Frequency sweep rate dependence on the dielectrophoretic response of polystyrene beads and red blood cells

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(Received 20 September 2013; accepted 11 November 2013; published online 10 December 2013)

Alternating current (AC) dielectrophoresis (DEP) experiments for biological particles in microdevices are typically done at a fixed frequency. Reconstructing the DEP response curve from static frequency experiments is laborious, but essential to ascertain differences in dielectric properties of biological particles. Our lab explored the concept of sweeping the frequency as a function of time to rapidly determine the DEP response curve from fewer experiments. For the purpose of determining an ideal sweep rate, homogeneous $6.08\text{ }\mu\text{m}$ polystyrene (PS) beads were used as a model system. Translatability of the sweep rate approach to $\sim 7\text{ }\mu\text{m}$ red blood cells (RBC) was then verified. An Au/Ti quadrupole electrode microfluidic device was used to separately subject particles and cells to 10Vpp AC electric fields at frequencies ranging from 0.010 to 2.0 MHz over sweep rates from 0.00080 to 0.17 MHz/s. PS beads exhibited negative DEP assembly over the frequencies explored due to Maxwell-Wagner interfacial polarizations. Results demonstrate that frequency sweep rates must be slower than particle polarization timescales to achieve reliable incremental polarizations; sweep rates near 0.00080 MHz/s yielded DEP behaviors very consistent with static frequency DEP responses for both PS beads and RBCs. © 2013 AIP Publishing LLC.

[<http://dx.doi.org/10.1063/1.4833095>]

I. INTRODUCTION

Microfluidic and dielectrophoretic (DEP) technologies enable a wide variety of particle polarizations with nonuniform electric fields on microchips^{1,2} to achieve particle manipulation, concentration, separations, and property-based identification. Particles^{3–5} include bioparticles (DNA,⁶ virus,⁷ and protein⁸) as well as cells (blood cell types,^{9,10} cancer,^{11–13} stem cells,¹⁴ and yeast¹⁵). The advantages to coupling DEP with microfluidics are small sample size (on the order of microliters), rapid analysis (approximately minutes to achieve results), minimal sample preparation, and minimal waste production.^{16,17} Traditionally, DEP experiments are completed at static, fixed frequencies such that maximum particle polarization can be achieved and measured. Multiple experiments are conducted, each at discrete frequencies over the range of interest to stitch together DEP response spectra; this is a labor-intensive approach. Further disadvantages are that extended field exposure times at fixed frequencies can change particle properties¹⁴ or cell viability.¹⁸ Prior research has used rapid frequency sweeping to increase cell separation efficiency,^{19–23} but to the best of our knowledge, frequency sweeping has not been explored to generate continuous DEP spectra. Therefore, a detailed study examining the effects of frequency sweeping on particle polarization is pertinent and timely. In this paper, we demonstrate that frequency can be swept with time in the β -dispersion region thus enabling interrogation of cells at multiple frequencies within a short time period. The benefits of using a frequency sweep technique are that nearly continuous DEP response curves, when coupled with automated

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response analysis, can be compiled in near real time and the number of experiments needed to obtain particle DEP spectra are greatly reduced.

DEP enables phenotypically similar biological cells to be discriminated based on dielectric properties including the conductivity and permittivity of the membrane, cytoplasm, and other structurally relevant organelles. Cell components and structure contribute to a cell's signature dielectric dispersion.^{2,15} A particle's complex permittivity is frequency dependent and characterized by dielectric dispersion regions (γ , β , and α , where $\omega_\alpha < \omega_\beta < \omega_\gamma$) specific to an applied frequency. This work explores 0.010 to 2.0 MHz in the β -dispersion region²⁴ because the Clausius-Mossotti factor, which governs sign and polarization strength, for polystyrene beads is nearly constant over this range. Maxwell-Wagner theory describes the polarization mechanism of particles in the β -dispersion region as interfacial polarization where moving charges build around the interface of a charged or charge-neutral particle and exchange ions with the suspending medium (Ref. 25, pp. 33–38). Interfacial particle polarization creates an induced dipole moment such that the particle experiences disproportionate forces in each half cycle of the alternating current (AC) field resulting in net particle movement (Ref. 25, pp. 8–11).

