19th Australasian Fluid Mechanics Conference
Melbourne, Australia
8-11 December 2014

A Microfluidic Device for Visualisation of Milk Fat Globule Alignment by the Acoustic Radiation Force – a High Particle Concentration case

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Abstract

A microfluidic device to study the dynamics and magnitude of the acoustic radiation force applied to milk fat globules (MFGs) is presented. Visual information at high resolution is provided by microscopy. This microscale system provides valuable feedback to the mechanistic description of batch-sized fat separation systems which today enable milk fat separation on litre-scale.

Introduction

The separation of fat in dairy products is usually performed by centrifugation or natural creaming. As an alternative method the separation of milk fat globules (MFGs) upon sonication has been demonstrated in a batch system [10, 8]. The suggested mechanism is alignment by acoustic radiation force subsequent to particle aggregation or coalescence and enhanced buoyancy rise [8], see Figure 1.

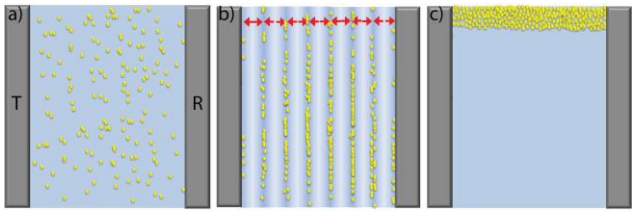

Figure 1. Simplified schematic model of large-scale ultrasonic standing wave particle separation systems (batch), with a transducer (T) and a reflector (R) wall respectively: a) Milk fat globules (MFGs) (yellow) are randomly distributed at the time of ultrasound turn on. b) An acoustic standing wave is created between the reflecting walls causing alignment of the particles by the acoustic radiation force (red arrow). The MFGs aggregate and then rise rapidly due to enhanced buoyancy force on the aggregates. c) Particles are gathered at the top surface.

We here present a microfluidic system for visualisation of the acoustic alignment by the acoustic radiation force.

The MFGs have a wide size distribution with a peak of about 4 µm diameter, see Figure 2.

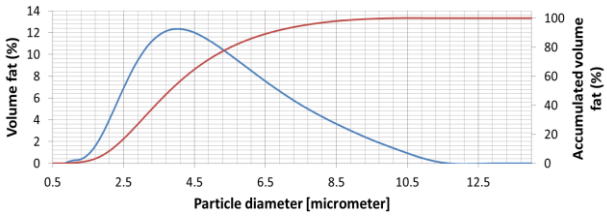

Figure 2. Milk fat globule particle size distribution in terms of volume fat (%) (blue) and accumulated volume fat (%) (red).

Theory

The Primary Acoustic Radiation Force

The time-averaged primary radiation force, F_{prim}, in an ideal standing wave field in the direction of the propagating wave was derived by Yosioka and Kawasima [13] and is here expressed by rearranging the expression by Coakley et al. [2] to be expressed in terms of frequency and with the spatial period as by Wiklund et al. [12],

$$F_{prim} = -V \frac{\pi}{2\rho_l c_l^3} p^2 f \Phi \sin\left(2\pi \frac{z}{\lambda/2}\right), \qquad (1)$$

where V is the particle volume, ρ is the density, c is the speed of sound, p is the pressure oscillation amplitude, λ is the wavelength of sound, Φ is the acoustic contrast factor ($\Phi=((5\rho_l-2_\rho)/-(\beta l/\beta_p))$, β is the compressibility ($\beta=1/(\rho c^2)$), and the subscripts l and p refer to the liquid and the particles respectively.

Hence, the acoustic radiation force is proportional to the volume of the particles and the acoustic contrast factor (ACF). The ACF for MFGs is about half the ACF of polystyrene particles[9]. Since polystyrene particles down to 1 µm diameter size are commonly manipulated in microsystems, it is expected that MFG sizes larger than 2 µm will be readily manipulated in a microsystem provided it has reasonably high energy density.

The Secondary Acoustic Radiation Force

In addition to the primary acoustic radiation forces there is short-range secondary acoustic radiation force, F_{2nd}, due to the scattering off neighbouring particles, which act to attract the particles further in the nodal planes. For the case of two particles, this is described as [4]

$$F_{2nd} = 4\pi a^6 \left(\frac{\left(3\cos^2\theta - 1\right)\left(\rho_p - \rho_l\right)^2}{6\rho_l d^4} v^2(x) - \frac{\varpi^2 \rho_l \left(\beta_p - \beta_l\right)^2}{9d^2} p^2(x) \right), \quad (2)$$

where d is the centre-centre distance between the particles with radius a, θ is the angle between the propagation direction and a line between the two particles, ω is the angular frequency of the ultrasound, v the oscillation velocity amplitude and p is the pressure oscillation amplitude. At the pressure antinodes of the ideal standing wave field, the velocity is zero (and vice versa).

Method

The microsystem is shown in Figure 3. The principal microsystem design constitutes a miniaturised transducer[11, 7, 3] and a square capillary fluidic channel [5, 6]. The transducers (approximately 2 × 2 × 0.25 mm^3) and the system operate in the frequency range of about 4-8 MHz.

Milk fat globules in diluted cream were stained with Nile red (Sigma Aldrich). The sample was transported through the channel by a manually operated syringe at a flow rate of a few mm/second.

