

Factors affecting particle collection by electro-osmosis in microfluidic systems

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Abstract

Alternating-current electro-osmosis, a phenomenon of fluid transport due to the interaction between an electrical double layer and a tangential electric field, has been used both for inducing fluid movement and for the concentration of particles suspended in the fluid. This offers many advantages over other phenomena used to trap particles, such as placing particles at an electrode centre rather than an edge; benefits of scale, where electrodes hundreds of micrometers across can trap particles from the molecules to cells at the same rate; and a trapping volume limited by the vortex height, a phenomenon thus far unstudied. In this paper, the collection of particles due to alternating-current electro-osmosis driven collection is examined for a range of particle concentrations, inter-electrode gap widths, chamber heights and media viscosity and density. A model of collection behaviour is described where particle collection over time is governed by two processes, one driven by the vortices and the other by sedimentation, allowing the determination of the maximum height of vortex-driven collection, but also indicates how trapping is limited by high particle concentrations and fluid velocities. The results also indicate that viscosity, rather than density, is a significant governing factor in determining the trapping behaviour of particles.

Introduction

The displacement of micron-scale (or smaller) particles in suspension when subjected to an AC electric field generated by planar microelectrodes may be attributable to one of several phenomena, of which two are directly attributable to direct interaction with the field (as opposed to, say, thermal heating or Brownian motion). One of these is alternating-current electro-osmosis (ACEO), an electrohydrodynamic force caused by the interaction between the field and the medium itself [1]. Electric fields within the electrical double layer at the electrode–fluid interface

contain tangential components, which interact with charges in the double layer to cause fluid motion across the electrode surface. This reduces pressure at the inter-electrode gap that causes suspending medium to be drawn in from overhead, which then moves across the electrodes, causing a vortex at the electrode edge [2]. Originally a process found to cause unexplained motion in particles in low frequency (below 1 kHz) non-uniform fields [3], the phenomenon was studied extensively from the early 2000s onwards [4-8], leading to the identification of the origin of the phenomenon. Since then, it

has been widely exploited for induced fluid pumping and stirring through the use of asymmetrical electrode designs [9-13].

In order to exploit ACEO for particle trapping, a novel electrode was introduced by Hoettges et al., dubbed the zipper electrode [14, 15]. This geometry consisted of interlocking teardrop shapes, which acted to focus all particles from across a large area towards a central spot, as shown in Fig. 1. The interlocking tear-drop shapes with separating gap electrodes have been successfully used to overcome the problems for surface detection technique to bring colloidal particles to the detection surfaces and used to concentrate a wide range of nanoparticles of biological interest [16]. When electrical potential is applied to zipper electrodes, fluid motion generated by ACEO forms vortices at the electrode edges, as observed in previous studies; however, the two electrode edges (on either side of the zipper “pad”) generate the opposing vortices that works together to force particles into the centre of the electrode. If the electrode is sufficiently small, the opposing vortices meet and create an updraft that will drive the particles back into the medium; if the vortices are much smaller than the electrodes, the particles do not reach the centre of the electrode and will form a ring. This is shown schematically in Fig. 2. The optimal size will deposit the particles at the centre of the electrode. The phenomenon acts at any electrode edge, and geometries such as parallel electrodes have been used in the past [4]; similar recent work with interdigitated electrodes [17] has demonstrated that the effect can be used to concentrate nanoparticles from flow, for example.

ACEO-driven particle trapping is sometimes compared with DEP, the force induced in particles suspended in non-uniform electric fields. Both phenomena have been used for trapping of particles from cell size to particles

