Magnetic microbead transport during resistive pulse sensing

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Tunable resistive pulse sensing (TRPS) experiments have been used to quantitatively study the motion of $1\text{ }\mu\text{m}$ superparamagnetic beads in a variable magnetic field. Closed-form theory has been developed to interpret the experiments, incorporating six particle transport mechanisms which depend on particle position in and near a conical pore. For our experiments, calculations indicate that pressure-driven flow dominates electrophoresis and magnetism by a factor of ~ 100 in the narrowest part of the pore, but that magnetic force should dominate further than $\sim 1\text{ mm}$ from the membrane. As expected, the observed resistive pulse rate falls as the magnet is moved closer to the pore, while the increase in pulse duration suggests that trajectories in the half space adjacent to the pore opening are important. Aggregation was not observed, consistent with the high hydrodynamic shear near the pore constriction and the high magnetization of aggregates. The theoretical approach is also used to calculate the relative importance of transport mechanisms over a range of geometries and experimental conditions extending well beyond our own experiments. TRPS is emerging as a versatile form of resistive pulse sensing, while magnetic beads are widely used in biotechnology and sensing applications. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4833075>]

I. INTRODUCTION

Use of magnetic beads is widespread in nano- and microscale biotechnological systems, particularly for biosensing applications.^{1,2} These beads can be manipulated in a magnetic field independently of other forces, while their small size enables efficient interactions with bioparticles and analytes.^{3,4} Resistive pulse sensing is used to study particles suspended in aqueous electrolyte as they move through a pore in a thin membrane under an applied potential. When the particle is within the pore, the flow of ionic current is momentarily blocked, giving rise to a “resistive pulse”. Recently,⁵ a variant known as tunable resistive pulse sensing (TRPS) was used to study $1\text{ }\mu\text{m}$ magnetic beads as they clustered into magnetic supraparticle structures (SPSs), then re-dispersed upon removal of the magnetic field. In another study, TRPS was used to detect magnetically clustered “barcode” rods with alternating gold and nickel sections in an aggregation assay which achieved femtomolar sensitivity.⁶ The combination of resistive pulse sensing with magnetic transport is attractive because of the ubiquity of magnetic beads, and also the advantages of magnetism over other particle transport mechanisms near the sensing pore. The applied field can be controlled, the magnetic force acts independently of other mechanisms, and the spatial dependence of magnetic transport differs from other mechanisms. Formation of SPSs provides both challenges (e.g., pore blockages) and opportunities (e.g., aggregation assays). The aim of this article

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is to develop our quantitative understanding of magnetic bead transport near and within pores using theory and TRPS experiments.

The motion of colloidal particles can be driven by a number of mechanisms, which are conventionally described using the Nernst-Planck framework for steady-state transport.⁷ Using this approach, in Sec. II we extend a closed-form analysis of transport in TRPS^{5,8–12} to encompass six transport mechanisms commonly present in resistive pulse sensing, namely diffusion, dielectrophoresis, electrophoresis, electro-osmosis, pressure-driven flow, and magnetism. We focus on the latter four mechanisms, studying dimensionless ratios of particle flux components in order to establish their relative importance. Competition between mechanisms is explored as a function of pore geometry and the position of the particle within (and outside) the pore. We present calculations which extend to experimental conditions (including geometry, applied potential, pressure, zeta potential, and magnetic flux density) well beyond those applicable to our own experiments, demonstrating the generality of the theory. The geometric model used is that of a truncated linear cone (Fig. 1(a)), because tunable pores (and others^{13–19}) are near-conical, with one larger opening tapering to a smaller opening on the opposite face of the membrane. Sections III and IV detail TRPS rate, size, and duration measurements for 1 μm superparamagnetic beads in a variable magnetic field, which are interpreted using the theoretical work.

Previous studies combining resistive pulse sensing with magnetic transport are limited to the TRPS work mentioned above^{5,6} and a recent report using an 80 nm wide pore etched in Si_3N_4 .²⁰ Resistive pulse sensing has become prominent in recent years due to single molecule sensing using sub-10 nm biological and synthetic nanopores.^{21–24} 10 nm diameter glass nanopores were recently used to study competition between electrophoresis, electro-osmosis, and pressure-driven flow.²⁵ TRPS is typically used to study particles from 50 nm to several micrometers in diameter, a size range relevant to many interesting particle types,²⁶ particularly in nanomedicine,²⁷ and including most commercially available magnetic beads. In this size range, other resistive pulse sensing systems have been used to address particle transport via diffusion,²⁸ electrophoresis,^{29,30} and pressure.¹⁶

In TRPS (Fig. 1),^{8,26,27,31–34} the geometry of a sensing pore can be continuously controlled on micrometre to nanometre scales by stretching and relaxing the membrane, which is elastomeric.^{32–38} This control can be achieved *in situ*, in real time. Protocols have been established for measuring particle concentration,^{8,39,40} size,⁴¹ and charge,¹¹ broadly based on the frequency,

FIG. 1. (a) is a schematic section through a conical pore with opening radii a and b in a membrane of thickness b . The half cone angle is α and the origin for cylindrical polar co-ordinates r and z is the centre of the smaller pore opening. A particle of radius R travels downwards along the z -axis, and the local pore radius is $\lambda(z)$. The potential applied between the upper and lower halves of the fluid cell is V_0 , and the pressure across the membrane is $\Delta P = P_2 - P_1$. The dashed box indicates the usual configuration for our experiments, in which particles are initially in the upper fluid cell and the directions of the four major transport mechanisms are as shown. Labels refer to electro-osmosis (EO), electrophoresis (EPH), pressure-driven flow (PDF), and magnetic transport (MAG). (b) shows the qNano apparatus, a tunable pore specimen, the fluid cell, and the bar magnet. The tunable pore is a distance ρ_0 from the centre of the magnet.

magnitude, and duration of resistive pulses, respectively. Charge measurement techniques have been used to measure ζ -potentials in several studies.^{10,11,42,43} TRPS has been directly compared with complementary techniques including light scattering^{10,26,36,44–46} and others,^{27,47–51} and has proved particularly successful for distinguishing multimodal mixtures.^{26,32,34,36,49}

