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6 Concluding remarks

6.1 General discussion and conclusions



In this thesis, we have evolved a microfluidic methodology for particle separation based on a continuous-flow phenomenon known as flow-induced electrokinetic trapping (FIET). The concept of FIET was originally introduced by Lettieri *et al.* as a novel microfluidic approach for particle trapping [1]. The approach involves the opposition of pressure-driven (PF) and electroosmotic (EOF) flows [2] in a straight narrow channel whose width expands linearly at both ends. The opposition of these two flows leads to the generation of a bidirectional flow profile along the straight segment, with the PF and EOF dominating at the center and near the channel walls, respectively. At the ends of the channel, the flow adopts an unusual recirculating profile that, under the right pressure and electrokinetic conditions, drives particles dispersed in the fluid to reside (mostly) inside the straight segment. The trapping potential of this approach thus lies in the continuous bidirectional motion of particles as a result of the combined hydrodynamic and electrokinetic effects acting within the straight segment and its expanding ends. This principle had been qualitatively exploited by Jellema *et al.* as a particle separation strategy on the basis of surface charge [3] and size [4]. Here, we present an empirical model based on particle velocity to optimize, characterize and accomplish high-performance fractionations of binary and ternary polymer microbead mixtures using the FIET principle.

This model is described in detail in **Chapter 3**, where the trapping process for a single-type polymer-particle dispersion (2.69- μm -diameter carboxylated polystyrene) is fully characterized. For this, the distribution of particles trapped throughout the separation channel was investigated under different bidirectional flow velocity conditions. This study was initially conducted at constant applied pressure while progressively tuning up the applied potential 10 V at a time in a stepwise manner. It was observed that under these conditions, particle distributions inside the straight channel segment exhibited a Gaussian distribution as a function of the applied voltage, determined by the acquired

velocity in the bidirectional flow. This Gaussian behavior ultimately defined the range of applied voltage within which the trapping process occurred, as well as the trapping efficiency expressed as the total number of particles captured with respect to the actual concentration in the fluid. The effect of the applied pressure on the distribution of beads along the channel length was explored for two particle sizes (2.69- and 3.90- μm -diameter carboxylated polystyrene beads). Applied pressures above a certain threshold value were found to significantly alter the homogeneity of the trapping process, leading to random and unpredictable trapping conditions. Lastly, the enrichment capacity of the employed FIET microchannel was evaluated at different concentrations of 2.69- μm -diameter carboxylated polystyrene beads.

The characterization of the trapping phenomenon based on a Gaussian particle distribution model was harnessed in **Chapter 4** to describe and perform separations of binary particle mixtures. In this chapter, we looked at two different types of separations based on differences in particle size and surface charge (expressed in terms of zeta potential, ζ_p).

FIET size-based separations bear close resemblance to the concept of hydrodynamic chromatography, where the separation of different-sized particles is achieved as a result of the acquired velocity in the different streamlines of the flow [5, 6]. In FIET, larger particles are able to sample the more central hydrodynamic flow streamlines, gaining a net velocity in the PF direction. This separation modality was studied using a binary mixture of 2.69- and 5.34- μm carboxylate polystyrene particles (same ζ_p). The distribution curves of the two different-sized particles were recorded at four different applied pressures (1.00, 1.25, 1.50 and 1.75 mbar) using the same stepwise voltage program as above. It was observed that at low applied pressures the distribution curves for both particle types were sufficiently offset that the vast majority of the small-size particle fraction (95% of 2.69- μm beads) exited the channel while the large-size beads still remained trapped. The purity of the collected fractions, however, diminished considerably as the applied pressure was increased. The charge-based separation, on the other hand, was performed using a mixture of 3.02- μm PMMA and 3.54- μm green fluorescent polystyrene particles ($\Delta\zeta_p = 50$ mV). Unlike the previous presented case (hydrodynamic in nature), the separation of particles with different ζ_p depends on the electrophoretic mobility of the beads themselves. The distribution curves for both particle types displayed better separated peaks at higher applied pressures, leading to the less-negative- ζ_p bead fraction (100% of the PMMA beads) to exit the channel segment while the entirety of the more-negative- ζ_p fraction remained trapped.

It has proven essential to establish the optimal trapping conditions individually for each particle type in both separation modalities in order to conduct high-performance

particle fractionations using FIET. This was one of the major difficulties encountered when the proof-of-principle experiments for size- and charge-based separations were first carried out [3, 4]. A nice advantage of this method is the ability to register individual distribution curves during a fractionation experiment, since distributions are determined using image analysis. Unlike other separation approaches employing optical techniques for particle detection such as light absorbance and fluorescence [7–9], particle imaging offers the possibility of individual particle tracking regardless of the physical or chemical properties of the particles.

The separation applicability of this methodology is taken one step further in **Chapter 5** with the simultaneous fractionation of ternary particle samples. Here we describe for the first time the orthogonal separation of particle mixtures having different size and surface charge, exploiting the two FIET mechanisms synergistically. For this, a mixture of 2.69- and 5.34- μm carboxylated polystyrene ($\Delta\zeta_p \approx -46$ mV) and 3.1- μm green fluorescent polystyrene beads ($\Delta\zeta_p \approx -68$ mV) was employed. Initially, the distribution curves for the three constituent particles were recorded at three different applied pressures so as to obtain the optimal fractionation parameters for each particle type. This characterization was then followed by the actual fractionation, which was carried out under increasing applied pressure and voltage in a controlled fashion. The fractionation process revealed well-defined separation dimensions. The first dimension, performed at lower applied pressure, consisted of the size-based separation of the carboxylated polystyrene particles. The second dimension emerged thereafter at higher applied pressure, and consisted of the charge-based separation of the 5.34- μm carboxylated and green fluorescent polystyrene beads.