of 10 nm diameter and smaller; but whilst DEP acts directly on the particle, ACEO acts on the medium. This means that the size of the particles that can be trapped is not limited by the size of the particle (as is the case with DEP); furthermore, since DEP is dependent on field non-uniformity local to the particle, trapping only occurs close to the electrode edges. ACEO-based trapping is limited by the depth of penetration of the vortex flow, rather than the field non-uniformity. This means that ACEO-driven systems have the potential to trap particles from significantly larger volumes than DEP electrode systems. For example, electrodes approximately 1 mm across were used to trap all of the nano-particles contained in a volume 100 μm high [16], whereas nano-particle trapping by DEP requires electrodes a few micrometers across and has a trapping limit of a few tens of micrometers from that gap [18]. ACEO trapping offers a number of other advantages over DEP-driven trapping, such as the fact that the force acts to focus particles onto the centre of the electrode (at which point the flow is symmetrical and particles collect at a null spot) rather than at the edge of the electrodes where the highest electric field resides. This is beneficial for biosensor enhancement, since most surface-based biosensors, such as surface plasmon resonance or quartz crystal microbalances, principally detect particles on the sensor surface. This was demonstrated by the enhancement of nanoparticles onto a quartz crystal microbalance electrode surface onto which a zipper pattern had been etched [19]. The practical application of such a system thus depends on the penetration depth of the induced vortices, and the way in which the vortices interact with the rest of the chamber. This is dependent on a range of possible sources of influence, including electrode size, inter-electrode gap size, voltage, frequency, particle size, particle surface charge, medium

conductivity, and chamber height. Of these, the chamber height is arguably amongst the most important, as this defines the volume of particle solution that can be interrogated.

In this paper, we examine factors affecting the ability of these devices to trap particles, defining the parameters underlying the efficacy of ACEO-driven trapping. In the first part of the paper, the effect of altering the chamber height on the efficacy of trapping using ACEO-driven zipper electrodes is considered. The chamber height is important in that it affects particle collection in two ways; in the event where the vortices reach the top of the chamber, it is expected that all the particles will be collected on the electrode. However, if the vortex diameter does not reach the chamber height, then collection is dependent on the interaction mechanism between the particles and medium in the overhead layer. We report that a simple model can be used to predict particle collection at a range of chamber heights, wherein particles are either collected on the electrode surface by the vortices, or moving into the vortices by sedimentation and collected at the electrode surface. In the second part, we examine the optimisation of the medium—in particular the viscosity and density, and demonstrate that medium viscosity plays a more significant role than density in determining whether particles become trapped. These results collectively allow the determination of conditions for optimum particle collection by this method.

Materials and methods

Electrodes were fabricated by conventional wet etching. Zipper-geometry electrodes (Fig. 1) with 500 μm diameter and two inter-electrode gap sizes (100 μm and 150 μm) were drawn using Solid Edge V20 (Siemens PLM Software, Texas). The design was reproduced in photo masks, manufactured onto Agfa 0.007" base high-resolution

polyester film at resolutions of up to 128 000 dpi by JD Photo-Tools (Oldham, UK). Electrodes were produced on glass slides coated with 4–8 Ω sheet resistance ITO (Delta Technologies, USA). Fluorescent polymer microspheres, 3.1 μm in diameter and with density 1.05 g/cm³ were purchased from Duke Scientific Corporation (Palo Alto, CA). They were supplied as dyed polystyrene microspheres in water with 6.7×10^8 beads/mL concentration. Electric potentials (10 Vp-p, 1 kHz) were applied using a function generator (Thurlby-Thandar, Huntingdon, UK); movement of particles was observed using a Nikon Eclipse E400 fluorescence microscope, Photonic Science Coolview HS cooled camera and PhotoLite. Experiments were conducted at an ambient temperature of 18–20°C.

To assess the effects of trap geometry on trapping, particles were diluted to 10⁴ and 10⁵ beads/mL using deionised water. The particle suspension (20–50 μL) was pipetted onto the electrode arrays and covered with a glass cover slip using a spacer fabricated from layers of polyamide of 130 μm thickness; layers were stacked to change the height of the chamber; spacers were used with one, two, three, five, seven and ten layers. The electrode substrate, cover slide and spacers were clamped together with bulldog clips prior to experimentation. During the experiments the field was applied for 1000 s, and captured images were analysed using the ImageJ software and plots of time against particles collection are plotted at 100-s intervals. Each parameter set was repeated 27 times and the results were averaged. Experiments were also run three times without a field applied in order to measure collection due to sedimentation.