These TRPS-based characterization techniques have now been applied to a wide variety of particle types. The ability to control and measure competing transport mechanisms during TRPS could be advantageous for any such applications. For the food and beverage industry, TRPS has monitored emulsion stability over a period of months,¹⁰ and detected wine protein aggregation in heating cycles.⁵² For environmental management, TRPS has detected a surrogate for waterborne pathogens⁵³ and biogenic particles which remove toxic Se from the water cycle.⁵⁴ Biological contaminants including baculovirus,⁴⁰ adenovirus,⁴¹ and bacteria^{40,55} have been detected. DNA has been studied as single molecules³¹ and on-bead,^{34,56} having been detected by change in apparent surface charge⁴³ and in an aggregation assay (to 5 pM sensitivity) for single-nucleotide polymorphism.⁵⁷ Particles studied for drug delivery applications include novel expansile particles,⁴⁷ lipid matrices containing superparamagnetic nanoparticles,⁵⁰ liposomes,^{27,44,45} and “lyophilisomes”,⁴⁶ which are 0.1–3 μm biodegradable proteinaceous capsules. Exosomes (and larger extracellular vesicles) have been characterized in studies seeking to understand the spread of cancer,^{58,59} including in nonpurified biofluids (blood and urine).⁵⁹ TRPS has also been applied to measure exosomes from endometrial epithelial cells, relevant to embryonic implantation,⁶⁰ and from human stem cells, which could be used in the treatment of Alzheimer’s disease.⁵¹

II. THEORY

In this section, we provide a theoretical framework to illustrate the relative importance of transport mechanisms. Parameters for our experiments in Secs. III and IV are pointed out, and magnetic transport is of most interest in those experiments. Our analysis here is more general, extending over a range of transport mechanisms and realistic experimental conditions for resistive pulse sensing, and the supplementary material⁶¹ includes further analysis. The expressions developed are closed-form, and most accurate for transport on and parallel to the central z -axis of a pore. Park *et al.*²⁰ considered competition between electrophoretic and magnetic transport for 30 nm diameter particles in a 30 nm long, 80 nm wide pore. German *et al.*²⁵ used a finite element model to study the balance between electrophoresis, electro-osmosis, and pressure using 8 nm gold nanoparticles in a conical glass pore with smaller opening diameter of ~ 10 nm. Neither of these studies considered transport as a function of particle position, nor of variable pore geometry.

To explore geometric variations, we use the truncated linear cone model (Fig. 1(a)), in which a and b are the pore openings and d is the length of the pore. We assume rotational symmetry about the z -axis, so that the circular cross-sectional area is $A(z) = \pi\lambda(z)^2$, where $\lambda(z)$ is the radius. The limits of a cylindrical pore ($a = b$) and an infinitely long cylinder ($d = \infty$) are covered by this model. We are most interested in pores with a relatively small opening angle α , so that $J_z \gg J_r$. This conical pore model is widely relevant beyond TRPS^{13–19} and extends the approximate approach to particle transport described previously.^{5,8–12}

A. General transport expressions

We use the Nernst-Planck approach to express the total steady-state transport of particles in a fluid as

$$\mathbf{J} = \sum \mathbf{J}_i, \quad (1)$$

where $\mathbf{J}$ (particles $\text{m}^{-2} \text{s}^{-1}$) is the total flux, and the subscript i refers to a specific transport mechanism. We are most interested in transport due to electrophoresis, electro-osmosis, pressure-driven flow, and magnetism. Initially, we introduce general expressions for each mechanism, before exploring specific experimental conditions.

For the electrophoretic and electro-osmotic contributions, we use the expressions (respectively)⁷

$$\mathbf{J}_{\text{eph}} = \frac{C\epsilon\zeta_{\text{particle}}}{\eta} \mathbf{E}, \quad (2)$$

and

$$\mathbf{J}_{\text{eo}} = \frac{-C\epsilon\zeta_{\text{pore}}}{\eta} \mathbf{E}. \quad (3)$$

Here C is particle concentration, ζ refers to the zeta potential of the subscripted surface, ϵ and η are, respectively, the absolute permittivity and viscosity of the electrolyte, and the electric field is $\mathbf{E}$. These expressions both use Smoluchowski's approximation, where the electrical double layer is small compared with (respectively) the width of a channel and the radius of the particle. Equation (3) strictly applies to a cylindrical channel without end effects.

For pressure-driven flow, we have

$$\mathbf{J}_{\text{pdf}} = \frac{C\mathbf{Q}}{A}, \quad (4)$$

where $\mathbf{Q}$ is the volume flow rate.

For a spherical bead of radius R in a magnetic flux density $\mathbf{B}$ ("B-Field") near a magnetic dipole $\mathbf{m}$, we use the approximate expression

$$\mathbf{J}_{\text{mag}} = \frac{2CR^2\Delta\chi}{9\eta\mu_0} (\mathbf{B} \cdot \nabla) \mathbf{B}, \quad (5)$$

where μ_0 is the vacuum permeability and $\Delta\chi$ is the susceptibility difference between the bead and the fluid. This expression uses the assumptions that $\nabla \cdot \mathbf{m} = 0$,⁴ that there is no initial magnetization of the beads, and that there is no significant demagnetization field due to particle shape.⁶²

B. Expressions for a conical pore

The spatial dependencies in Eqs. (2)–(5) for a conical pore are summarized here and explained more fully in the supplementary material.⁶¹ To specify the electric field when a voltage V_0 applied across the membrane, the geometry is assumed to be locally cylindrical ($E_z \gg E_r$). End effects are approximated by calculating the electric field within a cone in the half-space beyond the pore.⁹ The artificial cone follows the locus of points defined by a gradient $G = z/r$, which takes a value of 1.25, consistent with the electrical resistance between a circular pore opening and an infinite half space.⁶³ It is also assumed that the electrolyte resistivity is homogeneous and that the field is invariant across the pore width. This approach provides reasonable agreement with finite element simulations of E_z on-axis for TRPS.¹² The resulting form of E_z consists of a geometric term multiplied by V_0

$$\begin{aligned} E_z &= \frac{V_0 ab}{(d + 0.8(a + b))(a + Gz)^2} \quad z > 0, \\ &= \frac{V_0 ab}{(d + 0.8(a + b)) \left(a - \left(\frac{b - a}{d} \right) z \right)^2} \quad -d < z < 0, \\ &= \frac{V_0 ab}{(d + 0.8(a + b))(b - G(z + d))^2} \quad z < -d. \end{aligned} \quad (6)$$

