Isotachophoresis with emulsions

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An experimental study on isotachophoresis (ITP) in which an emulsion is used as leading electrolyte (LE) is reported. The study aims at giving an overview about the transport and flow phenomena occurring in that context. Generally, it is observed that the oil droplets initially dispersed in the LE are collected at the ITP transition zone and advected along with it. The detailed behavior at the transition zone depends on whether or not surfactants (polyvinylpyrrolidone, PVP) are added to the electrolytes. In a system without surfactants, coalescence is observed between the droplets collected at the ITP transition zone. After having achieved a certain size, the droplets merge with the channel walls, leaving an oil film behind. In systems with PVP, coalescence is largely suppressed and no merging of droplets with the channel walls is observed. Instead, at the ITP transition zone, a droplet agglomerate of increasing size is formed. In the initial stages of the ITP experiments, two counter rotating vortices are formed inside the terminating electrolyte. The vortex formation is qualitatively explained based on a hydrodynamic instability triggered by fluctuations of the number density of oil droplets. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4816347>]

I. INTRODUCTION

Isotachophoresis (ITP) is an analytical technique for the concentration and separation of ionic samples.¹ It is based on two different electrolytes, a leading (LE) and a terminating (TE) electrolyte, with co-ions of different electrophoretic mobilities. When an electric field is applied, the LE co-ions migrate in front of the TE co-ions, while a sharp transition zone between the two electrolytes is formed. Samples can be concentrated between these or stacked in the order of their electrophoretic mobilities, providing a method for analytical separation. Probably, the most important implementation of ITP is capillary ITP where the electric field is applied along a capillary filled with a suitable electrolyte system. A review of current activities in the field of capillary ITP is provided by Malá *et al.*²

Current technologies allow transferring ITP protocols into a microfluidic chip format. In that context, ITP has proven a powerful method for the concentration, separation, and detection of analytes. Microchip-ITP was demonstrated in conjunction with different detection methods.^{3,4} One important advantage of microchip ITP over conventional capillary ITP is the freedom to design tailor-made channel geometries or networks^{5–7} with channel diameters that may be reduced to the submicron or nanoscale.⁸ An important application of microchip ITP is the preconcentration of samples for subsequent processing in microfluidic assays.^{9–12} Furthermore, the freedom to design a large variety of channel architectures opens up novel application areas. ITP may as well be viewed as a technique to transport and manipulate minute samples in a manner similar to digital microfluidics,¹³ i.e., without any appreciable sample dispersion or dilution. In that context, it has been demonstrated how ITP samples may be split in a well-controlled manner at a Y-junction.¹⁴ Moreover, the contacting (mixing) of two ITP samples was established, together with hybridization reactions of single stranded DNA molecules during sample contacting.¹⁵

Yet another novel pathway of utilizing ITP opens up through protocols involving nano- or microparticles. Also, nanoparticles form a distinct ITP zone, an effect that may be employed to determine the concentration of nanoparticle solutions.¹⁶ Microparticles accumulate at an ITP transition zone, depending on their size.¹⁷ Bigger particles are transported along with the transition zone, while smaller particles are left behind. That way a size separation can be achieved.

The present paper is loosely connected with these studies of ITP with microparticles. It reports fundamental studies of the transport processes occurring when the LE is not a single-phase medium but an emulsion with droplets of micron-sized diameter. To the best of the authors' knowledge, such a system has never been examined before. Compared to the case with particles suspended in the LE, a system containing droplets is significantly more complex. Surfactants may readily adsorb to the liquid-liquid interface, electrophoretic droplet transport may be accompanied by a recirculating flow inside the droplet, and two droplets may coalesce. For these reasons, it appears necessary to establish a map of the qualitative behavior of such systems in different regions of the parameter space defining the properties of the fluids and the microchannel walls as well as the operating conditions applied in the experiments. The purpose of the present report is to take first steps in that direction.