To assess the effects of medium properties on collection, one electrode set was used; the electrode arrays were 500 μm in diameter when measured across the widest part of the

“bulb” orthogonally to the axis passing through the “stem,” as described elsewhere [14, 15]; separated by 150 μm inter-electrode gaps with 390 μm chamber height. The bead suspension was diluted to 105 beads/mL using each of six electrolyte media, prepared either for different density medium at a constant value of viscosity (isopycnic), or with a constant value of viscosity for different values of density (isoviscous). These six media are shown in Table 1. Medium density was determined using digital scales (Ohaus, New Jersey) to measure the mass of a defined volume, whilst viscosity was obtained from CRC the Handbook of Chemistry and Physics [20]. Aqueous solutions were chosen for two reasons—first, the nature of most applications of this technique (typically the concentration of biological particles) means that an aqueous solution is necessary and second, the fact that the driving force in ACEO is the electrical double layer limits the choice of solvent.

Results and discussion

As described previously [14-16], electrical signals applied to the electrode cause colloidal particles in solution to move over the electrodes edges and onward to the centre on the electrode. The fluid pumped by ACEO is replaced by fluid flows moving perpendicular to the electrodes through the inter-electrode gap, forming a vortex above the electrode edge.

Effect of physical dimensions on trapping efficiency

As particles were trapped onto the electrode plane (and entered the field of focus), they were counted at different time points; Fig. 3 shows the collection numbers for different chamber heights, concentrations and channel widths. As can be seen, in all cases the particle number began to increase following an exponential pattern; at longer time periods, this then either reached a maximum level (at

lower chamber heights), or entered a second phase of linear increase (for higher chamber heights). We estimated the number of particles in the “capture volume” of each electrode by considering the volume circumscribed by a line along the inter-electrode channel dividing each electrode from its adjacent electrode. However, as reported in previous studies [14, 15], the number of beads collected on the electrode surfaces was generally larger (by a factor of up to three for low concentration suspensions) than the number of particles within that capture volume, because the collected particles are gathered from a wider area horizontally than simply the immediate electrode area. There was some evidence of some pads occasionally emptying and distributing particles to adjacent pads, though this behaviour was not consistent and appeared to indicate some form of local instability.

Whilst the ACEO system of fluid dynamics and particle entrapment is highly complex, it is possible to analyse the system in order to draw out empirical descriptions of collection behaviour; this is of particular interest when designing ACEO-driven systems for biosensor enhancement or particle collection, where a “rule-of-thumb” allows for the optimisation of a given electrode geometry without in-depth recourse to an analytical model. Furthermore, such empirical models may also describe the behaviour of the system more clearly through a simplification to basics. From the plotted graph of collection of particles against time obtained from the experiment, curve fitting techniques were applied in MATLAB® using the exponential curve fitting tools. Curve fitting was employed in order to both maximise the correlation between data and model, and to remove operator bias.

It was found that the variation in number of particles as a function of time $n(t)$ can be

described using the following empirical formula:

$$n(t) = A \left(1 - e^{-\frac{t}{\tau}} \right) + B (Ct)$$

where the first component inline image is an exponential with time constant τ that represents collection due to vortex trapping, Ct represents a second collection mechanism that is linear with time and A and B are proportionality constants related both to the height of the chamber and the concentration of beads in the chamber. Examining the manner in which these parameters change for the different electrode and chamber geometries. The value of C was determined by examining the rate at which particles collected on the electrodes without the field applied, which was found to be linear with a gradient of 0.023 particles/s (Fig. 4). The results of best-fit values of A and B can be seen as lines through the data points in Fig. 3.

Analysis of the variation of these parameters gives clues to the mechanics of trapping behaviour; in order to perform this analysis, the variation in parameters A , B , and time constant can be seen plotted together in Fig. 5. For example, the component A relates to the number of particles trapped by the vortices. If the vortices extend a finite distance into the solution, then we would anticipate that value A would scale with the chamber height below this limit and would become asymptotic as chamber height increases beyond the height of the vortex. Similarly, we would anticipate that the value B of the contribution of sedimentation into the vortex from above would be zero where the chamber height is lower than the extent of the vortex and would increase as the chamber height moves beyond this.