Polarized particles can exhibit either positive dielectrophoresis (pDEP) or negative dielectrophoresis (nDEP) as a consequence of the frequency-dependent polarizability of the particle in the surrounding medium (Ref. 25, p. 10).²⁶ Particles that exhibit pDEP move to high electric field regions and particles that exhibit nDEP move to low electric field regions.^{2,15} This motion up and down electric field gradients is described by the Clausius-Mossotti factor for spherical particles,¹⁵

$$f_{cm} = \frac{\tilde{\epsilon}_p - \tilde{\epsilon}_m}{\tilde{\epsilon}_p + 2\tilde{\epsilon}_m}, \quad (1)$$

$$\tilde{\epsilon}_i = \tilde{\epsilon}_i + \frac{\sigma_i}{\omega j}, \quad (2)$$

where $\tilde{\epsilon}_i$ is the complex permittivity of the particle ($i=p$) and of the medium ($i=m$), which are both functions of conductivity (σ), permittivity (ϵ), and angular frequency (ω).²⁵

Polarization is not an instantaneous event;²⁷ charge transport into the interface takes a few microseconds in response to the electric field. Maxwell-Wagner dielectric relaxation is a physical phenomena related to the transport delay of cation and anion alignment in and around the interface of the dielectric particle.²⁸ At lower frequencies ($< \sim 10$ MHz), particle polarization is driven by this conductive polarization. At higher AC frequencies, charges do not have enough time to move into and around the interface double layer, so particles experience polarization lag time as a result of the rapidly modulating field and do not reach maximum polarization. Maxwell-Wagner dielectric relaxation is characterized by a time constant, τ_{MW} , which is unique to each particle or cell due to the time constant's dependence on the cell dielectric properties. The time required for a particle to reach maximum polarization is given by Ref. 25, p. 27:^{24,29}

$$\tau_{MW} = \frac{(\epsilon_p + 2\epsilon_m)\epsilon_0}{\sigma_p + 2\sigma_m}. \quad (3)$$

Typical relaxation times for particle polarization vary from pico to microseconds (Ref. 25, p. 37),^{24,29} and the calculated τ_{MW} for polystyrene (PS) beads in our Epure H₂O medium at 2.5×10^{-4} S/m is 3.5 μ s. Thus, a single AC cycle is on the order of 0.01 to 2 μ s; the time delay in ion transport within a static frequency field of 0.010 to 2.0 MHz is such that 2 to 350 AC cycles must be completed before the particle experiences full polarization.

The Maxwell-Wagner dielectric timescales for charge transport into and around the interface becomes important when the frequency is swept, i.e., changes as a function of time. Fig. 1(a) crudely cartoons the Maxwell-Wagner particle polarization at the interface under static frequency as well as slow and fast frequency sweep rates. At a static frequency in the β -dispersion region, the particle experiences a constant frequency field such that the relaxation time is not a factor and the particle fully polarizes. A particle in a field with a slowly changing frequency sweep has

FIG. 1. (a) Dielectric relaxation mechanism for PS beads showing cases when (i) particle polarization occurs at a static frequency, (ii) τ_{MW} is shorter than the slow frequency sweep rate ($\tau_{\Delta FS}$) allowing the bead interface time to polarize in response to the nonuniform AC field, and (iii) τ_{MW} is longer than the $\tau_{\Delta FS}$ for fast frequency sweep rates and the bead interface does not have time to fully polarize. (b) Schematic of the quadrupole electrodes micro patterned onto a glass slide, and (c) microdevice with PDMS fluidic layer bonded above the quadrupole electrodes silver epoxied to copper leads.