II. INSTRUMENTATION, MATERIALS AND EXPERIMENTAL PROCEDURE

A. Instrumentation

The experiments were performed using a cross channel microfluidic chip made from ultra precision milling of cyclo olefin polymer (COP) (Fig. 1). All channels have a cross section of $20\text{ }\mu\text{m}$ (depth) $\times$ $50\text{ }\mu\text{m}$ (width) and were covered with a $100\text{ }\mu\text{m}$ COP foil by solvent bonding. After bonding, the chips were tempered for 20 h in vacuum at $80\text{ }^{\circ}\text{C}$.

Imaging was performed in brightfield mode with an inverted microscope (NIKON eclipse TI) with $10\times/0.50$ or $40\times/0.93$ S Fluor NIKON objectives. A Lab Smith HVS 448 6000D HV power supply was used to apply the electric field. To establish electric connections, platinum wires of 0.3 mm diameter were dipped into the buffer reservoirs of the chip. The viscosities of the electrolytes were measured with a Brookfield DV III Ultra rheometer with cone CPE-40 at 100 rpm. Images were recorded with the Motion Pro Y4 camera (Imaging Solutions GmbH), with data acquisition at 12 fps. For higher frame rates, an ANDOR iXon DU8897D camera was used, with data acquisition at 200 fps. The experimental setup is shown in Fig. 2.

B. Materials

In all experiments, the leading electrolyte consisted of 0.01 M formic acid (Carl Roth 4724.3) and 0.005 M Histidine (Fluka 53330) dissolved in Milli-Q water (Millipore), pH 4.0.

FIG. 1. Schematic of the microfluidic chip. The channels have a $20\text{ }\mu\text{m}$ (depth) $\times$ $50\text{ }\mu\text{m}$ (width) cross section.

FIG. 2. Schematic of the experimental setup.

The terminating electrolyte (TE) consisted of 0.008 M MES and 0.005 M Histidine, dissolved in Milli-Q water, pH 6.0. The LE and TE solutions were filtered with $0.2\ \mu\text{m}$ pore size syringe filters (VWR International GmbH). In some experiments, 1% (w/v) polyvinylpyrrolidone (PVP) (Merck 1.07370.0100) was added to the solutions to suppress electroosmotic flow in the microchannels. The choice of electrolytes was dictated by the requirements that there should be a large jump in electric field strength across the ITP transition zone (also termed ITP interface), while the corresponding change in pH should be small. The latter is related to the fact that, in the experiments without PVP, the dispersion of the ITP transition zone due to differences in the electroosmotic flow (EOF) velocity between the LE and the TE should be suppressed as much as possible.^{18,19} For the given system, the electrophoretic mobility of the LE co-ions is about a factor of two larger than that of the TE co-ions. The viscosity of the LE was determined to be $0.91 \pm 0.01\ \text{mPa s}$ without PVP and $2.34 \pm 0.11\ \text{mPa s}$ with PVP. The corresponding values for the TE are $0.90 \pm 0.01\ \text{mPa s}$ without PVP and $2.43 \pm 0.10\ \text{mPa s}$ with PVP.

In most experiments, an oil-in-water emulsion was formed based on the LE. For that purpose, $1\ \mu\text{l}$ immersion oil (Merck 104699) was dispersed into $1000\ \mu\text{l}$ LE and ultrasonicated for 5 min using an ultrasonic cleaner (VWR International GmbH). This results in an oil-in-LE emulsion with droplet diameters between 1 and $10\ \mu\text{m}$. In some of the experiments in which only a single electrolyte solution is used, the same procedure is applied to an immersion oil droplet in TE, yielding an oil-in-TE emulsion with droplet diameters between 1 and $10\ \mu\text{m}$.

C. Experimental procedure

Two different classes of experiments were performed. In the actual ITP experiments, oil-in-LE emulsions were considered. Additionally, experiments with only a single electrolyte (either LE or TE) containing oil droplets were conducted.

