles of known size and concentration. A) Signal trace with resistive pulses. Each particle is represented by a blockade (purple line extending from the signal trace). Each pink dot represents a blockade event. B) Post analysis blockade data.

Calibrating Particle Size

As the software records blockades in nA, calibration particles of a known size are used to convert blockade magnitude (nA) into a diameter (nm).
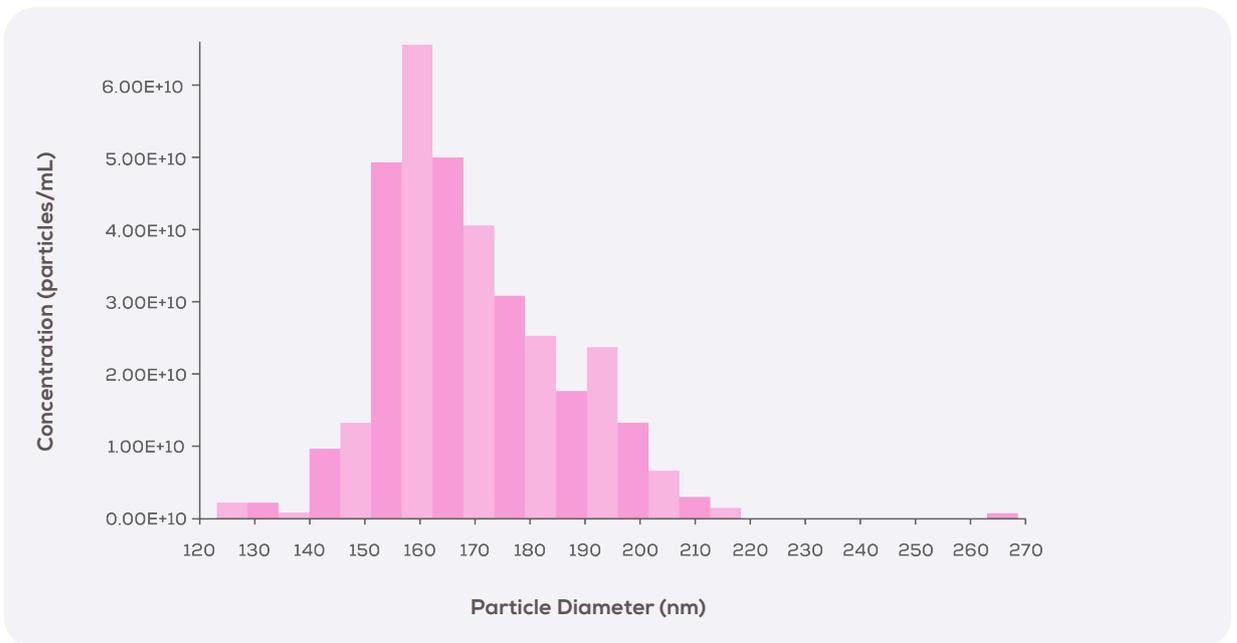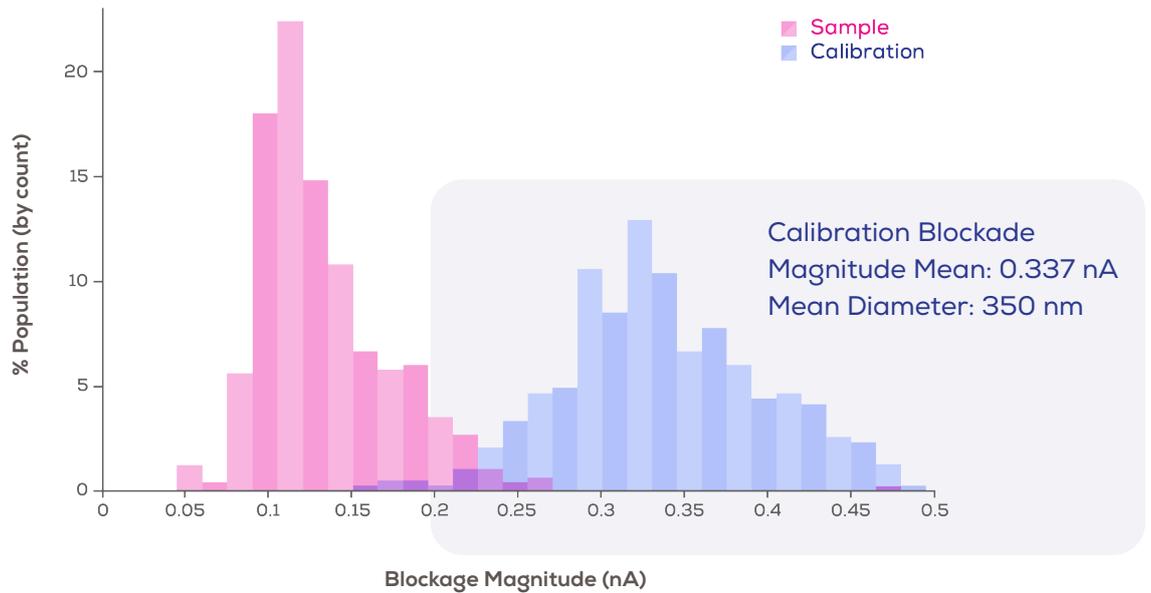

