Electrochemical sensing with nanopores

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Abstract

We discuss representative electrochemical nanopore sensing strategies, highlighting their underlying theoretical principles, and limitations.

Keywords
Nanopore sensor, resistive pulse sensing, Coulter counting, stochastic sensing

1. Introduction

The use of nanopores for chemical sensing generally narrows down to nanoporous membranes with straight-through pores of uniform size distribution and ultimately to single nanopore membranes. To understand what makes nanopores so unique in terms of their use for chemical sensing we must consider the extremely small volume defined by their interior. Thus species translocating or residing within a nanopore can effectively change the physical-chemical properties of the nanopore interior (e.g., conductance [1, 2] or refractive index [3]), which can be detected in a label-free manner. By having a single nanopore with a volume comparable to that of the targeted species, detection of single species becomes feasible. The use of nanopores for electrochemical sensing originates in the Coulter counter, best known for blood-cell counting in hematology [4]. However, the instrumentation and implementation of biological nanopores additionally benefited from studies on biological ion channels [5, 6]. Conventional Coulter counters use a single cylindrical pore to count and size particles suspended in an electrolyte. Pulsewise changes in the pore conductance are detected as
insulating particles passing through replace their own volume of highly conducting electrolyte 
(Fig. 1). The analytical information from a resistive pulse sensing (RPS) measurement is the 
pulse height (indicative of the volume of the target), pulse frequency (proportional to target 
concentration) and pulse length (depends on the mean translocation velocity and relative lengths 
of the pore and the target species).

A major strength of the method is the ability to determine particle concentration in a 
calibration-less manner by relating the number of pulses to the known volume of suspension 
flown through the pore. The classical apparatus detect species of ca. 2 to 60 % of the pore 
diameter [8] and since the smallest pore diameter is 10 µm the lower size limit of the assessable 
species is a few hundred nanometers. The reduction of the pore size is an obvious way to extend 
the applicability of the Coulter principle to species with characteristic dimensions in the lower 
nanometer range, e.g., nanoparticles of synthetic or biological origin, and macromolecules. 
However, such a scaling down proved to involve essential changes compared to micropores and 
to enable new detection methodologies.

2. Resistive pulse sensing with single nanopores

While in case of micropores the dominant transport form is the pressure driven flow through 
the pore, the volume flow rates established through nanopores are orders of magnitude smaller 
and therefore less efficient. Additionally, in case of charged species or pores the electrophoretic 
or electroosmotic contributions, respectively, should be considered. In practice, the transport 
through nanopores occurs through concurrent diffusive, hydrodynamic, electrophoretic, and 
electroosmotic mechanisms resulting in a mean translocation velocity. Generally, for larger 
diameter pores \(d>10\) nm) the dominant mechanism is the hydrodynamic transport owing to its 
quadratic dependence on the pore diameter. For \(d<10\) electrophoresis and electroosmosis 
dominate; with relative contributions depending on the surface charge density of the pore and 
the translocating species. Diffusive transport scales with \(1/d\) and becomes comparable to 
electrophoresis only for \(d<1\) nm,[9] because diffusion of smaller particles is faster while 
electrophoresis is practically independent of the pore diameter. Thus, a calibration-less 
concentration determination is challenging with nanopores unless the hydrodynamic transport 
prevails. In case of hydrodynamic transport while difficult to determine the minute volume flow 
rates experimentally, they can be calculated if the pore geometry is known [10]:
where, $P$ is the applied pressure, $\eta$ is the electrolyte dynamic viscosity, $l$ is the pore length, $d_b$ and $d_t$ are the base and tip diameters of the conical pore geometry, respectively. Thus the pore geometry, generally cylindrical or conical, clearly plays an important role in nanopore sensing, by determining the electrical resistance, the shape of the current pulses and the overall sensitivity of the detection. The uniform cross-section of cylindrical pores results in square wave pulses, while the growing cross-section in conical pores causes an asymmetric triangle-like pulse shape [11] (Fig.1).