In the ITP experiments, priming of the microfluidic chip was performed by filling the North (N) and West (W) reservoirs with TE, the East (E) reservoir with LE, and applying vacuum to the South (S) reservoir for 2 min. Subsequently, all reservoirs were emptied, after which the N and W reservoirs were refilled with $40\ \mu\text{l}$ TE, and the E reservoir with $40\ \mu\text{l}$ oil-in-LE emulsion. Then vacuum was applied at the S reservoir for 30 s, after which this reservoir was filled with $40\ \mu\text{l}$ TE to avoid differences in hydrostatic pressure. The state of the chip before electric fields are applied is shown in Fig. 1. To start the experiment, the electrodes are introduced into the reservoirs and a voltage is applied between the W (cathode) and E (anode). To follow the ITP transition zone, the xy-stage is moved with a speed compensating the electrophoretic velocity.

In the experiments in which only a single electrolyte (either LE or TE) is used, the following procedure was applied. First, all reservoirs were filled with either LE or TE, depending on

which electrolyte is considered. Then, vacuum was applied at the S reservoir for 2 min. Subsequently, all reservoirs were emptied, after which the N and W reservoirs were refilled with either 40 μl TE or LE, while the E reservoir was refilled with either 40 μl oil-in-TE or oil-in-LE emulsion. Then, vacuum was applied for 30 s at the S reservoir, and the S reservoir was refilled with either 40 μl LE or TE. To start the experiment, the electrodes are introduced into the reservoirs and a voltage is applied between the W and E reservoirs. In these sets of experiments, the *xy*-stage remains fixed.

III. RESULTS

A. Electrophoretic motion of single droplets

Before studying the phenomena occurring in oil-in-LE emulsions as a whole, the electrophoretic motion of single droplets was examined. This was done both for droplets suspended in a single electrolyte system (either LE or TE) and for droplets being observed during ITP experiments. In each case, the droplet velocity under the influence of an electric field was determined using the tracking tool of the NIS Elements AR software (NIKON).

1. Droplet motion in single electrolytes

Generally, the presence or absence of PVP in the solutions has a substantial influence on the observed droplet motion. For this reason, the results with and without PVP are reported in separate paragraphs.

a. Electrolytes without PVP. Tracking was performed over an average distance of about 700 μm per droplet. A voltage of 500 V was applied along the 35 mm long channel. Based on a sample of at least 10 droplets, the following velocities were determined (we report mean value $\pm$ standard deviation). LE: $223 \pm 47 \mu\text{m s}^{-1}$. TE: $177 \pm 37 \mu\text{m s}^{-1}$. In each case, the droplets migrate in the direction of the cathode. In this and the following, velocities with a positive sign indicate migration towards the cathode, while negative velocities denote transport towards the anode. In none of the experiments in which droplet velocities were measured, a significant dependence of the velocity on the size of the droplets could be identified.

b. Electrolytes with PVP. Tracking was performed over an average distance of about 300 μm per droplet. It was found that the droplet velocities are substantially smaller than in the case without PVP. Under the same electric field strength as above and based on a sample of 5 droplets, the following velocities were determined. LE: $5.4 \pm 0.6 \mu\text{m s}^{-1}$. TE: $6.4 \pm 1.5 \mu\text{m s}^{-1}$. In each case, the droplets migrate in the direction of the cathode. Hence, in electrolytes with PVP, the measured droplet velocities are only about 2%–3% of the velocities in systems without PVP. The main reason for this difference probably lies in the EOF that is generated if no PVP is present, although a substantial contribution from electrophoretic droplet motion cannot be ruled out. PVP acts as a surfactant coating the walls of a microchannel if the channel is flooded with a PVP-containing solution.²⁰ To determine the influence of EOF, the current-monitoring method was used, i.e., the electric current through a channel was measured as a function of time while displacing one electrolyte by a second one.²¹ In order to obtain a suitable signal-to-noise ratio for the current-measurement, a 1:1 dilution of the respective electrolytes with Milli-Q water was used as displacing electrolyte. In these experiments, all electrolytes were free of dispersed oil. In solutions without PVP, an EOF directed towards the cathode was determined. For the applied voltage of 500 V, EOF velocities of $336 \pm 13 \mu\text{m s}^{-1}$ for the LE and of $820 \pm 100 \mu\text{m s}^{-1}$ for the TE were measured (in each case averaged over three measurements in a single chip). In an order-of-magnitude manner, these velocities agree with the droplet migration velocities observed in electrolytes without PVP. In that context, it should be noted that the experiments reported in this article were performed with ten different microfluidic chips. Using a variety of chips is necessary because once the polymer surface was exposed to a specific solution, the low chemical and thermal resistance of the substrate usually does not allow to completely remove deposits