Expressions for pressure-driven flow through a truncated conical pore have been developed^{5,9,11,12} similar to other workers,^{16,40} and using a comparable approach to the electric field calculation. The geometry is assumed to be locally cylindrical so that $Q_z \gg Q_r$, and end effects are approximated by introducing artificial cones beyond the ends of the pore, now with a gradient of $16/9\pi$, consistent with the resistance to pressure-driven flow between a circular pore opening and an infinite half space.⁶⁴ The flow rate parallel to (and invariant along) the z -axis due to a pressure difference between half spaces ΔP is

$$Q_z = \frac{\Delta P}{\eta} \left(\frac{a^3 b^3}{\frac{3}{2}(a^3 + b^3) + \frac{8d}{3\pi}(a^2 + ab + b^2)} \right). \quad (7)$$

The magnetic flux density at the spherical-polar co-ordinate position $\mathbf{X} = (\rho, \theta, \phi)$ relative to a magnetic dipole $\mathbf{m}$ is

$$\mathbf{B}(\mathbf{X}) = \frac{\mu_0}{4\pi} \left(\frac{3\mathbf{X}(\mathbf{m} \cdot \mathbf{X})}{X^5} - \frac{\mathbf{m}}{X^3} \right), \quad (8)$$

where μ_0 is the vacuum permeability. $B_\rho = 0$ in a direction perpendicular to the dipole ($\theta = 90^\circ$), and the value used in calculations is the radial field along the dipole axis ($\theta = 0^\circ$ or 180°)

$$B_\rho = \frac{\mu_0 m}{2\pi \rho^3}. \quad (9)$$

C. Dimensionless comparison of transport mechanisms

Equations (2)–(5) are now used to explore variations with respect to experimental conditions. Our approach is to use dimensionless ratios of these particle flux terms to establish precedence of the transport mechanisms as experimental parameters are varied. These ratios are explored using six examples of conical pores from the literature, with openings ranging from hundreds of microns to a few nanometres, as detailed in Table I. The extreme values from these six examples (indicated in Table I) define values of R , V_0 , and ΔP for “upper” and “lower” limits used in our comparisons, with further data for calculations included in Table II. The “shape

TABLE I. Benchmark conical pore geometries based on the present work (case B) and previous studies, with some values rounded or inferred. TPU indicates thermoplastic polyurethane.

Case	Based on	Material	a μm	b μm	d μm	α	R μm	V_0 V	ΔP kPa
A ^a	Stober <i>et al.</i> ¹³	glass	5	165	1000	9.1°	1.1	≤ 0.5	≤ 7.50
B	Present work	TPU	3	30 ^b	200	7.7°	0.5	0.08	0.05
C	Somerville <i>et al.</i> ¹⁰	TPU	0.3	20	200	4.1°	0.2	0.8	0.05
D	Lan <i>et al.</i> ¹⁶	glass	0.21	9	50	10°	0.12	0.2	1.33
E	Heins <i>et al.</i> ¹⁴	polyimide	0.002	1	10	5.7°	$\sim 0.001^c$	≤ 0.6	n/a
F ^d	Kowalczyk <i>et al.</i> ⁶⁵	SiN	0.002	0.013	0.02	26.6°	$\sim 0.001^c$	≤ 0.2	n/a
Maximum							1	1	5
Shape only							0.1	0.05–1	0.5
Minimum							0.001	0.05	0.05

^aGeometry neglects the varied taper in Stober *et al.*’s study.

^bCalculated value of $29 \pm 4 \mu\text{m}$ for a cone with end effects⁹ using electrolyte resistivity $0.86 \Omega \text{ m}$ (0.1 M KCl, 293 K)⁶⁶ and measured baseline current $114 \pm 14 \text{ nA}$.

^cAnalytes were single molecules.

^dGeometry corresponds to half of the hourglass shape in Kowalczyk *et al.*’s study.

TABLE II. Experimental parameters not varied in calculations.

Input	Symbol	Default value	Data source
Temperature	T	293 K	Room temperature
Permittivity	$\epsilon_r \epsilon_0$	$7.1 \times 10^{-10} \text{ F m}^{-1}$	Water, 293 K ⁶⁶
Viscosity	η	1.002 mPa s	Water, 293 K ⁶⁶
Susceptibility	$\Delta\chi$	0.25	Dynabead measurements ^{67,68}
Dipole moment	m	1.52 A s ²	Section III

only” case in Table I enables comparison of cases A–D without varying any parameters other than pore geometry. These “shape only” values are arbitrary (within the upper and lower limits) and are used below if the lower limit gives insignificantly small values. Based on previous work using a range of synthetic colloids stabilized by negative surface charge at neutral pH,¹¹ we use the range $-40 \text{ mV} \leq \zeta_{\text{particle}} \leq -10 \text{ mV}$. Variation of ζ_{particle} depends on buffer and surface chemistry, so although we use central value of -25 mV as a default, the range indicates uncertainty for specific calculations. We usually express the ratio for mechanism i relative to electrophoresis ($D_{i,\text{eph}} = J_{z,i}/J_{z,\text{eph}}$), because electrophoresis must be present in any resistive pulse experiment in order to drive and measure the ionic current passing through the pore.

