MULTIPLE NODE ULTRASONIC STANDING WAVE SEPARATION IN MICROCHANNELS IMPROVES LIPID DISCRIMINATION FROM COMPLEX BIO-SUSPENSIONS

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ABSTRACT
A microchip for multiple node separation of particles using acoustic standing waves was developed. The combination of multiple nodes, with sheath flow, demonstrates for the first time a mode of operation that avoids lipid particle aggregation along the sidewalls with subsequent loss in separation performance, as compared to previously reported single node ($\lambda/2$) standing wave systems. The design also prevents separation performance loss due to Rayleigh streaming (RS) in systems with large differences in particle size [1]. The proposed methodology is developed for the separation of bacterial species from foodstuff samples to enable high-speed ($\leq 10$ min/sample) quality control in industrial food production.

KEYWORDS: Ultrasound, Acoustic standing wave, Microfluidic chip, Foodstuff analysis

INTRODUCTION
Acoustic particle manipulation is a gentle and robust way of handling particles in microfluidic networks. The acoustic radiation force induced on the particles allows for sorting, trapping and valveless switching of particles [2]. Successful cell culturing in the acoustic field and viability studies post exposure to the acoustic field has been performed [3] [4]. The method does however have some intrinsic difficulties when it comes to handling of small particles and high particle concentrations.

Trapping at surfaces with low shear rate and RS caused by the need of higher acoustic power will set the lower size limit for separation of particles in $\lambda/2$ systems. Both problems are caused by decreasing acoustic radiation forces coupled to decreasing particle size. An adequate example would be raw milk handling where the ability to remove the emulsified lipid fraction is important for rapid bacterial content analysis.

THEORY
When trying to separate the bacteria in a standard $\lambda/2$ separator the input acoustic power required to move bacteria would either cause the lipids to be trapped on the channel side walls or RS to disperse the bacteria laterally across the whole channel width, both cases leading to erroneous separation. This paper proposes the use of multiple standing wave node systems to solve these problems.
EXPERIMENTAL

Initially a $\lambda/2$ reference channel matched to 2 MHz was investigated. Subsequently channels matching $2\lambda/2$ and $3\lambda/2$ widths respectively at 2 MHz were employed to alleviate problems of lipid emulsion trapping. The channels were supplied with three in- and outlets. A test sample containing 3% milk lipid suspension and 0.1% small particles (red 3 µm polystyrene (rPS)) was used for the investigations. Later experiments consisted of lipid separation in Escherichia coli (E. coli) bacteria spiked consumer milk as well as E. coli bacteria spiked raw milk.

RESULTS AND DISCUSSION

The $\lambda/2$ channel rapidly clogged when running the test sample, Fig 1. The $2\lambda/2$ channel prevented clogging as lipids were focused in the channel centre with high flow rate. The additional nodes acted as acoustic barriers through which no particles could reach the sidewalls, Fig 2. However, when running 0.87 µm polystyrene particles in the $2\lambda/2$ channel, RS prevented separation, Fig 3. The final solution emerged with the $3\lambda/2$ channel, which managed to separate lipids from rPS without moving the rPS, thus making the system independent of small particle size, Fig 4. The $3\lambda/2$ channel, prepared at varying flow speeds and actuation voltages, allowed less than 1% E. coli to be erroneously separated. This is our first proof of concept of a microfluidic sample preparation chip for foodstuff bacterial content analysis.

![Figure 1](image1.png)

Figure 1. $\lambda/2$ channel. Left, no ultrasound. The rPS/lipid emulsion particles enter and leave the chip in a laminated centre stream. Right, 2 MHz, actuation voltage 15 V. As the ultrasound is activated the lipid emulsion (white) is focused to the antinodes along the side walls, leaving a pure rPS suspension (red) in the centre. The problem of lipid trapping (clogging) along the side walls and deteriorated flow lines becomes evident.

![Figure 2](image2.png)

Figure 2. $2\lambda/2$ channel. Left, no ultrasound. The rPS/lipid emulsion particles enter and leave the chip in a laminated centre stream. (Right), 2 MHz, actuation voltage 15 V. As the ultrasound is activated the lipids are focused in the centre antinode while rPS are focused in the nodes. The nodes act as acoustic barriers preventing particles to reach the sidewalls. The acoustic forces have thus maintained the clear sheath flows on both sides.
Figure 3. 2λ/2 channel. Left, no ultrasound. Same setup as in Fig 2. FITC labelled 0.87 µm particles viewed through a fluorescence microscope are hydrodynamically focused into the centre outlet. Right, 2 MHz, actuation voltage 15 V. As the ultrasound is activated a clear band broadening is seen as RS, in combination with a lower acoustic radiation force acting on these smaller particles, prevent the particles to focus in the nodes unlike the rPS in Fig 2. In order to focus these particles the actuation voltage needs to be increased, but that leads to increased trapping and RS, counteracting the system performance.

Figure 4. 3λ/2 channel. Left, no ultrasound. The rPS/lipid emulsion particles enter and leave the chip in a laminated centre stream. Right, 2 MHz, actuation voltage 15 V. As the ultrasound is activated the lipids are focused in the pressure antinodes closest to the channel centre thereby avoiding trapping along the side walls. Furthermore, as the small rPS particles (red) are hydrodynamically focused to the channel centre and further subject to a focusing standing wave force these are extracted via the centre outlet. The chip was operated for extensive time periods without previous lipid clogging interrupts.

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REFERENCES