relaxation time, $\tau_{\Delta FS}$, that is less than τ_{MW} and thus the particle interface fully polarizes. In contrast, a particle in a fast frequency sweep has a relaxation time, $\tau_{\Delta FS}$, that is larger than τ_{MW} and the particle interface does not have time to fully polarize in the field. Our new frequency sweep technique is able to take advantage of incremental particle polarization changes as the frequency changes, which is more time efficient. PS beads are lossy dielectric particles treated as homogeneous spheres and are thus an idealized particle to examine new techniques, devices, or approaches to dielectrophoretic characterizations. The homogeneous spherical DEP polarization model for PS beads ($\epsilon = 2.5$ and $\sigma = 9.4 \times 10^{-5}$ S/m) suspended in Epure H_2O displays only nDEP behavior over 0.010 to 2.0 MHz.

In this study, dielectrophoretic responses of PS beads (model system) were quantified at both static frequencies and frequency sweeps at rates ranging from 0.00080 to 0.17 MHz/s over the β -dispersion frequency range of 0.010–2.0 MHz in Epure H_2O medium at 2.5×10^{-4} S/m. PS bead motion in the electric field was imaged with video microscopy and analyzed using three techniques: intensity profiles, transient response, and particle velocities. Image intensity analysis has been used by other researchers to quantify the pDEP and nDEP behavior of particles representing particle concentration,³¹ voltage trapping,³² cell counting,³³ and

noncontinuous DEP spectra.^{34,35} We also utilize intensity analysis to capture DEP responses. Data showed that frequency sweep rates impact particle polarization due to dielectric relaxation limitations. This frequency sweep technique was further extended to negatively charged biconcave red blood cells (RBCs), which are an important cellular system for medical disease diagnostics.^{36–38}

II. MATERIALS AND METHODS

The microdevice shown in Fig. 1(c) was fabricated according to previously published microfabrication techniques,⁷ with the 100 μm wide electrodes spaced 200 μm apart aligned at 90° along the bottom of a 70 μm deep by 1000 μm wide microfluidic chamber as shown in Fig. 1(b). Polystyrene beads (Cat No. PP-60-10, Spherotech, Lake Forest, IL, USA), 6.08 μm in diameter were centrifuged at 1300 min^{-1} for 5 min to separate the beads from the liquid. The PS beads were resuspended in Epure H₂O (18 M Ω or $2.5 \times 10^{-4} \text{S/m}$) at a 1:10 (bead to water) volumetric dilution ratio and vortexed. Microdevice was pre-rinsed with Epure H₂O and Alconox precision cleaner (Cat No. 1104, Alconox Inc, White Plains, NY, USA) to prevent bead adhesion. PS bead-Epure H₂O suspension was pumped to the microchamber using a syringe. Time was allowed for inlet and outlet pressures to equalize and flow to stop. The function generator (Agilent 33250A, Agilent, Santa Clara, CA, USA) was connected via copper leads to produce a 10Vpp AC sine wave with frequencies ranging from 0.010 to 2.0 MHz at specific frequency sweep rates 0.00080, 0.0011, 0.0030, 0.0063, 0.013, 0.021, 0.028, 0.042, 0.056, 0.083, and 0.17 MHz/s. Frequency sweeps linearly increased the applied frequency as a function of time. Greater than five ($n > 5$) static frequency experiments were completed at each frequency 0.010, 0.020, 0.030, 0.040, 0.050, 0.20, 0.40, 0.60, 0.80, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 MHz by applying 10Vpp for 90 s. These DEP static frequency responses were compared to each frequency sweep rate DEP responses. For the static and frequency sweep experiments, the PS bead concentration was between 238 and 263 beads in the $t = 0$ field of view. Video recordings of experiments were taken at 30 fps at 640×480 pixels/image using LabSmith SVM Synchronized Video Microscope with a 10 $\times$ objective (LabSmith, Livermore, CA, USA).