The first dimensionless ratio to consider is $D_{eo,\text{eph}}$, which according to Eqs. (2) and (3) is just $-\zeta_{\text{particle}}/\zeta_{\text{pore}}$, independent of geometry. In general, ζ_{pore} will be significant unless charge is expressly removed from the pore walls. For example, in TRPS measurements of ζ_{particle} using polymer pores, ζ_{pore} has generally been found to be smaller (less negative) than ζ_{particle} , yet significant.^{10,11} Hereafter, any ratio $D_{i,\text{eph}}$ can be modified to $D_{i,eo}$ by replacing ζ_{particle} with $-\zeta_{\text{pore}}$. A ratio relative to the sum of electrophoretic and electro-osmotic transport can be obtained by replacing ζ_{particle} with $(\zeta_{\text{particle}} - \zeta_{\text{pore}})$. Equations (2) and (3) are appropriate for our experiments, because the double layer thickness of 0.1 M KCl is close to 1 nm.⁸ These equations become less valid for the smaller pores in Table I, especially if electrolyte of lower ionic strength is used, and a full numerical treatment is often used in such cases.

The dimensionless ratio between pressure-driven flow and electrophoresis is (Eqs. (2) and (4))

$$D_{\text{pdf},\text{eph}} = \frac{\eta Q_z}{\epsilon \zeta_{\text{particle}} E_z A}. \quad (10)$$

$D_{\text{pdf},\text{eph}}$ calculations are plotted for the six example geometries and the experimental case in Fig. 2, with input parameters specified in Table I. Discontinuities are observed in Fig. 2(a) at $z = 0$ and $z = -d$, the membrane surfaces. Calculated values span several orders of magnitude, indicating the wide range of realistic experimental conditions for resistive pulse experiments.

$D_{\text{pdf},\text{eph}}$ is independent of R , and (as shown in Fig. 2) does not vary with z within the pore, because E_z scales with A^{-1} . The ratio monotonically increases with ΔP (noting that $Q_z \propto \Delta P / \eta$), and decreases proportionally to ζ_{particle} and V_0 . Calculations in Fig. 2 show that for the larger pores (geometries A and B), pressure-driven flow will dominate even for the “lower” limit in which only 50 Pa is applied across the membrane, with strong electrophoresis. For our experiments, $D_{\text{pdf},\text{eph}} \sim 100$ within the pore, and the ratio increases with distance above or below the membrane. Dimensionless ratios close to 1 are typical for geometries such as C and D. This observation is consistent with TRPS charge measurements on 200 nm particles^{10,11,42} and recent experiments using 10 nm glass pores²⁵ in which electrokinetics and pressure are near balanced. Pressures in the kPa range are required for pressure to be significant in geometries such as E and F.

Using the Hagen-Poiseuille profile for pressure-driven flow, variation of $D_{\text{pdf},\text{eph}}$ across the pore width can be estimated as

$$D_{\text{pdf},\text{eph}}(r) = \frac{2\eta Q_z (\lambda^2 - r^2)}{\pi \lambda^4 \epsilon \zeta_{\text{particle}} E_z}. \quad (11)$$

FIG. 2. (a) plots the dimensionless ratio of pressure-driven to electrophoretic transport in conical pores, from Eq. (10), for a “lower” limit of $\Delta P = 50$ Pa, $V_0 = 1$ V, and $\zeta_{particle} = -40$ mV. Labels A–F refer to geometries from Table I, with other parameters specified in Table II. Insets show data for cases C–F with expanded scales. (b) summarizes $D_{pdf,eph}$ data at $z = 0$ (the pore constriction) and $z = 20 \mu\text{m}$ for the lower limit, along with an “upper” limit ($\Delta P = 5$ kPa, $V_0 = 50$ mV, and $\zeta_{particle} = -10$ mV). Data relevant to experiments in this paper ($\Delta P = 46$ Pa, $V_0 = 80$ mV) are also plotted for $z = 0$ and $z = 20 \mu\text{m}$ with $\zeta_{particle} = -25$ mV. The highlighted horizontal band indicates the range $0.1 \leq D_{pdf,eph} \leq 10$.

$D_{pdf,eph}$ falls parabolically from a constant value on the pore axis to zero at the pore wall, where the flow velocity is zero. In our experiments (Fig. 3(a)), $D_{pdf,eph} > 1$ over almost the entire pore width. However, under certain conditions (Fig. 3(b)), the dimensionless ratio drops below 1 mid-way between the pore axis and the wall. If electrophoresis and pressure act to drive the particles in opposite directions, the sum of these fluxes will change sign at $D_{pdf,eph} = 1$. Variation in $D_{pdf,eph}$ across the pore width is most extreme near the pore constriction, where resistive pulses are generated. This complication will be most relevant for experiments with closely competing transport mechanisms, and those in which off-axis trajectories are common.

Equation (11) must be used with the caveat that radial variation is not included for electrophoresis, nor for other transport mechanisms considered. Our assumption that the electric field (relevant to $J_{z,eph}$) is invariant across the pore width is not exact, especially near the pore entry.¹² However, pressure-driven flow (if significant overall) will strongly influence radial variations due to the zero velocity boundary condition at the pore walls. Electro-osmosis in a

FIG. 3. (a) plots the dimensionless ratio of pressure-driven to electrophoretic transport in conical pores as a function of radial position from the pore axis (Eq. (11)). These data correspond to case B from Table I ($\Delta P = 46 \text{ Pa}$ and $V_0 = 80 \text{ mV}$, as in our experiments), with further parameters specified in Table II. (b) is a similar plot for case C ($\Delta P = 500 \text{ Pa}$, $V_0 = 500 \text{ mV}$, $\zeta_{particle} = -40 \text{ mV}$) in which the horizontal line indicates a dimensionless ratio of 1.

cylindrical pore creates a plug flow⁷ invariant up to the slip plane. Magnetic field lines (below) are relatively unaffected by pore geometry due to the low permeability of the polymer membrane. The motion of any off-axis particle is affected by a correction to the hydrodynamic drag due to the proximity of the pore wall.⁶⁹

Following Eqs. (2) and (5):

$$D_{mag,eph} = \frac{2R^2 \Delta\chi B_\rho^2}{3\rho_0 \mu_0 \epsilon \zeta_{particle} E_z}, \quad (12)$$

where $\rho = \rho_0$ is the position of the tunable pore (Fig. 1(b)). Equations (9) and (12) suggest that $D_{mag,eph}$ monotonically increases with R^2 and with the magnetic interaction, via $\Delta\chi$ and m^2 . There is inverse dependence on $\zeta_{particle}$, the electric field, and strong dependence ($\propto \rho_0^{-7}$, see Eq. (9)) on the magnet position. Variation of $D_{mag,eph}$ is explored in Fig. 4, with $\Delta\chi$ and m held constant at the experimental values determined for the present work. The maximum variation of $D_{mag,eph}$ due to variation of ρ_0 between $-220 \mu\text{m} \leq z \leq 20 \mu\text{m}$ is only 5.7%, so a constant value (equal to the distance between the dipole and $z=0$) is used in the calculation. Lower limit ratios are smaller than 10^{-6} , so the “shape only” case is plotted instead. In this case, parameters other than pore geometry are held constant over cases A–D (see Table I, with $\zeta_{particle} = -10 \text{ mV}$ and $\rho_0 = 50 \text{ mm}$).

