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Design and FEM Simulation of an Asymmetric Pinched Flow Fractionation Microfluidic System for high-Throughput Screening Applications †

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Abstract: The separation of microscopic encapsulates is an area of increasing importance due to applications in a wide variety of fields ranging from the production of cosmetics and pharmaceuticals to the search for new molecules and genetically modified microorganisms. Major challenges are related to the proximity in physicochemical properties of the encapsulating material and the suspension media. We have recently started a research program to search for translocating peptides using a surface display system that locates the possible candidate molecules on yeasts' surface. At this point, the encapsulated yeasts need to be separated and collected for further analyses. An attractive route for separation is microfluidics, as they permit control over the flow rates and interaction times. Here, we explored *in silico* an asymmetric pinched Flow fractionation (AsPFF) microfluidic system for separating particles in the range of 50 and 500 μm. The simulations involved the particle tracing module of COMSOL Multiphysics intending to mainly separate yeasts of 40 μm and liposomes of 200 μm with the encapsulated yeasts. We investigated flow rate ratios in the range of 1:25 to 1:50 over the system's 11 different outlets. The results show separation efficiencies above 90%, which are very encouraging and open the opportunity to further explore this microfluidic system experimentally via low-cost manufacturing via the laser cut PMMA devices developed by us over the past few years. Moreover, this opens the opportunity for improving separation efficiencies in other biological and biomedical applications of interest.

Keywords: particle; separation; microfluidic; Comsol

1. Introduction

High throughput screening (HTS) is a technique commonly employed in pharmaceutical and biotechnology industries that allows identifying compounds with pharmacological or biological activity in a large-scale process. This ultimately facilitates the analysis of thousands to millions of samples in considerably short periods ranging from minutes to a few hours [1]. To achieve this, certain technologies have been developed recently that involve robotics, high-density microplates,

small volume liquid handling, and sophisticated detection schemes [2]. These must provide a compact, traceable, and addressable format to perform thousands of parallel reactions in low volumes of reagents. Moreover, they need to guarantee the ability to manipulate and analyze each sample based on its position, the nature and duration of response to a particular stimulus; acceptable sensitivity for the identification of reduced activity compounds; precise negative and positive controls; reproducibility and stability; and economic feasibility [3,4]. Once the reaction is complete, further manipulation of the sample for analysis or downstream applications is required, which involves the integration of complex collection or sorting methods such as dielectrophoresis (DEP) or fluorescence-activated cell sorting (FACS), incurring in additional fabrication costs [3]. Therefore, there is a need for new high-throughput screening technologies, which require only low sample and reagent consumption, have a low cost, support cell-based screening assays, and provide an easy detection mechanism for sorting and manipulation [2].

Microfluidics is an interdisciplinary area and has a wide variety of applications in medicine, chemistry, electronics, and the biomedical industry [5]. Due to the small volume of fluid used in the microfluidic devices and advantages such as high efficiency, low cost, and environmental compatibility, this approach has been the focus of attention in the last decades [6]. For these reasons, microfluidic devices have found application in precise transportation, preparation, separation, and low volume samples detection. Moreover, they can be exploited for the separation or purification of microscopic objects in samples intended for novel products such as additives for complex formulations in the food chemical, pharmaceutical and petrochemical industries [7]. Microfluidics systems for mixing purposes can be categorized into active and passive. The active separation methods require external force fields such as magnetic, electric, acoustic, or gravitational. In contrast, passive systems do not require additional external forces, need smaller sample volumes, and allow better system integration for point-of-care devices [8].