In fact, both of these behaviours appear to be generally true; whilst parameter A does not reach its asymptote during the 1000 s duration of the experiment, the trend is in this direction. However, at lower chamber heights a near-linear relationship between captured particles and chamber height can be observed at height up to approximately 650 μm before levelling off, indicating that this may be the extent of the limit of the vortices. Similarly, it is at approximately 650 μm that parameter B starts to rise, though there is a small component at that height indicating that the limit of the vortices is slightly lower than this, and an upper limit of approximately 500 μm on vortex height would be reasonable. Notably, in three of the four cases the value of B remains nearly constant from this limit upwards, indicating that the process of sedimentation is sufficiently slow that number of particles dropping into the vortices from overhead is substantially smaller than the resource of particles in this zone.

Analysis of the variation of time constant is also notable, though there appears to be considerably more scattered than for the other parameters, possibly indicating a weaker association between time constant and collection than for the other parameters. If we consider the average of the four, then for the lowest case (where the vortex is most severely constrained) the average is 52 s, rising to between 100 and 111 s in chamber heights between 260 and 650 μm . This near-constant plateau is indicative of the capture mechanism being entirely based around a vortex that reaches the full height of the chamber in all three cases. When the chamber height finally exceeds the limit in which the vortex can trap all particles, the vortex is no longer a closed fluid loop and particles take longer to be trapped (time constants of 155 and 189 for 910 and 1300 μm , respectively), once again pointing to a

trap limit of the order of 650 μm in height. However, scatter in the data prevents more definitive conclusions being reached.

The final parameters to be compared are those of the particle concentration and inter-electrode gap width. Considering concentrations first, as the two concentrations analysed were an order of magnitude apart, we would anticipate that this would result in a corresponding order of magnitude in particles trapped. In fact, this was not the case; parameter A varied between a $\times 3$ increase (100 μm inter-electrode gap) and $\times 5$ increase (150 μm gap). For parameter B, the difference for the larger gap was indeed an order of magnitude, but for the smaller gap there was little difference at all. Turning to inter-electrode gap, previous work [10] indicated that the range over which effective particle trapping could be engendered was relatively small—a large electrode gap produces insufficient flow for effective trapping, whilst electrodes separated a small gap produce a significantly higher DEP force, which overwhelms the ACEO flow and causes particles to trap at the electrode edges rather than at the centre. Nevertheless, in order to examine whether there is a dependency on this parameter, two widths which were known to produce effective trapping were examined. Comparing the values of A for low concentrations of particles showed little difference between the two inter-electrode gap sizes. The evidence discussed above regarding the maximum height of the vortex showed no dependence on the size of the inter-electrode gap, and similarly it showed no relationship to the time constant of collection; instead, the change in the magnitude of parameters A and B relate to the efficacy of trapping particles in higher concentration solutions. One possible explanation for this is that the smaller gap produces higher field strengths and

correspondingly faster vortices, leading to a smaller zone at the centre of the electrode where the particles can settle rather than be re-circulated into solution. A smaller trapping zone will become filled more rapidly, leading to a reduction in the maximum number of particles that can be trapped and may correspond to a reduction in the perceived values of A and B, where the former is limited by the filling of the trap and B by the fact that the trap is near-full at the point where sedimentation becomes important.

It is known from previous study [16] that the collection of particles on zipper-geometry electrodes is largely unaffected by particle diameter of several orders of magnitude, or variations in particle density. We therefore suggest that in low conductivity aqueous media typical of ACEO and DEP experimentation, there exists an optimum set of parameters for particle trapping. As stated before, and described in previous work, there is a fairly narrow range of electrode sizes over which the device is effective, leading to a configuration of 500 μm diameter pads. This work indicates that larger gaps are better, with 150 μm being more effective than 100 μm , but bearing in mind that gaps of 300 μm fail to drive fluid vortices. Within that configuration, our model indicates that the maximum effective height is between 390 and 650 μm ; chamber heights equal to or greater than 650 μm require sedimentation to assist. In non-sedimentation-based systems, all (or the significant majority of) particles are trapped within the first 300 s, giving a 5 min duty cycle for enhancing biosensor devices, for example. Such enhancement offers significant improvement for the detection of nanoparticles, such as viruses, present in small quantities and unlikely to reach a sensor surface by sedimentation alone.