Video recordings of PS beads DEP behaviors were analyzed with ImageJ (NIH, Bethesda, MD) using intensity, transient slope, and velocity measurements. Since PS beads only exhibit nDEP over the frequency range of interest, intensity data acquisition from images was partially automated by drawing, in the time 0 image, a rectangular box at the device center, I_{CTR} , and background box, I_{BK} measured in a location with no PS beads present (see Fig. 2(a)). ImageJ Z Project function was used to average the pixel intensities in the specified boxed region automatically for the remainder of the video. The initial background, $I_{BK}(t = 0)$ and center intensity, $I_{CTR}(t = 0)$ were subtracted from the center and background intensity at each time, $I_{CTR}(t)$ and $I_{BK}(t)$, and then a normalized intensity was calculated by dividing by the maximum intensity experienced by the PS beads via the following programmed calculation,

$$\bar{I}_{DEP,t} = \frac{[(I_{CTR} - I_{BK})_t + (I_{BK} - I_{CTR})_{t=0}]}{[(I_{CTR} - I_{BK})_t + (I_{BK} - I_{CTR})_{t=0}]_{MAX}}. \quad (4)$$

This normalized intensity tracked the real-time magnitude of the PS bead DEP response, which had two distinct regions: transient where beads moved with nDEP toward the center, and steady-state (SS) where beads achieved tight packing at the device center. These two responses were analyzed separately via transient slope and particle velocity calculations.

The transient slope of the PS bead responses was extracted from the normalized intensity using signal processing tools to quantify the delay and rise times. The PS bead delay time, t_d , was characterized as the time required for the intensity response to reach 50% of the final intensity response for the first time. The rise time, t_r , was determined as the time needed for the intensity response to reach 100% of the final intensity response for the first time (Ref. 39, pp. 517–518). This allowed the transient response to be segmented and a linear trend line was fit between t_d and t_r where $t_d < t_r$. A comparison of the transient slope for frequency sweep rates

FIG. 2. (a) nDEP behavior of 6 μm PS beads suspended in E-pure H_2O 2.5×10^{-4} S/m and 250 V_{pp}/cm 0.0063, 0.056, and 0.17 MHz/s sweep rates from 0.010 MHz to 1.0 MHz. (b) Raw intensity profile of PS beads in the center nDEP region (boxes shown at 0.20 MHz) at 0.0063 MHz/s sweep rate. Inset is a calibration of intensity per bead. (c) Clausius-Mossotti factor for the PS beads from 0.010 MHz to 2.0 MHz at three conductivities of 2.5×10^{-4} , 1.0×10^{-3} , and 1.0 S/m. PS bead assembly at slower frequency sweep rates track static frequency responses while 0.056 MHz/s illustrates transitional behavior and frequency sweeps above 0.17 MHz/s substantially lag the true static frequency DEP responses.

and static frequency measurements is given in Fig. 3(c). PS bead velocities were determined from the original video by tracking the x-, y-pixel position of individual PS beads from 0 to 50 s. PS beads located within 5 μm of electrode tips were selected to control for similar electric field gradients. This procedure was repeated for at least 10 beads in each specific frequency sweep rate and static experimental video.

For the experiments involving human RBCs, blood type O+ was obtained from a single donor and centrifuged at 1400 rpm for 5 min to separate the packed RBCs from the plasma and leukocytes. The packed RBCs were removed, then resuspended at 1:75 v:v in 0.10 S/m isotonic dextrose buffer doped with 0.75% BSA (Cat No. A7906, Sigma Aldrich, St. Louis, MO, USA) to prevent cell adhesion to surfaces. This RBC suspension was syringe-pumped to the micro-chamber, time allowed for flow to stop and the 10V_{pp} signal applied over 0.010–0.50 MHz (range reduced to avoid pDEP behavior) at frequency sweep rates of 0.00080, 0.0063, and 0.056 MHz/s ($n=7$). RBC static frequency experiments were completed at 0.010, 0.10, 0.25, and 0.50 MHz at 10V_{pp} for 90 s ($n=7$). Video microscopy at 25 $\times$ and 1 fps was obtained with a Zeiss Axiovert Inverted Light Microscope (Zeiss, Germany). The video images were analyzed as described above for the PS beads.

