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Efficient separation of small microparticles at high flowrates using spiral channels: Application to waterborne pathogens

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HIGHLIGHTS

- Spiral channels investigated for the first time for separating waterborne pathogens.
- Impacts of rigid particle concentration, size and velocity evaluated.
- Results compared to behaviour of viable and non-viable pathogenic *Cryptosporidium*.
- 100% separation efficiency observed for *Cryptosporidium* at 500 μL/min.

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ABSTRACT

Detecting waterborne pathogens is a challenging task because of their low concentration in water and their wide diversity. In order to ease this detection process, the potential of microfluidics is investigated in this paper. Spiral channels are designed for separating particles, in a single device and without any external forces or additional buffer, depending on their size at high flowrates. This paper focuses first on the impact of the channel length, flowrate, particle concentration and size on the separation efficiency of polystyrene beads of relevant sizes (4–7 μm). The system is then tested with viable and non-viable pathogens (*Cryptosporidium parvum*) with an average size around 4–5 μm.

1. Introduction

Access to safe drinking is 'a human right that is essential for the full enjoyment of life and all human rights' as recognized by the United Nations General Assembly resolution (A/RES/64/292-2010). However, despite the current available treatments, several outbreaks are reported each month across Europe. Between 2000 and 2007, 47,617 episodes of illness have been reported in Europe by the European Environment and Health Information System (2009) while the Drinking Water Inspectorate (2012) reported around 60 significant events caused by waterborne pathogens in England and Wales in 2012. *Cryptosporidium* is one well-known and highly resistant protozoa encountered in water systems (Bridle et al., 2012; Bridle, 2013), which has been detected in water despite the absence of the target microbiological parameters (*Escherichia coli*, or faecal/thermotolerant coliforms; total coliforms; enterococci, faecal streptococci; and *Clostridium perfringens*) designated by the European Union for monitoring the water quality (WHO, 2014). A specific standardized procedure (namely US EPA 1623) is thus required for detecting its potential presence relying on (i) a filtration allowing large volumes of water to be treated while retaining all the particles of the same size or bigger than *Cryptosporidium*, (ii) an elution step to remove *Cryptosporidium* from the filter while (iii) centrifugation and immunomagnetic-separation are used for concentrating and isolating captured *Cryptosporidium* from other particles for detection. Highly experienced staff are then required to perform the detection by (iv) fluorescent labelling and microscopy (Bridle et al., 2012). This procedure is long (several days) and non-automated, delaying detection and thus potentially increasing the number of people affected in case of an outbreak. This protozoa is only one of many waterborne pathogens and one could easily imagine how challenging detecting accurately all the potential harmful pathogens is. The development of new tools enhancing the separation of pathogens by kingdom (virus, bacteria and protozoa) after filtration is thus required to enable a more automated/rapid process. This is particularly important with the growing interest in molecular methods for detection, as optimal lysis methodologies vary between different pathogen kingdoms. Due to its appropriate scale, microfluidics represents an interesting

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approach for working with small biological material, including *Cryptosporidium* as recently reviewed (Bridle et al., 2012, 2014). Studies have proposed a direct miniaturization of the final stages of the current process for microfluidic filtration and immuno-magnetic-separation although clogging issues and the specificity to single pathogens are still limiting their practical use by water companies. Dielectrophoresis is another technique proposed in the literature for concentrating and separating *Cryptosporidium* but the working flowrates are usually small, while hundreds of millimetres need to be analysed after filtration. There is thus a need in developing intermediate stages to process the large volumes of water obtained after filtration for promoting the potential of these microfluidic-based detection techniques.

The purpose of this paper is to try to fill this gap by proposing an efficient sized-based separation of pathogens after filtration at high flowrates. There is indeed an interesting correlation between the size of pathogens and their kingdom. For instance protozoa such as *Cryptosporidium* can be characterized by an ellipsoidal shape of about 5 μm in diameter (one should note that the size of *Cryptosporidium* also depends on its specie. The 5 μm figure corresponds to *C. parvum* and *hominis*, which are common problematic human pathogenic species whereas *Cryptosporidium muris* can size up to ≈ 7 μm in diameter). Pathogenic bacteria (≈ 1–3 μm) and viruses (≈ 20–100 nm) are smaller. The shape of pathogens can also drastically differ from one kingdom/specie to another, this point will be discussed later in the paper. In the literature, two main microfluidic techniques have been proposed for size-based particle separation at high flowrates (*e.g.*, in the mL/min range): deterministic lateral displacement (DLD) and inertial focusing (IF). As recently reviewed (McGrath et al., 2014), DLD can perform efficient separation in complex biological media such as blood. Although this technique has been successfully scaled up for separating 'angry pathogens' with LEGO ® for outreach activities (Jimenez and Bridle, 2015), the presence of posts in the channel makes DLD devices prone to clogging and thus potentially not suitable for routine procedures. To overcome this limitation, inertial focusing using spiral channels is considered for the first time in the literature for waterborne pathogen separation. The first part of this paper focuses on the different mechanisms behind inertial focusing in straight and spiral channels. Impacts of the particle concentration, size, velocity and channel length on focusing behaviour are then investigated with rigid polystyrene beads. The system is finally tested with pathogens and its potential as an interesting alternative for water companies discussed.