Effect on medium composition on trapping efficiency

The dependency of the velocity to frequency of the applied signal and conductivity of the surrounding medium have been studied elsewhere [4, 14, 15]; similarly, the effect of trapping of different particles covering a range of sizes and material properties has shown that these factors have little impact on particle behaviour or particle velocity [16]. However, the trapping mechanism by which particles become entrapped in the vortex generated at electrode edges before leaving these to become trapped at the electrode centre has yet to be fully elucidated. It was proposed by Hoettges et al. [14, 15] that DEP pulled the particles from the vortex as they pass adjacent to the electrode edge before placing them in a boundary layer laminar flow across the electrode surfaces to collect in the centre of the electrode pad. However, later work [16] has shown that the effect is the same regardless of whether the particle is expected to undergo positive or negative DEP at the energising frequency, indicating that DEP plays no role in the collection by ACEO; however, if the DEP force is strong enough it can trap particles and prevent them from reaching the electrode centre.

If particles are trapped in a vortex, then in theory they can move towards the vortex centre or away to the periphery (and hence into the electrode pad) by centrifugal forces, that is, the vortex acts as a small, localised centrifuge. Centrifugal force states that:

$$F_c = \frac{mv^2}{r}$$

where F_c is the centrifugal force, m is mass, inline image is speed, and r is particle radius. The velocity in the formula is derived from [2]:

$$v = \frac{1}{8} \frac{\epsilon V_0^2 \Omega^2}{\eta x (1 + \Omega^2)^2}$$

where ϵ is the permittivity of the medium, V_0 is the potential applied to the electrodes, η is the viscosity of the medium, x is the distance from the centre of the inter-electrode gap, and Ω is a parameter given by [2]:

$$\Omega = \frac{\omega \kappa \epsilon \pi}{2\sigma}$$

where ω is the electric field frequency, σ represents the conductivity of the medium, and κ is the reciprocal double layer thickness. Based on the velocity formula, viscosity is shown to be one of the denominators. This means the higher the viscosity the less likely the particles move outward from the vortex, thus less collection at the centre electrode pad. Consequently, questions may be raised about whether density or viscosity plays a greater role in determining collection behaviour.

To that end, we have examined particle collection behaviour as a function of viscosity for fixed density, and vice versa. For each of the media in Table 1, the trapping behaviour of the particles was observed to see if particles trapped at the centre of the vortex (indicating particles move up the centrifugation gradient) or at the centre of the pad (indicating they had moved down the gradient), or had not been observed to trap. The behaviour of particles in media of different viscosity and density is shown in Fig. 6.

Notably, the variation in viscosity (Fig. 6A) for the same density (isopycnic media) exhibits a significant trend in the trapping behaviour; as the viscosity increased, particles were observed to go from trapping in a tight bundle

at the centre of the vortex to collection at the centre of the pad in a manner that indicated a relationship between these parameters. However, whilst the variation in density with the same viscosity (isoviscous media) showed some differences in particle motion, Fig. 6B does not show any significant pattern in particle collection, either at the electrode centre or in the vortices. Even when the medium density is held constant, trapping can be observed everywhere, either at the centre of the electrodes or trapped in the vortices.

This suggests that the medium density plays a less significant role in ACEO collection than that of medium viscosity. The only exception in the experiments was in solution I, where all the particles were collected at the edge of the electrode by positive DEP; there were no vortices developed at the edge of the electrode, suggesting that ACEO flow was not generated. The variation of viscosity as small as 0.363 (between solution II and IV) and 0.437 (between solution III and IV) made a significant change in the behaviour of particles collection in the zipper electrodes system, showing that a very small variation in medium viscosity can result in significantly different in ACEO collection behaviour. Indeed, the trapping behaviour was observed to change significantly between experiments in the same media, but conducted at substantially different ambient temperatures (data not shown); whilst Ramos et al. [2]

described the effect of temperature on ACEO pumping as negligible, our experiments has shown that this factor cannot simply be neglected.