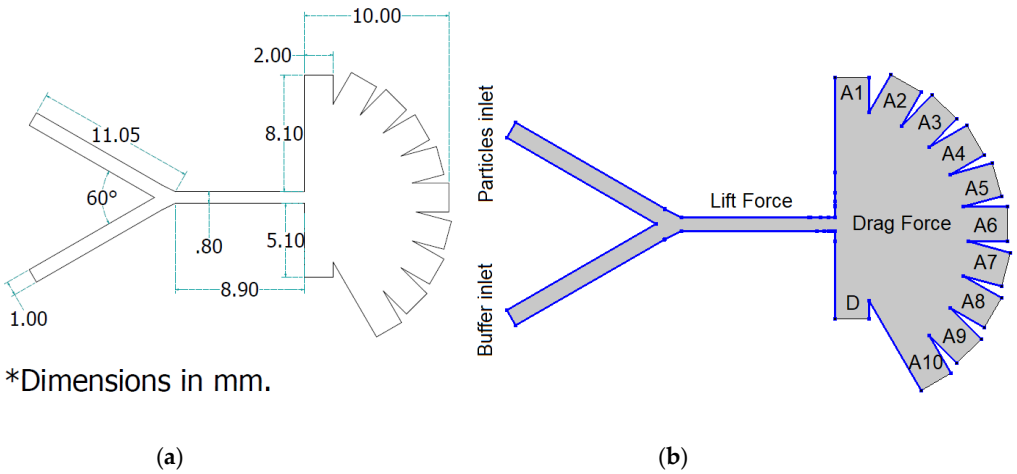
The pinched flow fractionation methods have been used for the continuous size separation of particles in microchannels by taking advantage of the laminar flow regime inside them[9]. One subcategory of these methods was recently reported by Tagaki et al. based on the asymmetric pinched flow fractionation (AsPFF) principle. In this approach, the device consists of microchannels asymmetrically arranged along multiple branches at the end of a pinched segment [10]. This type of separation is based on the behavior of the fluid at the pinched segment, which causes a difference in the particle position near one sidewall of the pinched segment due to differences in size, which is then significantly amplified through its end [10].

Currently, we are working within the advancement of a HTS platform for translocating peptides based on yeast surface display and microfluidics droplet-based encapsulation. Thus, yeast candidates and liposomes will be put together to interact, resulting in the encapsulation of those with membrane translocating activity, for further separation and analysis. Therefore, the purpose of this study was to design and simulate a microfluidic system based on the AsPFF principle for the separation of 40 μm (Yeast), 200 μm (Liposomes), and 500 μm (Droplets) particles mimicking the encapsulated yeasts. The simulation was conducted in COMSOL Multiphysics by setting up the particle tracing module to determine the hydrodynamic parameters to maximize separation efficiencies. Our results indicate that the proposed separation device offers the interest sample purification in high throughput screening of yeast displayed peptides in a microfluidic droplet-based approach.

2. Materials and Methods

2.1. Microfluidic System Design

The design for the AsPFF separation is based on the work by Tagaki et al. [10]. The design presented in Figure 1 shows a system with ten branches (A1-Upper), one drain channel, and a pinched segment of 800 μm width.



(**a**) (**b**)

Figure 1. AsPFF design dimensions (mm) and boundary conditions. The proposed system was based on the work of Tagaki et al. [10] (**a**) Dimensions of the AsPFF system (**b**) Boundary conditions used in the particle tracing module.

2.2. Simulation

The multiphysics simulation was carried out using COMSOL Multiphysics by implementing the particle tracing module on the microchannels as computational domains for laminar fluid flow. The governing equations for laminar flow were the conservation of momentum by the Navier-Stokes equations for incompressible fluids (1) and the conservation of mass by the continuity Equation (2) [11].

$$\nabla[-PI + \mu(\nabla u + (\nabla u)^{\mathrm{T}})] + F = 0 \quad (1)$$

$$\rho\nabla \cdot (u) = 0 \quad (2)$$

where P is the pressure, μ is the dynamic viscosity of the fluid, F the volumetric forces, and ρ is the fluid density. Also, the particle tracing module for fluid flow was coupled. In this case, the movement of the particles is governed by Newton's second law, according to Equation (3):

$$\frac{d(m_P v)}{dt} = F_t \quad (3)$$

where m_P is the particles mass, v the velocity and F_t is the sum of all forces acting on the particles, which in this case were the drag force as defined in (4) by the Stokes law and the lift force, which is described by Equation (5)

$$F_D = \frac{1}{\tau_P} m_P(u - v) \quad (4)$$

$$\tau_p = \frac{\rho_P\, d_p^2}{18\mu}$$

where m_ρ is the particle mass, u is the velocity field, v the particle velocity, ρ_P is the particle density, d_P is the particle diameter and μ the dynamic viscosity.