2.1. Electrical resistance of nanopores

The general expression of the pore resistance assumes a conical pore geometry (in fact truncated cone) and homogeneous conductivity (valid at high ionic strengths):

$$R_p = \frac{1}{\sigma} \int_{x=0}^{l} \frac{1}{A(x)} \, dx = \frac{4l}{\pi d_i d_b \sigma}$$

where $\sigma$ is the electrolyte conductivity, $x$ is the coordinate along the centerline, $A(x)$ is the cross-section at position $x$, $d_b$, $d_t$, and $\alpha$ are the base and tip diameters of the truncated cone, and the half-cone angle, respectively ($d_b = d_t + 2 \tan(\alpha) l$).

Since the electric field lines gradually converge into the pore orifice, the changing cross-sections of the ion flux can contribute significantly to the overall pore resistance. This additive component is called the access resistance ($R_a$) derived first by Hall [12]:

$$R_a = \frac{1}{2d \sigma}$$

Considering $R_a$ at both openings the total resistance of a conical pore is

$$R = \frac{1}{\sigma} \left( \frac{4l}{\pi d_i d_b} + \frac{1}{2d_i d_b} + \frac{1}{2d_b} \right)$$

while for cylindrical pores ($d_t = d_b = d$), $R = \frac{4}{\sigma d \pi} \left( \frac{l}{d} + \frac{\pi}{4} \right)$. The total resistance deviates with only 3% from values simulated at $l/d=5$ using Nernst-Planck/Poisson equation, as opposed to 20% when the access resistance is unaccounted. The discrepancy is even higher for pores with lower $l/d$ ratio.
2.2. Theoretical models to estimate pulse amplitudes

During particle translocation the maximal resistance change determines the current pulse amplitude or peak height for “triangular” shaped pulses. For simplified models, such as considering uncharged pores, insulating spherical targets and translocation along the pore axis the pulse amplitude can be calculated analytically. However, in many practical cases one or more of the above assumptions is not valid and therefore numerical solutions of coupled Poisson, Nernst-Planck and Navier-Stokes differential equations [13] are used to provide the pulse amplitudes (and shapes), but at largely increased computation times.

2.2.1. Cylindrical pores

There are four main models to calculate the pulse amplitude for cylindrical pores, each with different validity region depending on the relative particle size \( \frac{d_{\text{part}}}{d} \) (Fig. 2.) [14]. The earliest model uses the equation derived by Maxwell for the effective resistivity of a suspension of insulating particles [15]. The model introduced by Gregg and Steidley treats the pore as an ideal conductor containing an insulating sphere. Homogenous electric field is assumed although the electric field lines distort around the particle resulting in an unaccounted resistive contribution. Therefore, the model approximates the pulse amplitude from below [16]. Deblois et al. assumed a “bulging” pore shape that follows the distorted electric field lines around the particle. This modified shape enables to calculate the particle containing pore resistance exactly, but underestimates the resistance of the empty pore by neglecting the electric field inhomogeneity near the bulge. Therefore, this model provides an upper limit for the pulse amplitude [1].

Anderson and Quinn [17] developed the fourth model on the analogy to the numerical calculations of Smythe, who investigated the hydrodynamic resistance change in a cylinder caused by a sphere [18]. This model has the broadest validity covering the whole practically relevant particle size range (up to \( \frac{d_{\text{part}}}{d} = 0.9 \)).

\[
\frac{\Delta R}{R} = \left( \frac{d}{d_{\text{part}}} \right)^3 - 0.8 \left( \frac{l}{d + \pi/4} \right) \\
\frac{\Delta I}{I} = -\frac{\Delta R}{R} \frac{\Delta R}{R + 1}
\]
where $\Delta R$ is the resistance change, $R$ is the resistance of the empty pore with access terms, $\Delta I$ is the pulse amplitude.

2.2.2. Conical pores
To date there is no simple analytical expression for calculation of RPS pulse amplitudes in conical pores. The main approaches to relate the pulse amplitude to the particle size and pore geometry include:
- approximation of the very end of the conical pore with a cylinder [19],
- applying the model developed by Gregg to a conical pore geometry [20],
- calibration with nanoparticles of known size and assuming that the pulse height is approximately proportional to the particle volume [21].

These approaches are valid only at sufficiently high electrolyte concentrations because the number of counter ions shielding the surface charge of the nanopore [22] or the analyte [23, 24] should remain negligible in the pore interior with respect to the free ions of the electrolyte. The resistance calculations also assume continuum media which is valid until the smallest dimension of the pore is larger than 10 nm [25].