Concluding remarks

The trapping of particles using ACEO-driven vortices has been studied previously, but this work represents the first attempt to understand the mechanisms contributing to the trapping behaviour as a prelude to optimisation. It has been shown that for a 500 μm diameter zipper electrode, the optimum chamber height is between 390 and 650 μm for inter-electrode gaps of 100 and 150 μm . The inter-electrode gap was found not to affect the height at which the vortex-driven trapping behaviour gives way to sedimentation-based trapping, but the smaller gap was seen to limit the number of particles in the trap, possibly due to the higher field intensity producing greater fluid velocities, reducing the area on which particles can come to rest. When examining the effect of density and viscosity of the medium on the behaviour of particles, it was found that trapping in the pad occurred at a medium viscosity of 17 mPa s in a medium where the particle is neutrally buoyant.

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Tables and figures

Table 1. Media used in the density/viscosity experiment

I–III, isopycnic media; IV–VI, isoviscous media.

	Electrolyte	ρ	η
I	Sucrose 12%	1.05	1.429
II	Glycerol 20%	1.05	1.737
III	Ethylene glycol 36%	1.05	2.537
	Medium	ρ	η
IV	Sucrose 22%	1.01	2.1
V	Ethylene glycol 31%	1.03	2.1
VI	Glycerol 26%	1.14	2.1

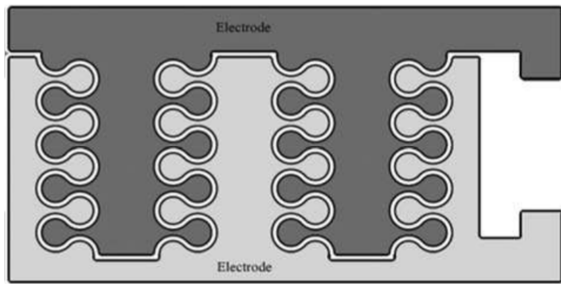


Figure 1. A schematic diagram of the electrode geometry used. Electrode “pads” were defined by a radius of $250\ \mu\text{m}$ from centre to edge, and the gap between adjacent electrodes varied between 100 and $150\ \mu\text{m}$ in different experiments.

image

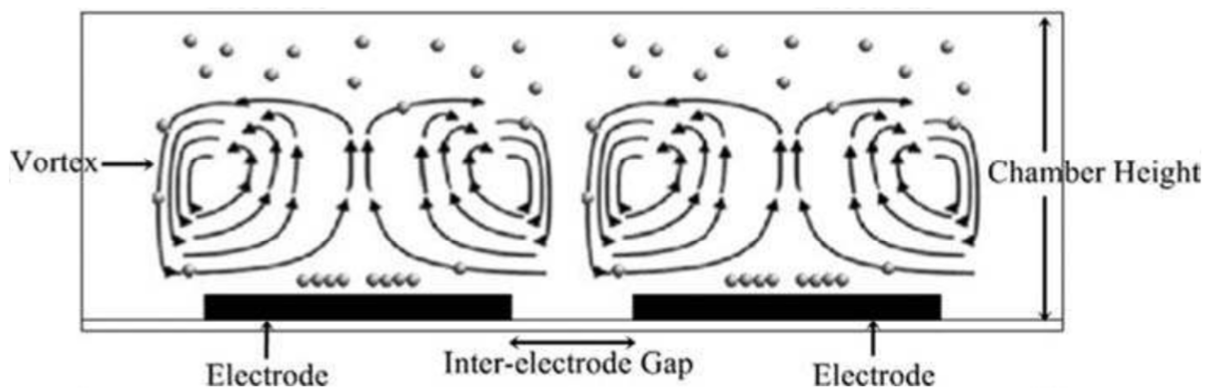


Figure 2. A schematic illustration of particle trapping using ACEO-driven vortices. Vortices are generated at the electrode surfaces nearest the inter-electrode gap. Particles trapped within the vortices are pulled to the neutral point at the electrode centre; those in the volume above the vortices are only trapped when they “fall” into the vortices by sedimentation.