Variation of $D_{mag,eph}$ as a function of axial position includes discontinuities at points of geometrical discontinuity—the pore openings. The key trend is that magnetic transport becomes increasingly significant further away from the pore constriction, because the magnetic force is reasonably consistent throughout the system, whereas the electric field is focussed at the constriction. In terms of magnitude, $D_{mag,eph}$ does not reach 0.1 at $z=0$ in any of the calculated cases, but can reach 1 or greater within a few tens of microns from the pore constriction in an upper limit of large particles, close approach of the magnet and weak electrophoresis. Notably, Fig. 4(b) demonstrates the strong dependence on experimental variables here, with the upper limit and the “shape only” case separated by a factor of 10^4 for pores of the same geometry. Under conditions matching our experiments, $D_{mag,eph}$ does not exceed 0.2 within $20 \mu\text{m}$ of the constriction, but is greater than 1 further than $48 \mu\text{m}$ from the membrane surface. Geometries E and F from Table I are not included in Fig. 4 because $D_{mag,eph} < 0.025$ at the upper limit and $z = 20 \mu\text{m}$ from membrane surface, although in these cases magnetic transport will also dominate electrophoresis at a relatively large distance from the membrane.

The analyses above indicate that pressure-driven (Eq. (4)) and magnetic (Eq. (5)) transport should be of most importance in our experiments, so the final dimensionless ratio here compares these two mechanisms

FIG. 4. (a) plots the dimensionless ratio of magnetic to electrophoretic transport in conical pores, from Eq. (12), for an “upper” limit of $R = 1 \mu m$ (or $R = a$ if $a < 1 \mu m$), $\zeta_{particle} = -10 mV$, $V_0 = 50 mV$ and $\rho_0 = 30 mm$. Labels A–D refer to geometries from Table I, with other parameters specified in Table II. Inset, the same data with expanded scale near $z = 0$. (b) summarizes $D_{mag,eph}$ data at $z = 0$ (the pore constriction) and $z = 20 \mu m$ for the upper limit, with a consistent “shape only” configuration for cases A–D ($R = 100 nm$, $\zeta_{particle} = -10 mV$, $\rho_0 = 50 mm$). Each vertical line joins values at $V_0 = 50 mV$ and $V_0 = 1 V$. Data relevant to experiments in this paper ($R = 500 nm$) are also plotted for $z = 0$ and $z = 20 \mu m$, with $\zeta_{particle} = -25 mV$ and values of the magnet position ρ_0 annotated. The highlighted horizontal band indicates the range $0.1 \leq D_{mag,eph} \leq 10$.

$$D_{mag,pdf} = \frac{2A^2 R^2 \Delta\chi B_\rho^2}{3\rho_0 \mu_0 \eta Q_z}. \quad (13)$$

As in Eq. (12), there is monotonic increase in the ratio with R^2 , $\Delta\chi$ and m^2 , and proportionality to ρ_0^{-7} . $D_{mag,pdf}$ is also inversely proportional to ΔP . Figure 5 shows that (similarly to $D_{mag,eph}$) $D_{mag,pdf}$ is smallest at the pore constriction, with discontinuities observed at the pore openings. For geometries A–D, lower limit ratios are smaller than 10^{-11} , so the “shape only” case is plotted instead, with parameters other than pore geometry held constant (see Table I, with $\rho_0 = 50 mm$). The magnitude of $D_{mag,pdf}$ for geometries A–D is approximately two orders of magnitude lower than in comparable cases in Fig. 4. None of our calculated ratios at $z = 0$ exceeded 10^{-4} , and the specific parameters used in our experiments do not yield a ratio greater than 10^{-3} within $20 \mu m$ of the pore constriction. Using the experimental parameters, and substituting $(\rho_0 - z)$ for ρ_0 in Eq. (13), we found that $D_{mag,pdf} = 1$ for $\rho_0 = 30 mm$ at $z = 0.95 mm$. At the height of the fluid in the upper fluid cell ($z = 4.7 mm$), we can expect a ratio of 1 when $\rho_0 = 51 mm$. Unlike $D_{mag,eph}$, calculations of $D_{mag,pdf}$ can be large for smaller pores (geometries

FIG. 5. (a) plots the dimensionless ratio of magnetic to pressure-driven transport in conical pores (Eq. (13)) for an “upper” limit of $R = 1 \mu m$ (or $R = a$ if $a < 1 \mu m$), $\Delta P = 50$ Pa, $V_0 = 50$ mV, and $\rho_0 = 30$ mm. Labels refer to geometries from Table I, with other parameters specified in Table II. Inset, data for cases E and F with expanded scale near $z = 0$. (b) summarizes $D_{mag,pdf}$ data at $z = 0$ (the pore constriction) and $z = 20 \mu m$ for the upper limit, with a consistent “shape only” configuration for cases A–D ($R = 100$ nm, $\Delta P = 500$ Pa, $\rho_0 = 50$ mm). Data relevant to experiments in this paper ($R = 500$ nm, $\Delta P = 46$ Pa) are also plotted for $z = 0$ and $z = 20 \mu m$, with values of the magnet position ρ_0 annotated. The highlighted horizontal band indicates the range $0.1 \leq D_{mag,pdf} \leq 10$.