2.3. Noise during RPS measurements
After electrical shielding RPS measurements are affected by $\Delta I_T$ thermal noise stemming from the thermal motion of charge carriers, the $\Delta I_D$ dielectric noise due to the energy dissipated by the dielectric pore substrate, the $\Delta I_A$ amplifier noise generated by the headstage and the $\Delta I_F$ flicker (or 1/f) noise, arising only when voltage is applied. [26, 27] These noise components are independent and the total noise level is:

$$\Delta I_{total} = \sqrt{\Delta I_T^2 + \Delta I_D^2 + \Delta I_A^2 + \Delta I_F^2}$$

The noise is attenuated by electrical shielding, analog/digital noise filtering and using low-noise/low-capacitance materials for the nanopore membrane. The bandwidth of the measurement can influence both the noise level and the shape of the current pulse. While a high bandwidth increases the noise, a bandwidth lower than the highest frequency component of a translocation pulse results in signal attenuation/distortion. Considering this trade-off the cut-off frequency during RPS experiments is generally 10 kHz. Commonly, thermal noise dominates when $R$ is less than ca. 10 M$\Omega$, while at ca. 100 M$\Omega$ resistance and 20 kHz cut-off frequency the amplifier and dielectric noise also become comparable. Above 100 M$\Omega$ pore resistance
usually amplifier or dielectric noise sets the total noise level while the flicker noise is typically not dominant because of signal filtering.

2.4. RPS for selective detection

Solely size and shape information are not sufficient to identify target species in a complex matrix. Therefore, selective receptors either immobilized to the nanopore environment or added to the sample solution have been used to induce target-specific changes in the RPS signal. Selective receptor is added to the sample generally to increase the size of the target species and consequently the pulse amplitudes. This principle is illustrated in Fig. 3A through selective detection of viruses by adding capsid-binding antibodies into the sample [28]. An alternative approach is to monitor the translocation of a receptor the conformation (size) of which is altered upon binding the target species. A relevant example is the detection of cocaine through the blocked translocation of the cocaine–specific aptamer, which suffers a conformation change upon cocaine binding that prohibits its translocation through the pore [23]. Using solid-state nanopores through which double-stranded DNA strands translocate, but not their complexes with restriction enzymes, allowed the identification of single-nucleotide polymorphism by detecting the increase in the threshold voltage, i.e., the minimum voltage required to drive the DNA strands through the nanopore by releasing the restriction enzyme[29].

In the simple case of having immobilized receptors that on the time scale of the analysis bind their target reversibly with 1:1 stoichiometry the pulse duration can be related to the dissociation rate constant \( k_{\text{off}} \) of the analyte-receptor complex:

\[
\Delta t = \frac{1}{k_{\text{off}}}
\]

The mean time between successive binding events is a function of both the concentration and the association rate constant \( k_{\text{on}} \) [30]:

\[
\Delta t_{\text{on}} = \frac{1}{k_{\text{on}} c}
\]

Thus, for selective stochastic sensing low affinity receptors can be used both for quantitative determination of the target as well as to determine the kinetics of single molecule binding events [31, 32]. In case of “irreversible” target binding permanent blockage events are observed [32, 33], which can be used for a “Yes-No” type identification of a given species. Quantitative detection is also feasible by measuring the mean time elapsed until the target binds to the receptor, which is inversely proportional to its concentration [33]. In the case when the single nanopore possess multiple binding sites (Fig. 3B), characteristic to receptor
functionalized solid-state nanopores, the time elapsed between the first and second binding can be used as a more convenient modality for quantitative analysis [34]. Multipore membranes can be also used for quantitative sensing, in which case a cumulated change of the membrane resistance is detected, without the possibility to differentiate single binding events [35].

2.5. Detection limit of RPS
While RPS has single species detection capability, as the signal is due to individual species translocating through the nanopore, the detection limit is in fact determined by the target throughput. At low concentrations the probability of a species encountering the pore becomes very small; the limiting situation being the undirected, Brownian motion of a single particle in a volume $V$, that requires a mean encounter time of $t_e = \frac{V}{2Dd}$ ($D$ is the diffusion coefficient of the target) [36]. Thus, in RPS the translocation frequency decreases with the analyte concentration, but for statistical analysis there is an $f_{\text{event}}^{\text{min}}$ minimal frequency that results in a practical measurement time (e.g. 100 pulses in 10 minutes). It is possible to increase the event frequency (e.g. by applying hydrostatic pressure), but at the expense of a higher translocation velocity, that shortens the duration of the current pulse. Shorter pulses than the electronic filter rise time $\tau_{\text{rise}}$, will be attenuated and thus useless for analyte sizing. The salt gradient method used by Wanunu et al. is to date the only approach to simultaneously boost translocation throughput and increase translocation time [37].