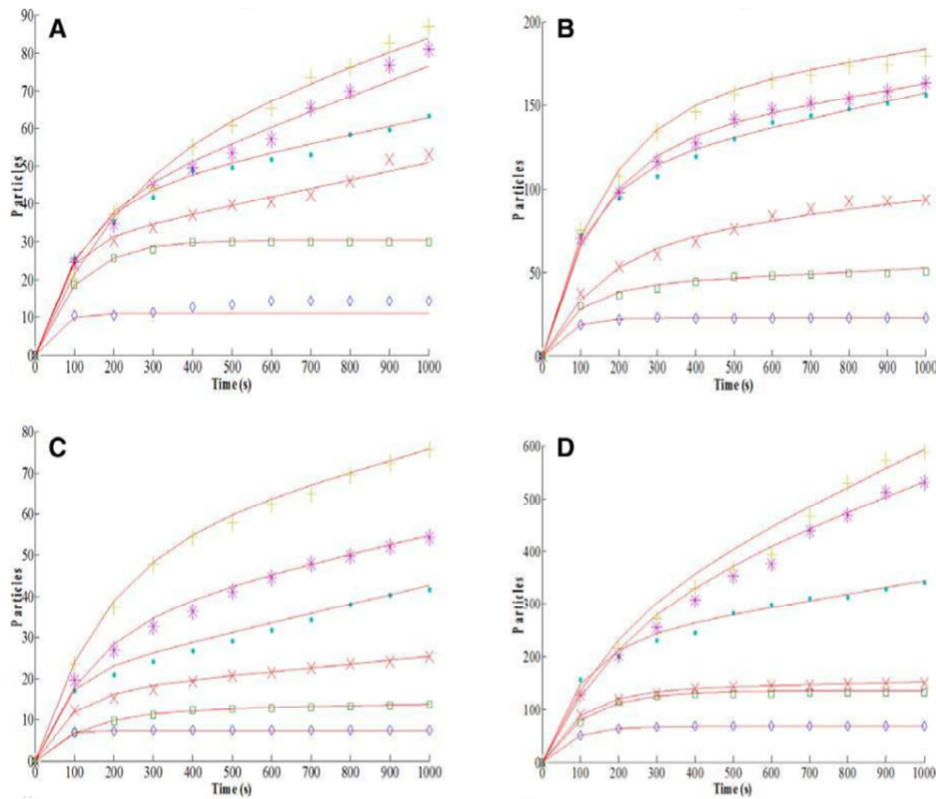


Figure 3. Particles collected on 500 μm diameter electrodes over a 1000-s period, for different chamber heights, gap sizes and concentrations. For all figures, \diamond corresponds to a chamber height of 130 μm; \square , 260 μm; \times , 390 μm; \bullet , 650 μm; $*$, 910 μm; $+$, 1300 μm. The four subfigures correspond to the following combinations of inter-electrode gap and particle concentration: (A) 100 μm/104 beads/mL, (B) 100 μm/105 beads/mL, (C) 150 μm/104 beads/mL, (D) 150 μm/105 beads/mL. The solid lines represent the best fit using Eq. (1) and the parameters in Fig. 3.

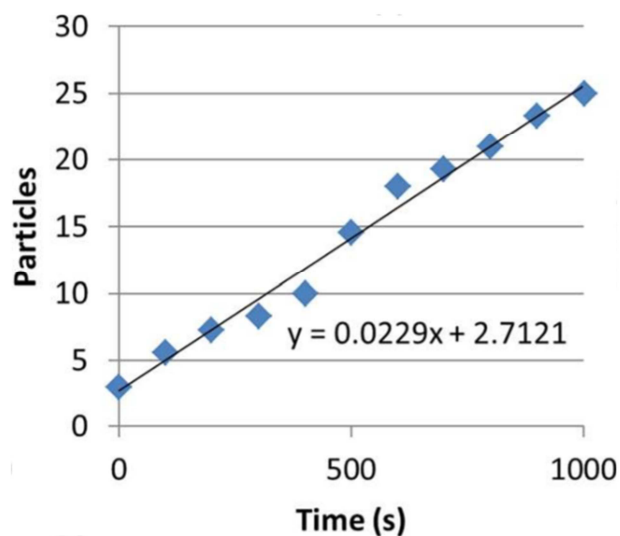


Figure 4. An example of particle sedimentation. Particles were suspended in an electrode chamber with 1300 μm height at a concentration of 104 particles/mL, and observed over 1000 s. The graph

represents the average of three experiments and shows near-linear collection of particles at the bottom of the chamber.