E and F) because the resistance to pressure driven flow scales with the fourth power of the pore radius. However, the influence of magnetism in the pore constriction is limited in these cases due to the requirement for particles to fit within the pore opening.

Finally, we briefly summarise results for diffusion and dielectrophoresis, which are not directly relevant to the experiments below, but are explored further in the supplementary material.⁶¹ Although diffusion is often an important consideration for studies of single-molecule ($R \sim 1$ nm) nanopore dynamics,²⁴ it is heavily dominated by electrophoresis in resistive pulse sensing experiments on scales on tens of nanometres or greater. In our experiments, $D_{diff,eph} < 10^{-3}$. This analysis does not account for stochastic Brownian motion (as opposed to bulk transport),²⁸ which can be expected to introduce some noise into any detailed analysis of resistive pulses. The importance of DC dielectrophoresis increases sharply around the pore constriction, with a discontinuous change in sign at $z = 0$. The dielectrophoretic force acts opposite to the direction of ∇E^2 (in practice, away from $z = 0$). Dielectrophoresis can comfortably dominate electrophoresis under realistic experimental conditions, and approaches 1 for our

experiments. However, this is only the case in the immediate vicinity of the pore constriction, within a distance comparable to the size of the pore opening a . In the present work, we are primarily concerned with resistive pulse rates, and the dynamics within one opening radius of the pore should not prevent particles moving through the constriction. We note the importance of DC dielectrophoresis for work elsewhere regarding the shape of resistive pulses.

III. EXPERIMENTAL

His-tagged Dynabeads of specified diameter $1.1\ \mu\text{m}$ were obtained from Invitrogen ($<5\%$ CV, $\sim 4 \times 10^9\ \text{ml}^{-1}$, catalogue 101.04D). 780 nm carboxylated polystyrene spheres (Thermo-Fisher, $<5\%$ CV) were used for calibrating size measurements. As-received particles were diluted at 1:1000 in 0.1 M KCl (aqueous), buffered with 0.01 M tris(hydroxymethyl)aminomethane (tris, pH 8), and $<0.1\ \text{w/w}\%$ Triton X-100 and 0.1 w/v% ethylene glycol tetraacetic acid (EGTA) were added. Freshly-prepared suspensions were ultrasonicated for 15 min, and sonication was repeated for 1 min prior to each measurement. All water used was deionised to a resistivity of $18.2\ \text{M}\Omega\ \text{cm}$ using a MilliQ system (Millipore Corp., USA).

TRPS was carried out using the *qNano* instrument with tunable pore specimens, both supplied by Izon Science (Christchurch, New Zealand). Descriptions of the apparatus^{8,27,31,32} (Fig. 1(b)) and images of pore specimens^{8,26,32–34} are available. Each pore is located near the middle of the circular membrane ($\sim 200\ \mu\text{m}$ thick) at the centre of a specimen, which is made from a thermoplastic polyurethane (Elastollan 1160D, BASF). Pores are formed by mechanical puncture of the membrane, and the pores used in this work were designated “NP1000” by the manufacturer, indicating that they are most suitable for measurement of $1\ \mu\text{m}$ particles. Specimens are held on the *qNano* device by teeth that fit into holes in each of the cruciform’s four arms. Mechanical “tuning” is achieved by moving these teeth in order to stretch and relax the membrane *in situ*, and thereby to optimise the experiment. The specimen was held with opposite pairs of teeth 47 mm apart, relative to a resting separation distance of $\sim 42\ \text{mm}$. Previously, material properties and actuation have been described, measured, and modelled,^{32,33,35–37} pulses have been recorded at varied applied stretch,^{34,36} and fast micropore actuation has recently been reported.³⁸

Tunable pores are near-conical, and measurements were carried out with the small opening facing upwards. Pores were filled and surrounded by electrolyte contained within a fluid cell. $80\ \mu\text{l}$ of buffer was loaded into the lower fluid cell, and $40\ \mu\text{l}$ of buffer containing particles of interest was added to the upper cell, generating a gravitational pressure head of 46 Pa.¹¹ A potential of 0.08 V was applied between the Ag/AgCl electrodes embedded in each half of the fluid cell. The ionic current through the pore was measured and analysed using the *qNano*’s customised electronics and software (v2.2.2.64), with a lower limit for the pulse magnitude (ΔI) of 0.05 nA.

For each measurement, a stable current was established over 30 s before data were recorded, typically consisting of several hundred to 1000 pulses over 3 min. The minimum number of events for any individual run was 78, and the minimum duration was 146 s. Between measurements, the specimen was rinsed with buffer solution and the upper half of the fluid cell was flushed with deionised water and dried in a stream of nitrogen. When pore blockages occurred, they were identified due to step changes in the current. Affected data were disregarded, and blockages were removed by mechanical agitation.

Pulse rate data were collected from 5 independent measurements at each magnet position (see below) with the applied stretch, applied voltage and specimen unchanged. Uncertainties represent the standard error in the mean across the repeated measurements. The full-width half maximum (FWHM) duration and ΔI of each pulse were recorded, so that each measurement produced a distribution of FWHM and ΔI values. Particle size was established following the previously published method,⁴¹ in which calibration particles are used and there is a known relationship between ΔI and particle volume. We use the mode of the ΔI distribution to represent the calibration particle size distribution. Although the distribution mean can provide a sensitive indication of change in the system,⁵ the mode is more appropriate for calculation of absolute values due to possible thresholding, outliers, selection effects, and distribution asymmetry. In calculations, we

FIG. 6. Radial magnetic flux density magnitude ($|B_\rho|$) plotted as a function of distance from the centre of a cubical bar magnet (ρ_0) in a direction perpendicular to the surface of each of six faces. Uncertainties in the measurement of $|B_\rho|$ and ρ_0 are smaller than the symbol size. Inset: plots of $|B_\rho|$ against ρ_0^{-3} for directions perpendicular to the two polar faces. Linear fits with $R^2 > 0.999$ are plotted for both faces.

conservatively attribute an error of 5% to the process of finding the central value of the calibration particle distribution. This uncertainty is systematic across all size measurements, and does not affect comparisons between results. The uncertainty presented in figures is a combination of this calibration uncertainty (5%), the bin size (conservatively 8%, determined by the variation of the result with bin size), and the standard error in the mean of repeated measurements (<10%).