Calibration Blockade Magnitude Mean: 0.337 nA
Mean Diameter: 350 nm

Figure 3: The Izon Data Suite converts blockade magnitude into particle diameter by calibrating with particles of a known size.

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Blockade magnitude is proportional to the volume of the particle passing through the nanopore, giving very high resolution of spherical particle diameter. For each sample particle passing through the nanopore, the following equation is used to calculate particle volume (where ΔI = change in current):

$$\text{Particle Volume} = \frac{\Delta I \text{ (sample)}}{\Delta I \text{ (Calibration, mean)}} \times \text{Calibration volume (mean)}$$

Calibrating Particle Concentration

Particle concentration is proportional to the change in blockade rate per unit of applied pressure. Accurate concentration values are derived from between one and three different pressure steps (depending on nanopore size) using calibration particles of a known concentration.
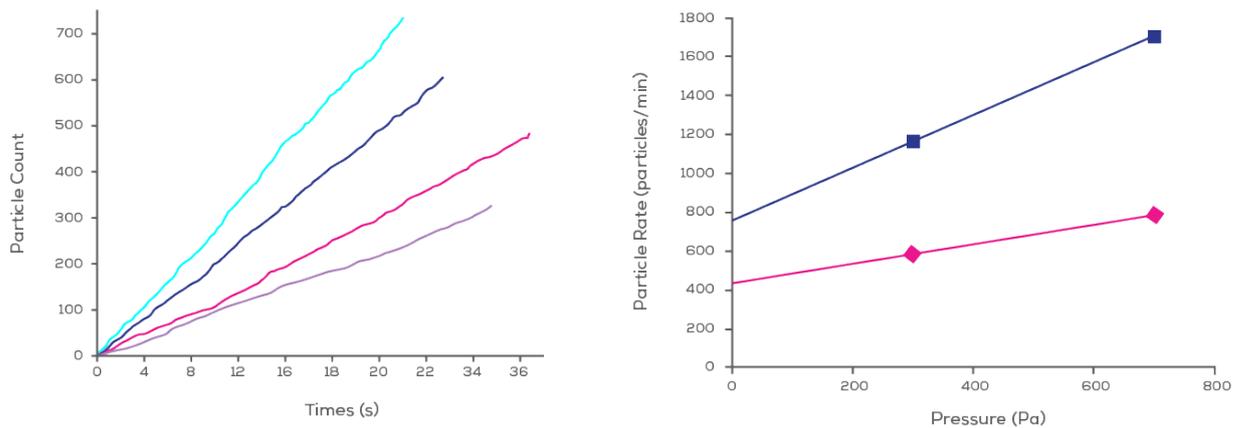

Figure 4: For each particle count measurement, particle rate is calculated and plotted against pressure. Left: Number of particles counted over time (s) for calibration particles (light and dark blue lines) and samples (purple and pink lines). Right: Particle rate (particles/minute) versus pressure (Pa). The gradient produced by plotting particle rate against applied pressure is used to calculate sample concentration. The observed particle rate should increase linearly with applied pressure.

This rate plot is used to check for system stability. This is crucial as the concentration value is measured by comparing the gradient of the rate plot of sample to calibration measurements.

$$\text{Sample concentration} = \text{Calibration concentration} \times \frac{\text{Sample gradient}}{\text{Calibration gradient}}$$

If concentration is calculated at a single pressure the software will calculate a gradient based on the fitted line passing through the origin. This can introduce significant errors, especially with smaller nanopores (NP300 and below) where a large proportion of particles are driven through the nanopore due to applied voltage. For this reason, multiple pressure recordings are recommended for NP300s and smaller while single pressure measurements are acceptable for NP400s and larger.