from the channel walls. However, replacing a used chip by a fresh one also introduces some scatter due to potential variations on the polymer morphology (chain length) or effects of material ageing. In fact, when measuring EOF velocities with different chips, substantial relative variations of the order of $\pm 40\%$ are observed. In addition to that, the EOF measurements were done without oil droplets. It cannot be ruled out that some coating of the channel walls with oil during the droplet velocity measurements has altered the level of EOF in the channel. In that sense, the measured EOF velocities in systems without PVP are roughly consistent with the corresponding droplet migration velocities. In other words, in these cases, much of the observed droplet transport appears to be due to droplets advected along with the underlying EOF and superposed by some electrophoretic droplet motion.

In PVP containing solutions, no EOF was measureable with the current-monitoring method. Consistent with what is stated in the previous paragraph, the corresponding droplet velocities are much smaller than in the cases without PVP. The actual electrophoretic droplet velocities are only of the order of some micrometers per second and are directed towards the cathode. Owing to the superposition of three different effects, it is difficult to predict how exactly the addition of PVP influences the electrophoretic droplet motion. First, PVP can be adsorbed to the oil-water interface and change the charge of a droplet. Second, this adsorption can lead to an immobilization of the interface, which in turn changes the hydrodynamic resistance. Third, the viscosity of the electrolytes is increased by roughly a factor 3 when adding PVP.

2. Droplet motion during ITP

a. Electrolytes without PVP. Again, experiments were performed with an applied voltage of 500 V, but now using the experimental procedure described in Sec. II C, i.e., employing ITP. In such cases, it has to be taken into account that even for a fixed applied voltage, the values of the electric field strength inside the LE and TE are a function of time, depending on the position of the ITP transition zone.¹⁷ To render droplet-velocity measurements well-defined, it needs to be specified where the ITP transition zone is located when the velocity is measured. All droplet velocities reported in the following were evaluated while the ITP transition zone was within the first 10 mm after the injection point at 7.5 mm of the 35 mm long microchannel.

Oil droplets inside the LE migrate in the direction of the cathode. Based on a sample of 10 droplets, the migration velocity was determined to be $49 \pm 7 \mu\text{m s}^{-1}$. Within the TE, no droplets were found. Compared to the experiments with only a single electrolyte, the droplet velocity is reduced by about a factor of 4.5. A potential reason for this reduction is the increased tendency of droplets to be deposited at the microchannel walls compared to a situation with only a single electrolyte. Corresponding oil films at the walls diminish the underlying EOF driving the droplets. Further details related to the formation of oil films will be given below in Sec. III B. As part of the same experiments, the velocity of the ITP transition zone was measured, resulting in $-94 \pm 4 \mu\text{m s}^{-1}$ (based on a series of three independent measurements).