III. RESULTS AND DISCUSSION

Frequency sweep rates ranging from 0.00080 to 0.17 MHz/s were explored to see if the nDEP response of PS beads would vary and/or correspond to static frequency measurements. Static frequency experiments were completed at fixed values in the range from 0.010 to 2.0 MHz. This range was chosen to more cleanly compare frequency sweep responses to static frequency responses; the Clausius-Mossotti factor, $\text{Re}(f_{\text{CM}})$, varies only slightly from 0.26 to 0.48 for a homogeneous lossy polystyrene sphere (see Fig. 2(c)) over this range. This design of experiments allowed comparison of response, lag times, and velocities without additional error from rapidly changing f_{CM} . It should be noted that under the experimental conditions employed, AC electroosmosis, which occurs in the low kHz range, was not observed. Electrothermal flow normally occurs in ~ 1 S/m or greater conductivity media and was also not a factor.³⁰ Fig. 2(a) shows still images from both static frequency experiments and the frequency sweeps at 0.20,

FIG. 3. (a) $6\text{ }\mu\text{m}$ PS beads nDEP intensity profiles for 0.00080, 0.0063, and 0.056 MHz/s and static steady state measurements (black diamonds). Intensity analysis captures bead assembly to the quadrupole center with transient and SS regions. The slowest frequency sweep rate of 0.00080 MHz/s best predicts the static DEP responses. (b) Bead assembly profiles for 0.0063 ($n=8$) and 0.17 MHz/s ($n=7$) with 95% confidence upper and lower limits shown as dashed lines. (c) Transient slope comparison for static frequencies (0 MHz/s) as well as frequency sweeps. (d) Comparison of static frequency and frequency sweep PS bead velocities from 0 to 50 s. 0.00080 MHz/s results are consistently similar to the static frequency results.

0.60, and 1.0 MHz. For static frequencies, the response 45 s after field application is shown while for frequency sweeps of 0.0063, 0.056, and 0.17 MHz/s, the image is shown at the time stamp when the specified frequency is reached. The electrodes are visible as black shadows in the images and the PS beads assemble due to nDEP forces at the central electric field gradient minima. Data were examined to determine the sweep rate that most closely approximated the static frequency response. Frequency sweeps 0.00080 and 0.0063 MHz/s (shown) tracked static frequency, or true, DEP responses while the slightly faster sweep of 0.056 MHz/s lagged the true DEP responses and at 0.17 MHz/s and faster, particles were unable to achieve sufficient polarization to respond sufficiently in the electric field.

nDEP responses were quantified via intensity analysis as described in materials and methods for all sweeps and all static frequency experiments. Fig. 2(b) illustrates the frequency (and time) dependent intensity for the 0.0063 MHz/s sweep rate images shown in Fig. 2(a). This quantification of the PS bead nDEP response was correlated to total bead packing via the calibration shown in the inset. The 188-bead count at the center deviates slightly from the initial, field off, bead count of 245 because PS beads also move down the electric field gradient to regions outside of the image field of view.

Normalized intensities, Eq. (4), were compiled in Fig. 3(a) for SS (i.e., 45 s) static frequency nDEP responses and 0.00080, 0.0063, and 0.056 MHz/s frequency sweep rate nDEP responses. The time for sweep responses to achieve the true nDEP static response decreases as the sweep rate decreases. Frequency sweep rates 0.00080 and 0.0063 MHz/s are within the 95% confidence intervals ($n=7$) of the static steady-state responses. Fig. 3(a) inset shows that the slowest 0.00080 MHz/s sweep rate more quickly aligns closely with the static frequency responses. Fig. 3(b) compares average 0.0063 MHz/s ($n=8$) to 0.17 MHz/s ($n=7$) with the

dashed lines signifying the upper and lower limits of the 95% confidence intervals for I_{DEP} . The confidence intervals around the transient 0.0063 MHz/s sweeps are smaller than for 0.17 MHz/s over much of the frequency range indicating greater reproducibility at slower sweep rates. Faster sweep rates either do not reach SS or have a lag before reaching SS (cf. Fig. 2(a)) suggesting the bead interface does not fully polarize and thus displays attenuated nDEP motion.