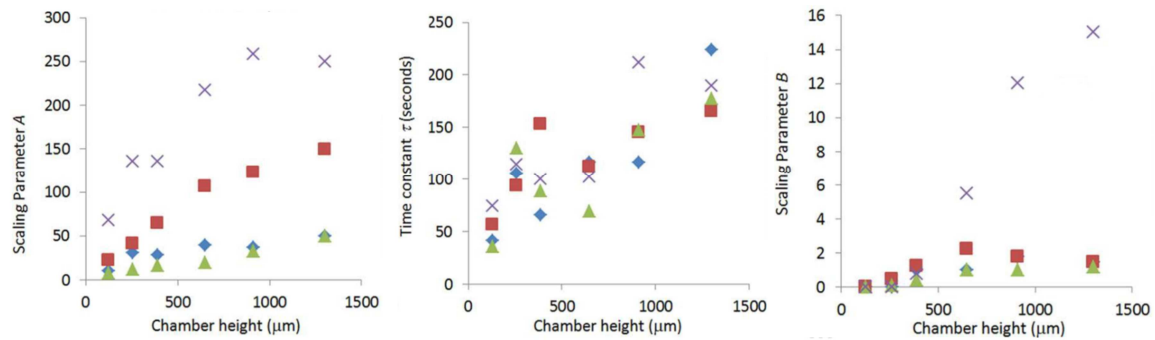


Figure 5. The best-fit parameters to the data using Eq. (1) for various chamber heights. (Top) scaling parameter, A; (middle) time constant, τ ; (bottom) scaling parameter, B. Vertical axes are in arbitrary units except for (B) in seconds. (◆) 100 μm /104 beads/mL, (■)100 μm /105 beads/mL, (▲) 150 μm /104 beads/mL, (×) 150 μm /105 beads/mL.

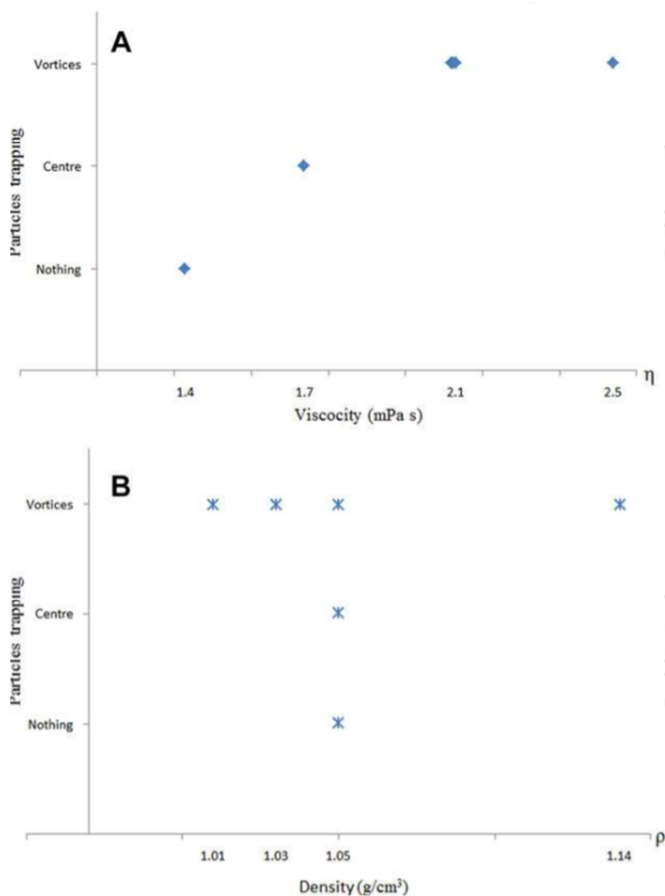


Figure 6. Pattern of particles trapping in zipper electrodes system as a function of (A) viscosity and (B) density for five solutions detailed in the text.