b. Electrolytes with PVP. When PVP is added to the system, the droplet velocities get substantially reduced. Under an applied voltage of 500 V, oil droplets inside the LE migrate in the direction of the cathode, with a velocity of $14 \pm 3 \mu\text{m s}^{-1}$ (based on a sample of 10 droplets). This value is larger than the velocity previously determined for a system with only a single electrolyte but of the same order of magnitude. The difference could result from residual advective velocities due to the incomplete suppression of the comparatively large (without PVP) EOF of some hundred μm per second. In any case, the results indicate that the droplet velocities in ITP experiments without PVP are to a substantial part due to the underlying EOF. The corresponding velocity of the ITP transition zone was determined to be $-175 \pm 16 \mu\text{m s}^{-1}$ (based on a series of three independent measurements). Apparently, the ITP interface travels faster into the direction of the anode than in systems without PVP. This can be explained by the extensive suppression of EOF which counteracts the electrophoretic transport of ions and therefore reduces the velocity of the ITP interface.

The droplets change their direction of motion when they meet the ITP interface. They get collected at the interface, forming a plug with an increasing number of droplets and move along with it. Further details about this process are given below in Sec. III B. Occasionally, a droplet is left behind (e.g., because of interaction with the channel wall), penetrating into the TE. Droplets inside the TE are found to migrate in the direction of the anode, with a velocity similar to that of the ITP transition zone, i.e., the velocity magnitude is much higher than for droplets inside the LE. Such high droplet velocities seem surprising in systems in which EOF is largely suppressed. Moreover, droplet velocities in single electrolytes containing PVP have already been determined. The corresponding results reported above do not give any evidence for such high droplet velocities. However, it has to be noted that in an ITP experiment, the composition of the TE is different from the TE solutions as prepared, a fact that has already been explained by Kohlrausch.²² For this reason, the concentration and pH profiles forming during an ITP experiment were computed using the open-source simulation software SPRESSO (Stanford Public Release Electrophoretic Separation Solver). From the simulations, the MES and histidine concentrations dynamically arising were obtained, and an adjusted TE electrolyte was prepared using the same concentrations. Again, measuring droplet migration in experiments with corresponding single electrolyte systems did not give any evidence for velocities as high as the ITP interface velocity. Furthermore, the droplets inside the adjusted TE still migrate into the direction of the cathode.

To explain the unexpected behavior of droplets inside the TE in the ITP experiments, two additional effects that may occur in such systems were considered. On the one hand, it had previously been observed that ambient carbon dioxide may get dissolved and hydrated in aqueous electrolytes, forming carbonic acid.²³ Via dissociation of carbonic acid, carbonate and bicarbonate ions are formed that may concentrate between the LE and TE, meaning that droplets could find a chemical environment significantly different from that of the prepared electrolytes. Therefore, additional experiments were conducted in which 7 mM Ba(OH)₂ was added to the TE. Ba(OH)₂ reacts with potential carbonate and bicarbonate ions, producing barium carbonate that precipitates from the solution. Even within the accordingly modified TE, droplets still migrate towards the anode, with a speed comparable to that of the ITP transition zone. Therefore, a potential contamination with carbonate and bicarbonate ions cannot explain the unexpected droplet migration.

As will be detailed in Sec. III B, during the ITP experiments, an agglomerate of droplets forms at the ITP interface. For a dense enough packing of droplets, it is expected that the agglomerate acts like a piston, pushing the liquid in front of it through the channel and dragging the one behind it. The corresponding flow would drag along droplets inside the TE with a velocity comparable to the ITP interface. However, at the same time, also the droplets inside the LE would be advected towards the anode. A corresponding motion of droplets inside the LE is not observed. Therefore, the flow due to a droplet agglomerate acting like a piston can be ruled out as a possible reason for the unexpected droplet motion inside the TE. At the current stage, no explanation for the droplet motion inside the TE in ITP experiments with PVP seems to be available. To cast some light on the underlying phenomena, detailed studies going beyond the scope of the present article are needed.