The transient behavior was quantified for all static frequencies and frequency sweeps via a transient slope analysis as compiled in Fig. 3(c). Four static frequency measurements 0.010, 0.60, 1.0, and 2.0 MHz are shown compared to 0.00080, 0.0063, 0.028, 0.056, and 0.17 MHz/s frequency sweep rates. Static frequency transient slopes range between 0.023 and 0.095 and are within the 95% ($p < 0.05$) confidence intervals of 0.00080, 0.0063, and 0.028 MHz/s frequency sweep transient slopes. These slower sweep rates and 0.056 MHz/s differ at $p < 0.001$ from the fastest sweep rate of 0.17 MHz/s, which is also significantly different at $p < 0.001$ from the static measurements (except for 1.0×10^4 Hz with $p < 0.01$).

Individual bead velocities were compiled for static as well as frequency sweeps in Fig. 3(d). PS bead velocity corroborates the intensity profile and the slope analysis that 0.00080 MHz/s frequency sweep rate closely tracks the bead velocity at static frequencies. The 0.056 MHz/s sweep gives good estimations of static frequency bead velocity at times greater than 20 s. Based on intensity, transient slope, and velocity analysis, the slow frequency sweep rate of 0.00080 MHz/s is most consistent with static frequency DEP responses.

There is an observable inverse relationship between the frequency sweep rate and particle polarization, slower sweep rates result in comparable particle polarization characteristics to static frequency responses. Dielectric relaxation is the driving force of this relationship; the calculated dielectric relaxation time Eq. (3) for PS beads in E-pure H_2O at 2.5×10^{-4} S/m is $3.5 \mu\text{s}$, which corresponds to ~ 0.28 MHz. There are two timescales that influence this behavior: the frequency itself and the change in frequency per time. The Maxwell-Wagner, conductivity-driven interfacial polarization mechanism occurs below ~ 0.28 MHz; above this frequency threshold the interfacial polarization of the PS beads gradually decreases and the particle permittivity increasingly influences the DEP force. The experimental frequencies tested were within the range dominated by Maxwell-Wagner polarization such that maximum particle interfacial polarization was possible. However, since the frequency sweep enables incremental polarization of the particle, the frequency sweep range could be extended into the MHz range.

The second timescale of interest is the frequency change per time or frequency sweep rate, which determines how many consecutive cycles a particle experiences a specific frequency. At slower sweep rates, the PS beads experience a specific frequency for a large number of cycles and thus the beads have time to polarize because the timescale of the frequency change is slower than the dielectric relaxation time. In the conductivity polarization regime, a particle must experience a single frequency during the sweep for a minimum of $3.5 \mu\text{s}$ for maximum interfacial polarization to be achieved. Upon polarization, the particle, with its current DEP force, has to overcome inertia and Stokes drag to achieve observable particle motion down the electric field gradient. At static frequencies, it takes roughly 5 s for maximum velocity to be attained (see Fig. 3(d), AC field applied at $t = 5$ s) and as much as 45 s for final SS at the field gradient minima to be reached. As the sweep rate increases, the dielectric relaxation time and the rate of change of the frequency approach the same order of magnitude. Results suggest that 0.056 MHz/s is a transitional sweep rate because the DEP behavior roughly corresponds to the static behavior of the PS beads. With further increases in frequency sweep rates, the timescale for frequency change surpasses the dielectric relaxation timescale such that particles are unable to fully polarize resulting in an attenuated DEP response as shown with data in Figs. 2, 3(a), and 3(b). Fig. 3(b) also demonstrates that the transient behavior of the PS beads is more reproducible at slower frequency sweep rates, which can be attributed to the interfacial polarization timescale of the beads. Implications of the intensity, slope, and velocity analysis compared with static frequencies are that slow frequency sweep rates accurately predict the DEP response of PS beads because the changes in frequency are slower than the characteristic Maxwell-Wagner dielectric relaxation.