A small bar magnet was used to apply a magnetic field to particles within the resistive pulse sensing fluid cell. This magnet was cubical with side length 12.05 mm, and in TRPS experiments, the magnet was held with one of its faces parallel to the membrane surface (Figure 1(b)). The magnetic flux density around the magnet ($\mathbf{B}$) was characterised using a digital Teslameter (Group 3, model D-151). The radial value B_ρ in directions parallel to the six surface normals is plotted in Fig. 6.

The inset to Fig. 6 shows that B_ρ values are consistent with the form of Eq. (9), with the dipole located at the centre of the magnet. Using this equation, data for the two polar faces indicate $m = 1.51$ and 1.52 A s^2 . In interpreting TRPS results, we infer $\mathbf{B}$ near the bar magnet by assuming that the magnet is a dipole with $m = 1.52 \text{ A s}^2$ located at the centre of the cube and oriented parallel to the normal of polar faces. B_ρ is non-zero along the normal from non-polar faces, indicating some errors in the modelled location, orientation, and dipole form of $\mathbf{B}$. We include an estimated (conservative) uncertainty in calculations of $\mathbf{B}$ components of $\pm 10\%$, based on the maximum ratio of B_ρ for non-polar faces to the equivalent value for polar faces. This ratio does not exceed 9.0% for any data in Fig. 6.

IV. RESULTS AND DISCUSSION

A. Rate and duration

Experimental pulse rate data in Fig. 7(a) are qualitatively consistent with expectations. As the magnet is moved closer to the fluid cell, the B-field in the vicinity of the pore increases, and the beads experience an increasing force parallel to the field lines. When a polar face of the bar magnet is facing the fluid cell, beads in the upper fluid cell are attracted towards the magnet, away from the pore, and fewer pulses are recorded. When the polar face was 30 mm above the membrane, the recorded rate for His-tagged Dynabeads fell to 20% of the case with no magnet present. In Sec. II, we showed that pressure-driven flow should dominate electrophoretic and magnetic transport by a factor of at least 100 within $20 \mu\text{m}$ of a pore. However, because the B-field is not strongly focussed at the pore entrance, magnetic transport should be

FIG. 7. Average pulse rates (a) and FWHM durations (b) as a function of distance from either a polar face or a non-polar face of the bar magnet. Data are normalised relative to the value when no magnet is present (equivalent to the dashed horizontal lines), and error bars represent the standard error in the mean across $n = 5$ measurements. Inset to (b), the standard deviation σ of pulse FWHM distributions, with error bars indicating the standard error in the mean over $n = 5$ measurements.

comparable to pressure driven effects ~ 1 mm from the pore. Therefore, we can attribute the lower pulse rate to a lower concentration of particles moving into the region closer than $\sim 100 \mu\text{m}$ from the pore, where pressure-driven flow will dominate.

There are various reasons for the rate data not following a smooth trend with ρ_0 . Random variation is expected due to Brownian motion and steric interactions near the pore constriction. There may be variation in the concentration and distribution of magnetic particles within the upper fluid cell, notwithstanding efforts to mix and load the particles into the fluid cell consistently. Another random uncertainty arises from exact positioning of the magnet, discussed above. There is a particular jump in the rate data for the polar face between $\rho_0 = 60$ and 70 mm. Here, we note the estimate (Sec. II) that $D_{\text{mag.pdf}} = 1$ at the height of fluid in the cell ($z = 4.7$ mm) when $\rho_0 = 51$ mm. The observed jump could coincide with the point at which $D_{\text{mag.pdf}} = 1$ throughout the electrolyte in the upper fluid cell.

The pulse rate is also slightly reduced when a non-polar face of the magnet is facing the fluid cell, falling to 66% of the case with no magnet present at $\rho_0 = 30$ mm. In this case, field lines are predominantly perpendicular to the pore axis. Particles are not forced directly away from the membrane surface, although they can still move horizontally away from the area close to the pore. Misalignment of the magnetic dipole could also produce a component of force parallel to this axis, reducing the rate in a similar way to the polar face case.

Pulse duration data (Fig. 7(b)) also suggest a change in particle dynamics as the polar face of the magnet approaches the fluid cell, but this observation is more difficult to rationalise. The particles move more slowly through the pore than when no magnet is present, and the clearest difference is observed at $\rho_0 \leq 60$ mm, consistent with the observed decrease in pulse rates. There is only a marginal increase (if any) in the mean FWHM when the non-polar face of the magnet is used. An observable difference in the pulse duration is not expected, because pressure-driven flow should dominate in the vicinity of the pore. Particles within the pore should not be slowed by the fact that magnetism dominates far from the pore, because the distance over which a particle accelerates from stationary (in the laboratory frame) to the same velocity as the flow field is $\leq 10^{-9}$ m, where this distance is $\sim Mu^2/2F$, the particle mass is M , and F is the drag force ($\sim 6\pi\eta Ru$).

We suggest two explanations for the increase in pulse durations, noting that because we have used mean FWHM data to increase sensitivity to change, the data are affected by outliers. The uncertainty in FWHM data and the standard deviation of FWHM distributions (σ , inset of Fig. 7(b)) both increase at $\rho_0 \leq 60$ mm. The first explanation is that formation of SPSs increases the likelihood of outlying, large duration events caused by steric hindrance at the pore entrance. However, size data (below, including distribution means) suggest that no such clusters were detected. The second explanation is that the model is most accurate for particles travelling along the z -axis. When the particles experience competition with magnetism as they move into the region near the pore, there should be

FIG. 8. Particle size measurements as a function of magnet position. Error bars represent the combination of the standard error in the mean across $n = 5$ measurements, and errors associated with determining the modal ΔI for calibration particles (5%) and magnetic beads (8%). Data include both the mean and mode of size distributions, and the data points to the right of the vertical dashed line were obtained with no magnet present. Inset, size histograms for individual measurements at $\rho_0 = 30$ mm (lower left, 128 events, bin size 100 nm) and $\rho_0 = 100$ mm (lower right, 386 events, bin size ~ 33 nm, $< 0.3\%$ of events with diameter > 1.6 μm not shown).

a greater distribution of particle trajectories, and in particular, a greater proportion travelling off-axis where pressure-driven transport is less important (Fig. 5). This result indicates the need for future work to understand the dynamics of particles in the half-space above the pore. Park *et al.*²⁰ also observed changes in dwell time with magnetic field in experiments, and used this to characterise particle magnetization, albeit under different experimental conditions.