2. Principle of inertial focusing

The purpose of this section is to understand how a spiral channel as depicted in Fig. 1 can separate particles without any external forces. To start with focussing in straight channels is discussed.

2.1. Principle in straight channels

Considerable effort has gone into understanding why particles randomly distributed at the inlet of a straight channel tend to focus at some specific equilibrium positions at the outlet. This phenomenon has been attributed to the equilibration of two main effects: (i) a shear induced lift directed towards the channel walls due to the parabolic profile of velocity and (ii) a wall induced lift directing particles towards the channel centreline when the particle approaches the wall. The balance of these forces causes particles to equilibrate at a specific distance from the wall (Di Carlo, 2009). In square or rectangular channels, a third mechanism is involved, pushing particles towards the middle of channel faces,

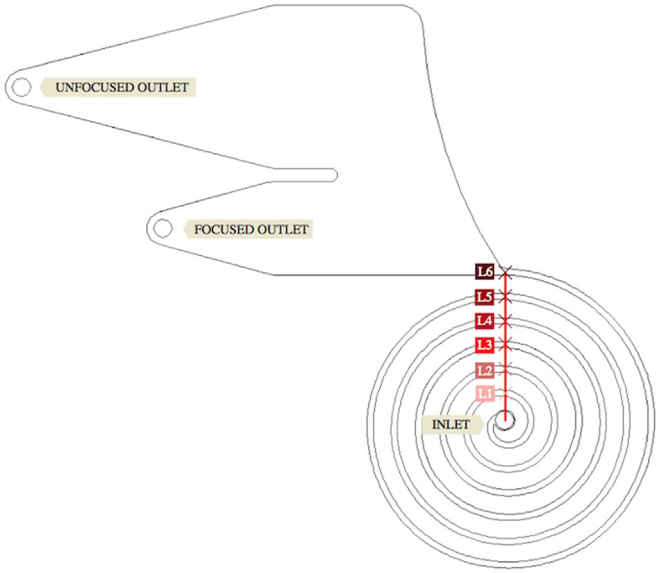

Fig. 1. The spiral microfluidic channel used for pathogens separation comprises 1 inlet in the centre of the spiral and 2 outlets. The depth of the device is 30 μm, the width 170 μm, and the pitch 500 μm.

and attributed to a rotation-induced lift (Zhou and Papautsky, 2013) or wall effects (Di Carlo, 2009; Amini et al., 2014). The net lift force F_L experienced by particles can be expressed as

$$F_L = C_L \times G^2 \times \rho \times a^4, \tag{1}$$

with C_L the lift coefficient, G the shear rate ($G = 2\overline{U}/D_h$, with $\overline{U}$ the average fluid velocity and D_h the channel hydraulic diameter), ρ the fluid density and a the particle diameter. Other formulations for this net lift force are proposed in the literature near the centreline ($\propto \rho \overline{U}^2 a^3/D$, D being the characteristic channel dimension) or the wall ($\propto \rho \overline{U}^2 a^6/D^4$) respectively. For further details, the interested reader is invited to consult the recent review of Amini et al. (2014).

As a consequence of these forces, particles tend to focus in the middle of the four faces in a square cross-section channel. In a rectangular cross-section channel, the velocity profile is sharper along the channel smallest dimension. The resulting shear lift is thus stronger along this direction leading to particles pushed towards the channels' longest faces. Similar to the behaviour in square channels, particles tend to focus in the middle of the channel faces resulting in two equilibrium positions in the middle of the longest faces.

2.2. Extension to spiral channels

In curved rectangular channels, the position of fluid maximum velocity shifts from the centre towards the concave wall of the channel due to a centrifugal action. In order to compensate this phenomenon, secondary rotating flows, namely Dean flows, appear in the channel (Nivedita et al., 2013). Particles flowing in a curved channel will thus experience a supplementary force, the Dean drag F_{DD}. Assuming the average Dean velocity proposed by Ookawara et al. (2004) ($\overline{U}_{DD} = 1.8 \times 10^{-4} De^{1.63}$), F_{DD} can be expressed as (Kuntaegowdanahalli et al., 2009)

$$F_{DD} = 5.4 \times 10^{-4} \mu De^{1.63} a, \tag{2}$$

μ being the fluid viscosity and De the Dean number defined as

$$De = \frac{\rho \overline{U} D_h}{\mu} \times \sqrt{\frac{D_h}{2R}}, \tag{3}$$

with R the radius channel curvature.