$$F_L = \rho r_P^4 \beta\big(\beta G_1(s) + \gamma G_2(s)\big)n \quad (5)$$

$$\beta = |D(n \cdot \nabla)u|$$

$$\gamma = \left|\frac{D^2}{2}(n \cdot \nabla)^2 4\right|$$

$$u_{||} = (1 - n \otimes n)u$$

where ρ is the particle density, r_p is the particle radius, s is the normalized distance to the first parallel boundary, n is the unit vector from the nearest point on the first boundary, and D is the width between the walls.

The laminar flow simulations were carried out in a stationary study with a mesh composed of 25,474 domain elements and 1269 boundary elements (Based on the mesh convergence showed in Figure 2). A bi-directionally coupled particle tracing with 400 s study with the multifrontal massively parallel sparse direct Solver (MUMPS) was used in particle tracing module with 1500 particles per specie (Yeasts, Liposomes and droplets). The boundary conditions used for this module are the lift force in the pinched segment, the drag force in all the microfluidic system domain and the upper inlet as the particles inlet of the system.

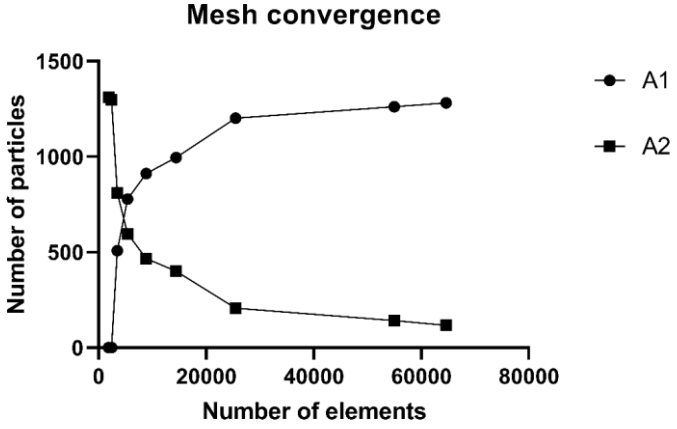


Figure 2. Mesh convergence analysis of the number of particles collected in outlets A1 and A2 at 120 s for nine different meshes: Extremely coarse (1902 elements), Extra Coarse (2409 elements), Coarser (3503 elements), Coarse (5378 elements), Normal (8884 elements), Fine (14,434 elements), Finer (25,474 elements), Extra Fine (55,020 elements) and Extremely Fine (64,644 elements). Finer mesh is selected due to changes in the number of particles collected in both outlets do not exceed 5%.

3. Results

Separation results are shown in Figure 3. It can be noticed that by using the proposed design, the separation of yeasts, liposomes, and droplets was feasible as particles with different sizes could be collected at separate system outlets, thereby indicating a high separation efficiency. All yeast particles in the study were effectively recovered in outlets one and two (A1 and A2) in which particles between 1 μm and 100 μm in diameter can be recovered. In the case of liposomes, the outlet four (A4) allowed the recovery of particles between 180 μm and 220 μm in diameter. Finally, for the droplets (i.e., encapsulated liposomes and yeasts), the preferred exits were outlets five and six (A5 and A6), where particles larger than 420 μm could be collected.