Assuming that the flux of target species is the constant through every cross-section of the analyte flow, $c_{\text{min}}$ can be introduced as the minimal concentration, which is measurable without significant attenuation of the pulse amplitude:

$$c_{\text{min}} = \frac{1.36}{d^2 \pi l_{\text{rise}} N_A f_{\text{event}}^{\text{min}} / f_{\text{cyl}}^{\text{max}}}$$

where $d$ is the diameter of the pore orifice (the tip diameter for conical pores), $N_A$ is the Avogadro-constant, $f_{\text{cyl}}^{\text{max}}$ is the maximal cut-off frequency where the analyte is still detectable irrespective of the current noise. The parameter $l_{\text{rise}}$ is the distance between the analyte positions where the current pulse starts to deviate from the baseline and where it starts to deviate from the peak value (Fig. 4). The approximations $l_{\text{rise}} = d_{\text{part}} / 2 + l$ for cylindrical,
\[ I_{\text{rise}}^{\text{cone}} = \frac{d_{\text{part}}}{2} \] for conical geometries were used with \[ \tau_{\text{rise}} = \frac{0.34}{f_c} \] as typical in RPS measurements [7].

Larger pores require lower analyte concentration because the detection limit scales inversely with the pore volume (cylindrical pores) or with the third power of the tip diameter (conical pores). According to Fig. 4 the detection limit of cylindrical pores is always lower than of conical pores at equal pore/tip diameters. Generally, this is not true because conical pores are more sensitive than cylindrical pores that enables to use a wider orifice for the same particle size.

3. Potentiometric sensing

Owing to the small diameters of nanopores the chemical-physical properties of the surface can selectively alter the transpore flux of ions through the nanopore (Fig. 3C). An early potentiometric study showed that membranes with charged nanopores rejected ions of the same charge sign and transported those of opposite charge [38]. The potentiometric response of such permselective nanopores can be described well by using the Nernst-Planck/Poisson equations [39]. Further increasing the transport selectivity of the nanopores by restricting it to a single ion is possible by using a selective complexing agent (Ag\(^+\) ionophore) and a hydrophobic compound grafted to surface of the nanopore, in addition to ion-exchanger sites. Simple potentiometric measurement of the membrane potential resulted in Ag\(^+\) -selective electrodes with nanomolar detection limit and selectivity coefficients exceeding six order of magnitudes for common ions [40].

4. Acknowledgement

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5. References


Figure captions

Fig. 1. Schematics of a single nanopore sensor (left) and typical current responses for cylindrical and conical pores (right). The ionic current through the nanopore is maintained by applying a transmembrane voltage between two Ag/AgCl electrodes. The full width at half maximum (fwhm) is a measure of the pulse duration while ΔI of the pulse amplitude [7].

Fig. 2. Relative current changes calculated with different models in a cylindrical pore with $l/d=20$ as a function of the relative particle size. Regions in brackets indicate approximate validity range of the models.

Fig. 3. Schematics of nanopore sensing methods and resulting signals. (A) RPS measurement with a selective reagent added to the sample, (B) analyte binding by a functionalized nanopore, (C) nanopore-based ion-selective membrane.

Fig. 4. Detection limit for particle sizing as function of pore diameter for a conical pore and cylindrical pores with various lengths ($d_{part}/d = 0.5; f_c^{max} = 10$ kHz ).
Figure 1
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Figure 2

- **Deblois**
  \( \left( \frac{d_{\text{part}}}{d} \leq 0.4 \right) \)

- **Smythe**
  \( \left( \frac{d_{\text{part}}}{d} \leq 0.9 \right) \)

- **Maxwell**
  \( \left( \frac{d_{\text{part}}}{d} \leq 0.35 \right) \)

- **Gregg**
  \( \left( 0.9 \leq \frac{d_{\text{part}}}{d} \right) \)