B. Particle size

Measurements of particle size (Fig. 8, see Sec. III for details) were independent of the magnet position. Data are also consistently within experimental error of each other, regardless of ρ_0 or whether they represent the mean or mode of size distributions. The specified diameter of the beads (1.1 μm) is close to the measured central values (consistently ~ 1.0 μm), providing further general evidence for the accuracy and utility of the sizing technique.⁴¹ The small disparity that is present could be due to inaccuracy in the specified value, or because error in the calibration (while random in nature) applies systematically across the size data.

There is one exceptional data point, for the data representing distribution modes at $\rho_0 = 30$ mm. When the magnet is close to the fluid cell, fewer events are recorded in a measurement, bin sizes become larger, and the mode is more uncertain. The insets to Fig. 8 demonstrate how a lower mode was identified at $\rho_0 = 30$ mm (left, blue) when compared with a measurement at higher ρ_0 (right, green). Three of the five measurements at $\rho_0 = 30$ mm yielded a lower mode, resulting in the stray data point. These observations raise the interesting point that some size distributions have a second mode—even at higher ρ_0 , there is a slightly increased population around 800 nm. These data may be generated by signal noise close to the lower threshold for ΔI measurement, and are only significant here when the overall pulse rate is low.

Size data are expected to vary with B_ρ if beads aggregate into SPSs, which form due to induced magnetic dipoles and align along B-field lines.^{4,5} Aggregation can be detected with high sensitivity by monitoring the mean particle size,⁵ while the mode may be unchanged if the majority of beads remain disaggregated. In Fig. 8, there is no evidence for aggregation comparable to data obtained using TRPS previously.^{5,6,10,45–47,52,57} Additionally, there was no change in the calculated dispersity, a measure of particle homogeneity in solution. Dispersity is calculated as $D' = M_w/M_n$, where M_w is the mass-average particle mass and M_n is the number-average particle mass.⁷⁰ For the experiments in Fig. 8, we found $1.2 < D' < 1.4$ with no

obvious trend with respect to ρ_0 and a characteristic standard error of 10% across the five repeat measurements.

Aggregation of 1 μm magnetic beads in a B-field has been observed in previous TRPS experiments,⁵ albeit with ~ 100 times greater bead concentration. Despite this lower concentration, aggregates are expected to form due to magnetic dipole interactions in the bulk solution. Our configuration is closest to the “reverse” configuration in the previous study, in which the applied pressure and potential were such that particles were transported downwards from the upper cell. In both our study and that “reverse” case, there is no evidence of aggregation, and the pulse rate decreases when the magnet is present. It is possible that aggregates form, but do not move through the pore due to steric effects, or because magnetic transport is stronger for clusters, as $\mathbf{J}_{\text{mag}}$ scales with both R^2 and m^2 (Eqs. (5) and (9)). Using a scaling argument, it can also be shown that SPSs should be separated by hydrodynamic forces near the pore constriction in our experiments. The hydrodynamic (F_h) and magnetic (F_m) forces between two nearby spheres can be expressed as⁵

$$F_h = 6\pi\eta R\Delta u, \quad (14)$$

where Δu is the flow velocity difference at a separation equal to the distance between adjacent bead centres (1 μm), corresponding to $\Delta u = 1.6 \text{ mm s}^{-1}$ at the pore constriction in the model for our experiments, and

$$F_m = \frac{3\pi\mu_f R^2 \beta^2 B_\rho^2}{2\mu_0 \mu_p^2}, \quad (15)$$

where $\mu_f (\sim 1)$ and $\mu_p (= 1 + \Delta\chi)$ are the relative permeabilities of the fluid and the particle (respectively) and β is equal to $(\mu_p - \mu_f) / (\mu_p + 2\mu_f)$. For our experiments, we have $F_h/F_m \sim 35$ for $\rho_0 = 30 \text{ mm}$, and greater for higher values of ρ_0 .

V. CONCLUSION

In this paper, the dynamics of colloidal magnetic beads have been studied in the context of TRPS experiments. The effects of an applied magnetic field on rate, size, and duration measurements have been quantified. Taken together with other studies of magnetic transport^{5,20} and aggregation^{5,6} in resistive pulse sensing, the record now covers a range of experimental conditions. However, the accessible range of conditions is very broad, as is the associated variation of particle transport. For instance, resistive pulse sensing research currently spans roughly three orders of length scale magnitude. A variable applied potential is common to all experiments, while significant pressure or magnetism can also be introduced and controlled. We have also noted the potential for DC dielectrophoresis to significantly affect the details of resistive pulse shape.

The theoretical analysis introduced, while containing approximations, provides a useful closed-form approach for studying competition between transport mechanisms as a function of the experimental parameters, and (crucially) the position of the particle in the system. In a conical pore, the geometric discontinuity at the smaller pore entrance is particularly important, because the resistive pulse is generated as the particle passes through this region, and the transport mechanisms are also discontinuous there. A key observation supported by both theory and experiments (and observed previously⁵) is that the magnetic field in TRPS should dominate transport over the majority of the fluid cell, but is not important when compared with an electric field or pressure-driven flow focussed in the pore constriction. This work also reveals the importance of future work into off-axis particles, and the trajectory and distribution of particles in the half-space adjoining the pore. There was an observed inconsistency in that pulse durations increased in a configuration for which pressure was expected to dominate, and clusters were not observed. Future experimental work could more precisely resolve competition between transport mechanisms. Precise control of this competition will enable development of new strategies for use of resistive pulse sensing in on-bead sensors and related technologies.

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