B. Accumulation of droplets at the ITP interface

1. Systems without PVP

Initially, all of the oil droplets are inside the LE. During ITP, the transition zone migrates into the direction of the anode and the droplets into the direction of the cathode. When the droplets meet the ITP interface, they get collected, reverse their direction of motion, and are transported along with the interface. This process is shown schematically on the left side, and as a series of snapshots from the experiments on the right side of Fig. 3. The forces acting on the droplets close to the ITP transition zone are of the same nature as the forces on polymeric microparticles.¹⁷ Considering that the dielectric permittivity of the electrolyte is much larger than that of oil, the force can be approximated as¹⁷

FIG. 3. (a) Schematic of oil droplet accumulation at the ITP transition zone. (b) Micrographs taken at different times showing the accumulation of oil droplets at the ITP transition zone in a system without PVP.

$$F_{ITP} \approx -2\pi r^3 \varepsilon \frac{E_{LE}^2 - E_{TE}^2}{\Delta x}, \quad (1)$$

where r is the droplet radius, ε is the dielectric permittivity of the electrolyte, Δx is the width of the ITP transition zone, and E_{LE} , E_{TE} are the values of the electric field strength inside the LE and TE, respectively. F_{ITP} should be understood as the maximum force an ITP interface can exert onto a droplet. The actual force a droplet experiences depends on its relative position with respect to the ITP interface. On top of that, there is at least one additional effect that may play a role. If the electrophoretic mobilities of droplets inside the LE and TE are different and such that droplets inside the TE move into the same direction as the ITP interface, but with a higher speed, the result will also be that droplets are picked up by the ITP interface and move along with it. The nature of this electrophoretic force is very different from the one appearing in Eq. (1), the latter being the sum of the dielectrophoretic force and the force due to electrohydrostatic pressure. Modeling the electrophoretic force onto droplets traveling between two different electrolytes is quite challenging, since the description will have to take into account memory effects due to the finite time it takes to recharge a droplet. Therefore, a detailed and complete analysis of the forces acting on droplets close to ITP transition zones is beyond the scope of the present experimental work.

As more and more droplets get collected at the ITP interface, a multi-droplet agglomerate of increasing size is formed, as can be seen on the right side of Fig. 3. There is, however, a limit to the growth of the droplet cluster. Quite frequently, two droplets inside the cluster merge and form a larger droplet. It was also observed that especially smaller droplets stick to the channel walls. Fig. 4 displays three different time series showing the progression of droplet coalescence and subsequent oil film formation. In part (a) the encircled droplets merge and form a larger droplet. In part (b), a droplet (indicated with an arrow) increases its size by multiple coalescence events. After having reached a specific size, the droplet disintegrates and forms a film at the channel wall. Part (c) shows the film formation more clearly.

FIG. 4. (a) Time series showing the coalescence of two droplets. (b) Time series showing the growth of a droplet by successive coalescence events. (c) Time series showing the formation of an oil film after droplet growth.

Effectively, in the absence of surfactants, ITP with an emulsion as LE induces a transformation between configurations in which the oil phase exists as droplets and as a film at the channel wall. The transformation is induced by coalescence events inside the droplet agglomerate close to the ITP interface, leaving an oil film behind. This process has been studied in more detail by following the evolution of 12 individual droplets inside the cluster. Fig. 5(a) shows the increase of the droplet diameter as a function of time as the average taken over the sample of 12 droplets. The data are nicely fitted by a linear curve. The maximum diameter achieved is roughly $20\text{ }\mu\text{m}$, after which the droplets disintegrate and form a film at the channel walls. The distribution of droplet growth rates is displayed in part (b) of the figure, giving an average of $0.87 \pm 0.22\text{ }\mu\text{m s}^{-1}$. Part (c) shows the distribution of the maximum droplet diameters achieved right before film formation takes place. The corresponding average is $18.9 \pm 1.1\text{ }\mu\text{m}$. The fact that the droplets reach a maximum diameter of around $20\text{ }\mu\text{m}$ before they get deposited as a film at the channel walls can be simply explained by the cross-sectional geometry of the micro-channel, having a depth of $20\text{ }\mu\text{m}$. When the droplet diameter reaches the channel depth, the droplet starts touching the upper and lower channel wall. With no surfactants at the walls and at the oil-water interface, there is little repulsion that could prevent wetting of the walls by the oil phase, and film formation takes place.