Thus, a frequency sweep approach can be utilized to attain accurate DEP behavior of PS beads, provided the sweep rate is slower than conductivity mediated interfacial polarization timescale. This result is reliable over frequency ranges where particle polarization is dominated by the conduction of free charges from the media. The charges are moving around the PS beads through the particle-liquid interface inducing a dipole, which causes PS bead movement down the electric field gradient to the center region of the quadrupolar electrodes. At different sweep rates, the rate of movement of the charges varies, which varies the rate of the dipole being induced, observed as dielectric relaxation. Each sweep rate has a unique dielectric relaxation time and our results are consistent with Maxwell-Wagner interfacial polarization theory. 0.00080 MHz/s is the optimal sweep rate necessary to predict the true DEP behavior of PS beads because it allows for full or partial (when the frequency is above 0.28 MHz) polarization. However, once the particles are polarized and the frequency shifts, the particle only experiences incremental polarization whose timescale is much faster. This suggests that frequency sweeps can also be utilized into the permittivity polarization frequency regime.

Given that the sweep methodology yielded accurate DEP responses for the ideal system of PS beads, the same methodology and frequency sweep rates were explored with human RBCs. The three most successful PS bead frequency sweep rates were reproduced with human red blood cells: 0.00080 MHz/s, 0.0063 MHz/s, and 0.056 MHz/s. Static frequency experiments were also performed at 0.010 MHz, 0.10 MHz, 0.25 MHz, and 0.50 MHz. Fig. 4(a) illustrates $25\times$ microscope images taken of the $t=45$ s final static frequency frames aligned above the sweep time points that correspond to those four static frequencies. Qualitatively, the only sweep rate that accurately matches the static frequency behavior of the human RBCs is 0.00080 MHz/s. This behavior was further verified by the same intensity analysis as for PS beads. In Fig. 4(b), the scaled intensity is plotted for 0.00080, 0.0063, and 0.056 MHz/s experiments ($n=8$) as compared to the static frequency intensities. After the initial 10 s transition for the red blood cells to polarize and overcome drag, the slowest frequency sweep of 0.00080 MHz/s accurately predicts the static frequency behavior and is highly reproducible with a very narrow 95% confidence interval range (Fig. 4(c)). The fastest sweep rate of 0.056 MHz/s does not predict the static behavior of the human RBCs and is much less reproducible, as evidenced by the large 95% confidence interval in Fig. 4(c). From these experiments, we conclude that

FIG. 4. (a) nDEP behavior of RBCs suspended in 0.1 S/m dextrose buffer and 250 V/cm at 0.00080 MHz/s, 0.0063 MHz/s, and 0.056 MHz/s sweep rates from 0.010 MHz to 0.50 MHz. (b) RBCs nDEP intensity profiles for 0.00080, 0.0063, and 0.056 MHz/s and static measurements. (c) 0.00080 and 0.056 MHz/s RBC assembly profiles $n=8$, with 95% confidence interval upper and lower limits shown as dashed lines.

the optimal frequency sweep for determining the accurate DEP behavior of RBCs is 0.00080 MHz/s. Due to the complex dielectric properties of cells, it is necessary to carefully compare frequency sweep rates with static frequency behaviors to ascertain optimal frequency sweep rates that accurately interrogate the cell of interest.

An inverse relationship between the frequency sweep rate and particle polarization exists for RBCs. RBC dielectric relaxation is the driving force; the calculated dielectric relaxation time is $4.6 \mu\text{s}$ corresponding to $\sim 0.21 \text{ MHz}$. By choosing to sweep at a rate slower than this relaxation time, the frequency sweep response is as accurate, to 95% confidence, as the static frequency response. Since red blood cells experience incremental polarization as the frequency changes, it is feasible to extend this approach into frequencies in which particle polarization is permittivity-driven.