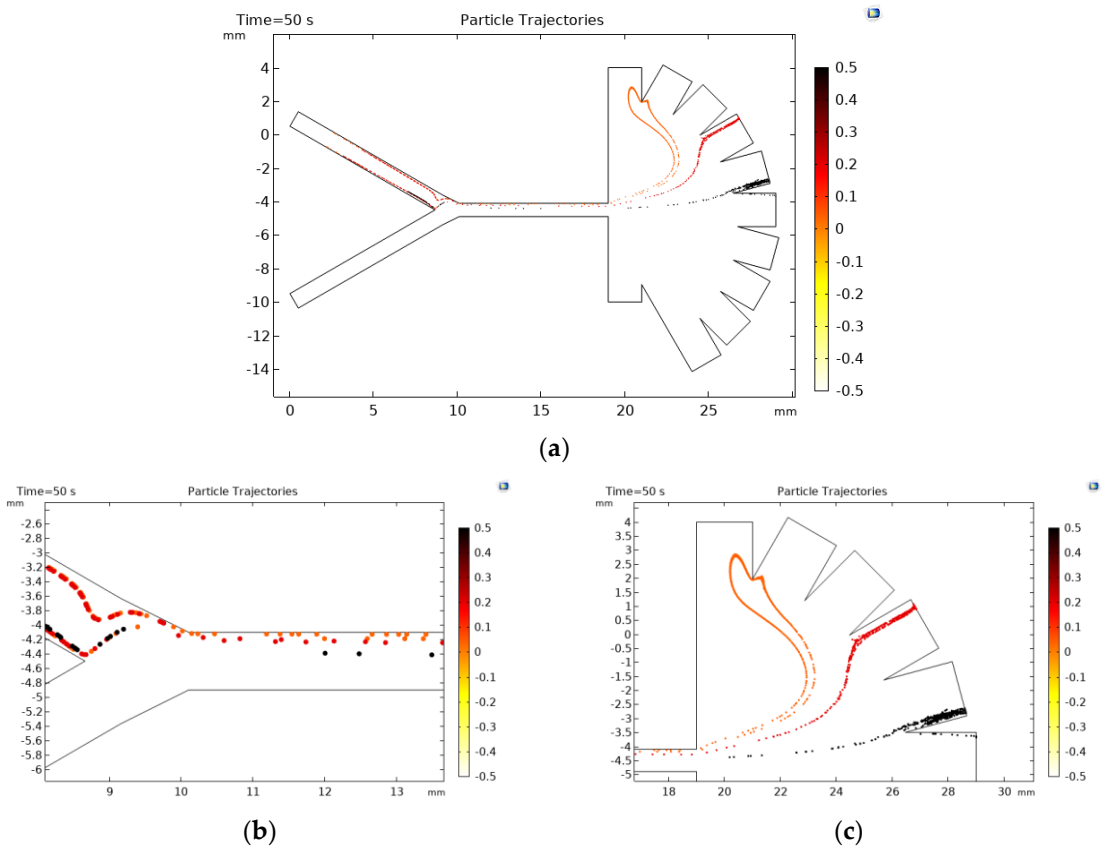


Figure 3. Separation results of the AsPFF system for Separation of the yeast of 40 μm (Orange), Liposomes of 200 μm (Red) and droplets of 500 μm (Black) (**a**) Particle trajectories in the AsPFF system (**b**) Visualization of the particles behaviour in the pinched segment (**c**) Visualization of the separation outlets of the system.

4. Discussion

The combination of Flow Rate Ratios (FRRs) and the Total Inlet Velocities (TIVs) for the designed AsPFF system led to separation efficiencies close to 95%. For all cases, each type of particle trajectories ended up at specific outlets of the system without a mixture between them. Further improvement in outlet selectivity can be achieved by increasing FRR and decreasing TIV, which opens the possibility of exploiting this design to separate even more complex mixtures of particles. These results demonstrated that the separation principle reported by Tagaki et al. for particles under 5 μm could be extrapolated for larger particles by modifying the pinched segment width [10]. For the specific case of the droplet-based screening of the yeast displayed peptides, this system provided the possibility of separate the droplets with the liposomes and encapsulated yeast to examine their interaction in a controlled media with the aid of additional analytical tools. In parallel, the system facilitates the separation of liposomes and unencapsulated yeasts, which opens the possibility of recycling them and potentially reducing costs. For future work, we are planning to increase the number of outlets further and adjust the pinched segment's width accordingly. Also, we expect to manufacture and test the device by low-cost manufacturing via laser cutting of polymethyl methacrylate (PMMA) sheets [12].

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