In this regard, the versatility of FIET combined with the extensive characterization of the trapping process using the presented distribution model has proven to be an accurate approach for high performance particle fractionations. One of the major (and novel) applications of this approach thus lies in the possibility of performing simultaneous particle fractionations based on more than one physical property of the beads themselves in the same microfluidic device. L.C. Jellema, who introduced the proof-of-principle of FIET particle separations, stated in his PhD thesis: *“Another interesting question is whether a sample containing particles of different sizes and/or charges can be separated”* [10]. With our approach, we evince that the answer to this question lies in the fact that the orthogonal (simultaneous size- and charge-based) separation of complex particle mixtures implies two mechanisms, very different in nature, that have to be handled in lockstep. Nonetheless, an initial characterization and optimization of particle trapping conditions is absolutely essential to actually harness these two mechanisms synergically.

Lastly, a reference should be made to the accomplished fractionation efficiency. Generally, the microfluidic sorting approach introduced in this thesis is comparable to many other reported so far intended for size- [11–13] and charge-based [14–16] particle separations in terms of performance standards. Although many of these strategies are capable of high efficiency separations, it is worth highlighting again that very few of them, if any, combine this attribute with the capacity of performing simultaneous fractionations based on multiple particle properties [17].

6.2 Outlook and future perspectives

As mentioned above, the unique sorting mechanisms displayed and great performance make FIET an especially reliable technique in the realm of particle separations. Given the versatility of this technique described throughout thesis, it would seem reasonable to assume that it could be employed for the separation of living cells in the same range as the particles employed in this thesis. Preliminary experiments were done in this regard by Jellema, albeit further characterization was suggested by the author [18]. Unlike colloidal particle systems in which the beads are monodisperse by definition, cells of the same type may exhibit different hydrodynamic sizes, as demonstrated in recent studies [19]. But then, would it be possible to distinguish between two cells of the same type even though they are slightly different in size? Indeed, multiple passive separation approaches have been actually reported so far for cell isolation. Among all of them, a loose analogy can be drawn with the approach reported at Di Carlo’s lab, based on the generation of flow vortices for the isolation of different-sized cancer cells (HeLa ($a_{ave} = 12.4 \mu\text{m}$), MCF7 ($a_{ave} = 20 \mu\text{m}$) [20] and CTCs ($a_{ave} = 10 - 30 \mu\text{m}$)) [21] from red blood cells ($a_{ave} = 7 \mu\text{m}$)¹. This proves that hydrodynamic isolation of cells of the same kind is actually possible despite slight size variation between them. In chapter 4, for instance, we have reported the optimal separation of 2.69- and 5.34- μm carboxylated polystyrene particles at 1 mbar, which was found to be the minimum pressure at which particles exhibited a Gaussian (and therefore predictable) behavior in the trapping channel. If we considered a suspension of cells instead, this experimental outcome would suggest a rough estimation of at least 3- μm -difference needed between the two cell types in the same size range to be collected in different fractions (optimal separation at 1 mbar). Thus, the cut-off size between cells having similar sizes to the particles used in this study should not be greater than 3 μm so as to be collected in the same fraction. Nevertheless, this difference in cell size cut-off value could vary with the individual size of each component of the mixture. We also should keep in mind that

¹ a_{ave} = average diameter.

the capacity of FIET to distinguish between two bead sizes depends not only upon the difference in diameter between one particle and another, but also upon the actual size of each type of bead. All in all, a tailored optimization of the individual trapping conditions must be necessarily done for each cell type to precisely assess the size cut-off size variation below which they can still be isolated in the same collected fraction. A reference should be also made to cell surface charge. Similarly to the particles used in this thesis, chemical groups attached to the membrane make most of cells negatively charged, and so subjected to experience electrophoretic force towards the positive electrode (anode) [22]. This rises the question of how different surface charge can be for two cells of the same type to yet be still collected in the same fraction using FIET. Examples of charge-based separation of different cells have been reported to date by means of electrophoresis [23,24], although no significant difference in charge between cells of the same type has been yet reported in literature to the best of our knowledge. If any, only an experimental characterization could reveal the answer to this question. We believe that cell trapping characterization using the distribution model presented in this thesis could give good insight as to the feasibility of a potential separation of these systems (either size-, charge-based or both). Additionally, cells could be eventually manipulated on-demand as a pretreatment step for other different events to happen thereafter, such as lysis and subsequent analysis of cellular compounds of interest, both physiological and molecular [25–27]. The latter would require the integration of an immunoassay platform inside the microchannel itself, which could be potentially useful for the analysis of a wide range of secreted compounds such as metabolites or biomarkers [28,29].

The applicability of this methodology for cell separation is still to be investigated. Additionally, other particle- (and particle-like-)based systems such as droplets [30] and macromolecules could be also addressed in the future [31].

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