IV. CONCLUSIONS

Traditional DEP measurements are completed at single static frequencies in order to compile frequency by frequency, the DEP spectrum for a particle or cell system. This method is laborious, and, as illustrated in this work, requires time for particles to fully polarize for accurate observed DEP responses. This work investigated the use of frequency sweeps as a means to more efficiently interrogate multiple frequencies in a single experimental run and systematically compared the responses to the nDEP response at fixed frequencies between 0.010 and 2.0 MHz. It was observed that frequency sweep rates influence the DEP response of PS beads and RBCs and further, the permissible frequency sweep rate is particle or cell dependent.

The underlying mechanism appears similar. At slower sweep rates, particles have more time to polarize in the electric field and therefore a more accurate and reproducible DEP spectrum can be obtained. At faster frequency sweep rates, the particles are unable to achieve maximum interfacial polarization because of the dielectric relaxation time scale so the observed DEP response does not match the true DEP behavior of the particle.

For polystyrene beads at frequency sweep rates below 0.0063 MHz/s, responses correlate closely with dielectric responses of particles subjected to a static frequency potential. In the PS bead system, 0.056 MHz/s is the transitional sweep rate where the particle dielectric relaxation is approximately the same order of magnitude as the shifts in frequency within the sweep. Dielectric responses continue to track the static frequency responses, although reproducibility is diminished. However as this sweep rate is increased further, conductivity dominated interfacial polarizations cannot be established and the PS bead frequency sweep data does not coincide with static frequency measurements.

For full utility in DEP experiments, this frequency sweep rate methodology must be translatable to cell systems. Results illustrated that only 0.00080 MHz/s accurately predicted the static frequency DEP responses of human RBCs. Red blood cells are substantially more morphologically and dielectrically complex than polystyrene beads. Calculation of the dielectric relaxation time, taking into account only the membrane permittivity and conductivity of 4.4 and 10^{-7} S/m , respectively⁴⁰ yields a dielectric relaxation time $\sim 4.6 \mu\text{s}$ roughly corresponding to 0.21 MHz. This relaxation time is larger than the PS bead relaxation time of $3.5 \mu\text{s}$, so the optimal frequency sweep rate for red blood cells would be slower than that for PS beads. This result suggests that for each new cell system of interest it is imperative to determine the optimal frequency sweep rate to accurately and reproducibly interrogate the behavior of that cell. This work outlines a systematic technique to make comparisons between frequency sweep rate and static frequency. For all cell systems, sweep rates that are too fast will not allow the cell adequate time to polarize and will result in inaccurate and less reproducible DEP responses. An optimal frequency sweep rate can be estimated by calculating the Maxwell-Wagner dielectric relaxation time for the particle/cell of interest, provided the cell's permittivity and conductivity is known. The frequency sweep rate chosen for the DEP study should then remain at frequencies below the inverse dielectric relaxation time ($1/\tau_{\text{MW}}$) for 5–45 s (longer times spent below the threshold give better DEP predictions). This is necessary because frequency sweep rates allow for particles/cells to be polarized incrementally. Faster

sweep rates do not allow sufficient polarization time. Slower sweep rates, which have smaller frequency step sizes, allow particles to attain full particle polarization consistent with static polarization measurements. Further, since the polarization that a particle needs to achieve as the frequency changes is incremental, this frequency sweep technique could be used to quantify DEP responses over a broad frequency range without any a priori knowledge of the DEP response.

Since the cell's permittivity and conductivity are determined from the frequency dependent DEP spectrum, this presents a cyclical situation. However, this work has demonstrated that frequency sweep rates slower than 0.00080 MHz/s can yield accurate DEP response of PS beads as well as RBCs. This sweep rate may therefore be translatable to other cell systems. In addition, at higher frequencies where the polarization mechanism is more heavily influenced by charge permittivity effects through the membrane and cell cytosol, it is possible that slow frequency sweep rates can still accurately capture DEP response spectra. Lastly, this frequency sweep rate technique will enable researchers to obtain accurate and continuous DEP response spectra in shorter experiment times.

ACKNOWLEDGMENTS

The authors gratefully acknowledge funding support from the National Science Foundation via CBET 1041338 and IIP 1340126.

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