2. Systems with PVP

The ITP dynamics in systems with PVP differs from what is described above mainly by the suppression of droplet coalescence. The droplets are much more stable, and coalescence or attachment of droplets to the channel walls followed by film formation is largely suppressed. This is most likely due to PVP getting adsorbed at the oil-water interface, thereby stabilizing

FIG. 5. (a) Droplet diameter as a function of time owing to successive coalescence events, based on a sample of 12 droplets. (b) Corresponding distribution of droplet growth rates. (c) Corresponding distribution of maximum droplet diameters before merging with the channel walls.

the droplets. A time series showing the droplet accumulation is displayed in Fig. 6. Initially, when only a small number of droplets have been accumulated at the ITP interface, two counter rotating vortices appear inside the TE close to the interface. The vortices were identified through the rotational motion of droplets inside the TE. While in the absence of PVP virtually no droplets are found in the TE, here a few droplets pass over from the LE. Subsequently, an increasing number of droplets accumulate in the vortices (Fig. 6(b)). As more and more droplets get collected at the ITP interface, the two-vortex pattern becomes unstable and transforms into an intermittent configuration with only a single, transient vortex structure (Figs. 6(c) and 6(d)). Subsequently, more and more droplets get collected at the ITP interface. Since coalescence and attachment of droplets to the channel walls are largely suppressed, there is no transition between a dispersed oil phase and a film. Instead, the collection of droplets results in a cluster of increasing size at the ITP interface (Figs. 6(e) and 6(f)).

At this point, the question arises which effect could be responsible for the formation and propulsion of a pair of counter rotating vortices behind the ITP transition zone. A potential mechanism giving rise to advective and transient structures at an ITP interface is the electrokinetic instability occurring in ITP experiments at high values of the electric field strength.¹⁴ The reason for this electrokinetic instability is the space charge density ρ forming in the ITP transition zone, being proportional to the jump in electric field strength across the transition zone. The corresponding body force density acting on the fluid is given by

$$F_{el} = \int \rho E dV, \quad (2)$$

where the integral extends over the whole microchannel, and E is the local value of the electric field strength. The jump in electric field strength across the ITP transition zone is given by $\Delta E \propto \int \rho dx$, where x is parallel to the microchannel, and the integration interval encloses the transition zone. Then simple scaling arguments show that

$$F_{el} \propto U^2, \quad (3)$$

where U is the voltage applied between the E and the W reservoir. For a droplet or a liquid volume inside a vortex, the electric body force is balanced by the Stokes drag which scales

FIG. 6. (a)–(f) Time series showing the formation of a droplet agglomerate in a system with PVP.

linearly with the rotational velocity u_{rot} . For the measured velocity scales, inertial effects are unimportant. Therefore, assuming the electrokinetic instability of the ITP interface being the origin of the observed vortex structures, it follows that

$$u_{rot} \propto U^2. \quad (4)$$

To test this hypothesis, individual droplets inside the vortices were tracked using the NIS Elements software (NIKON). A corresponding droplet trajectory in the lab frame is shown in Fig. 7. The rotational velocities were determined as follows. The x - and y (spanwise)-components of the droplet velocity were determined from the trajectory, after which the ITP velocity was subtracted from the x -component. The instantaneous rotational velocity is then given as $u_{rot} = \sqrt{(u_x - u_{ITP})^2 + u_y^2}$. The idea behind subtracting the ITP velocity lies in assuming that the driving force propelling the vortices is different from the mechanism advecting the droplets along with the ITP interface. The latter has already been discussed in Sec. III B 1. The experimental results for the averaged rotational velocity as a function of applied voltage are displayed in Fig. 8. The data are based on a sample of three different droplets per applied voltage. Apparently, the data are well described by a linear relationship, and there are no indications for a scaling with U^2 . Therefore, based on the available data, the hypothesis formulated in Eq. (4) has to be dismissed.