2.2 Size and Zeta Potential Measurements

The speed at which a particle passes through a nanopore is reflective of its zeta potential. Here, the blockade duration is used as part of the calculation. The blockade duration is measured by the Full Width Half Maximum (FWHM) duration, which is the width (ms) measured at half of the maximum value of the blockade magnitude.
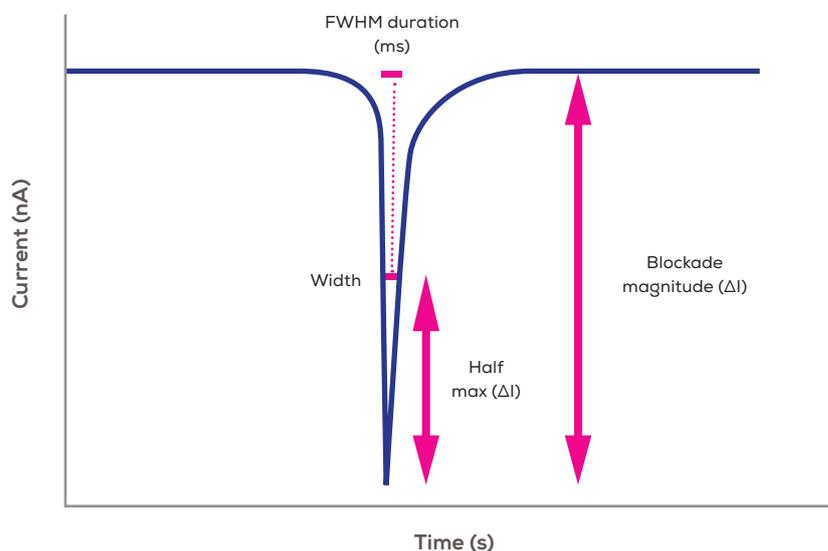

Figure 5: Components of a blockade. Blockade Full Width Half Maximum (FWHM) duration values are converted into particle zeta potential values by calibrating with particles of known size and zeta potential. The FWHM is shown as the horizontal pink bar in the centre of the blockade above. ΔI = change in current, nA.

A particle with a large negative surface charge will be strongly attracted towards the positively charged electrode in the lower fluid cell and will travel through the nanopore at a greater speed than a more neutral particle. In contrast, a particle with a small negative surface charge will be less attracted towards the positively charged electrode and will travel through the nanopore at a slower speed.

Because applied pressure has an effect on the relative particle speed, users should only use minimal pressure, if any, for high quality zeta potential measurements. This is because the convective force from the Automatic Pressure System (APS) will be much greater than any differences in speed due to particle surface charge and electrophoresis.

3 / FORCES IN THE NANOPORE

1. Convective Force: Pressure-dependent force. There is always a static pressure head due to gravitational force on the fluid. An additional positive or negative pressure can be applied to the system using the APS. Convective forces tend to dominate particle velocity in larger nanopore systems.

2. Electrophoresis: Voltage-dependent force. Electrophoretic mobility relates to the movement of charged nanoparticles through an electrolyte solution towards an oppositely charged electrode. It is proportional to particle surface charge (ζ-potential) and the applied voltage. Electrophoretic forces can dominate particle velocity in small nanopore systems when the applied voltage is increased.

3. Electro-osmosis: Voltage-dependent force. Electro-osmosis relates to the fluid flow caused by currents of solvated ions moving along the surface of the nanopore. It is proportional to the nanopore surface charge (ζ-potential) and the applied voltage. Electro-osmosis is stronger in some nanopore systems (non-zeta capable nanopores), but weak in others (zeta capable nanopores).
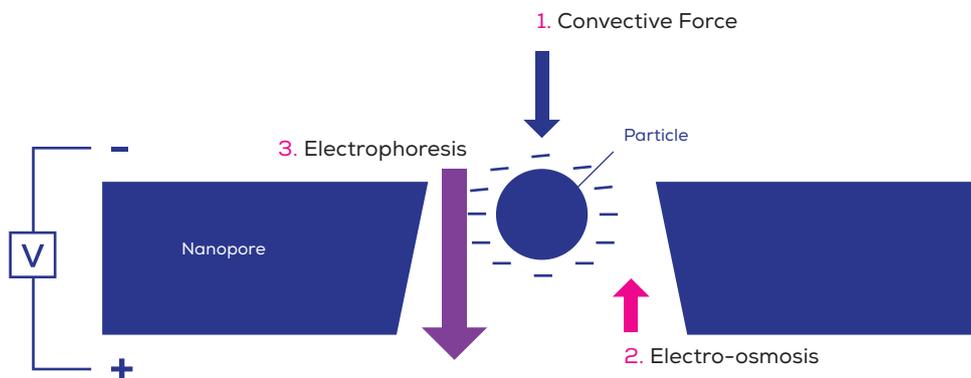

Figure 6: Main forces involved in a nanopore system.