An alternative qualitative explanation for the formation of vortices can be given based on the schematics of Fig. 9. In the upper frame of the figure, droplets having been accumulated between the LE (dark blue) and TE (light blue) are sketched. For simplicity, it is assumed that

FIG. 7. Trajectory of a single droplet trapped in a vortex behind the ITP transition zone.

FIG. 8. Rotational velocity of droplets inside the vortex structures as a function of applied voltage.

the transition zone is infinitely thin, and that the droplet distribution is homogeneous. In addition, the electric field line distribution is shown schematically. It is now important to note that owing to the much higher dielectric permittivity of the electrolyte compared to the oil phase, the electric field lines do not penetrate into the droplets. Therefore, a fluctuation of the droplet

FIG. 9. Pictorial representation of a possible mechanism explaining the formation of vortices behind the ITP transition zone.

density translates to a fluctuation of the electric field that is focused into regions of lower droplet density. In the center frame of Fig. 9, this is exemplified by a fluctuation that increases the droplet density close to the walls and reduces the density along the centerline of the channel. As a result, the droplets in the center experience a higher-than average electric field strength, while those close to the walls are exposed to a reduced field strength. Consequently, the latter are lagging behind the former, as indicated by the white arrows. Through the y -components of the electric field, the droplets lagging behind are driven to the center of the channel and finally back to the ITP interface, leading to the formation of vortices (lower frame of Fig. 9). If the droplet velocity scales as u_{ITP} (which itself scales as U), it is expected that this mechanism results in a rotational velocity scaling as U , in agreement with the experimental results.

It should be emphasized that this attempt to explain the formation of vortices is still of very preliminary nature and needs further corroboration. Besides the fact that up to now the effort is quite qualitative, several questions are left open. The picture drawn above is that of a hydrodynamic instability driven by fluctuations of the droplet density. For example, the nature of the instability (linear or nonlinear) is not known, and it is unclear how the mode selection takes place, i.e., why exactly the rotational pattern found in the experiments (and sketched at the bottom of Fig. 9) is selected and not a pattern with a different number of vortices or direction of rotation. An analysis of these problems goes beyond the scope of the present article but will be worth tackling in future work.

IV. SUMMARY, CONCLUSIONS, AND OUTLOOK

The phenomena occurring when isotachopheresis is performed with an emulsion as leading electrolyte were studied experimentally. The different types of phenomena were described, and it was explained under which conditions they appear. Generally, oil droplets (diameters between 1 and 10 μm) dispersed inside the leading electrolyte are picked up by the ITP transition zone and carried along with it.

How exactly the accumulation of droplets at the ITP interface proceeds depends on whether or not surfactants are present in the system. The experiments were performed both with and without PVP, a surfactant that both adsorbs at the channel walls and at the oil-water interface. In systems without PVP, coalescence between droplets having been accumulated at the ITP interface is observed. Via coalescence, the droplet size increases until it reaches the scale of the microchannel depth. After that, the droplets merge with the channel walls, leaving an oil film behind. Effectively, the ITP interface triggers a transition between an emulsion and an oil film covering the channel walls.

The situation is different when PVP is added to the electrolytes. In that case, droplet coalescence and the transformation of droplets into an oil film are largely suppressed. The droplets being accumulated at the ITP interface stay intact and form an agglomerate of increasing size. In the initial stages of droplet accumulation, a pair of counter rotating vortices is formed inside the terminating electrolyte close to the ITP interface. The dynamics of these vortices was analyzed, and a qualitative explanation for vortex formation was offered which is based on a hydrodynamic instability triggered by fluctuations of the droplet density.

To the best of the authors' knowledge, this is the first analysis of the phenomena occurring when isotachopheresis is performed with an emulsion as leading electrolyte. It is hoped that this article helps paving the way to further studies in that area and, potentially, also to applications of this novel type of ITP.

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