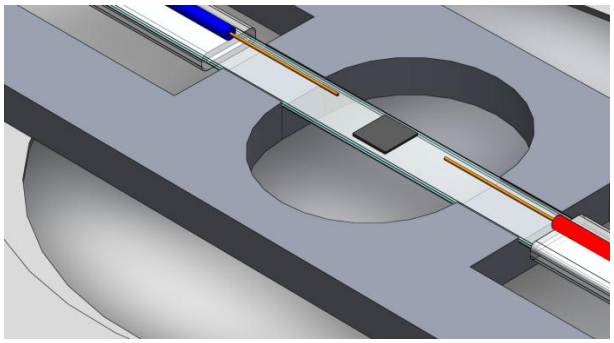

Figure 3. Schematic design of the microfluidic system with miniaturised transducers and a square capillary channel.

We characterise the milk alignment case by an estimation of the particle concentration in the nodal plane.

Results and Discussion

Milk Separation Analysis – a case of High Particle Concentration in the Nodal Planes

The approximate intermodal distance and number of multiple layers for fat content in the milk of around 4% can be estimated in the following way. For simplicity we assume i) all fat is present as 4 µm diameter particles. Further assume that ii) the milk fat globules are positioned close side-by-side in the nodal plane. For homogenous particle distribution throughout the volume the number of sub-layers in one nodal plane is 20 for the case of 1 MHz ultrasound and 4.5 µm particle-particle spacing, d_p, see Figure 4.

Hence, we conclude that for the case of milk it is crowded in the pressure anti-nodal planes. The secondary acoustic radiation force, equation (2), will act in addition to the primary acoustic radiation force, equation (1), once the particles are gathered in the pressure anti-nodal planes. However, we here suggest that for the case of high particle concentration samples the particles will gather into particle contact range mainly by the primary acoustic radiation force. This is different from a more dilute sample where the particles initially align into the nodal plane by the primary acoustic radiation force, but close contact is mediated by the secondary acoustic radiation force, lateral primary acoustic radiation force due to acoustic field inhomogenities and possibly also by acoustic streaming.

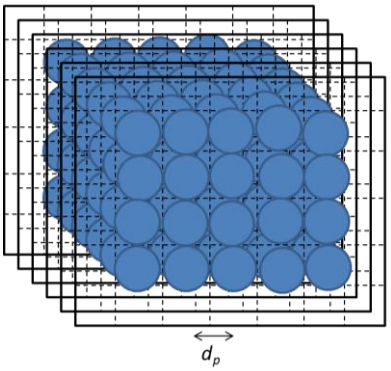

Figure 4. Illustration of the high particle concentration in the nodal planes due to the close inter-particle distance, d_p, in the nodal planes. The number of sub-planes in a nodal plane for 4.5 µm inter-particle spacing of 4 µm particles at 1 MHz is 20.

Alignment in the Microfluidic System

Alignment of MFGs is demonstrated in Figure 5. The acoustic potential field is observed to be dominated by cavity modes perpendicular to the transducer axis. This orientation enables visual observation of the particle alignment into nodal regions. The previous microsystems of the same principal design have demonstrated particle trapping in a horizontal plane [5], in some cases with acoustic near field effects [11, 3, 7] and the generation of an acoustic streaming pattern [6]. The system presented here increases the application versatility of these types of microfluidic systems.

The milk fat globules are observed to align into a narrow plane region. Video data show the particles in the flow are observed to be attracted into the nodal plane region next to previously trapped particles and form grape-cluster shaped aggregations.

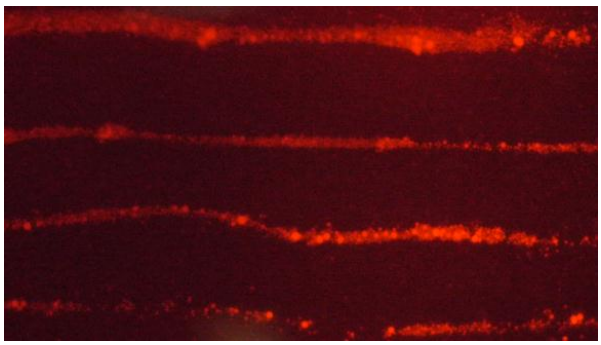

Figure 5. Alignment of milk fat globules in a diluted cream sample stained with Nile Red (top view and 20x objective) for a fluid flow from the right. The distance between the particle alignment nodes corresponds to half a wavelength. Observe that some MFGs stick to the walls.

We demonstrate the magnitude of the acoustic radiation force in one evaluated system by the alignment of small particles of only 1 µm diameter at about 6 Vpp into hot spots in the microfluidic field (not shown). Note that the acoustic radiation force required to manipulate particles of 1 µm diameter is 1000 times higher than required to manipulate particles of 10 µm diameter, as observed from equation (1). Manipulation of 1 µm diameter polystyrene beads corresponds to manipulating all MFGs larger

than 2 µm diameter, which according to Figure 2 is 99 % of the fat content present in the milk sample.

By applying a flow the speed of alignment for particles entering the acoustic zone can be estimated. It can be observed that all the MFGs large enough to be easily observed align within one to a few seconds. However, some of the smaller MFGs can be observed to pass through the acoustic field zone without being trapped in the nodal planes.

In the batch-scale system particle size measurements of the ultrasound separated cream indicate that the smallest particles sizes actively separated by the ultrasound might be around 2.9 µm diameter (after 20 minutes sonication at 2 MHz). Manipulating all particles larger than 2.9 µm corresponds to 96% of the volume fat present, see Figure 2. In centrifuges, semi-skimming separation starts at about 1.2 µm and upper FWHM (full-width-half-maximum) of the skimmed-milk peak is about 2 µm [1]. Theoretically the acoustic radiation force magnitude required to manipulate 2 µm particles is 3 times larger than for 2.9 µm particles.

Conclusions

We have presented a microfluidic device for *in situ* study of the dynamics and magnitude of the acoustic radiation force on milk fat globules. It is the first time vertical alignment has been demonstrated in these types of systems consisting of a miniaturised transducer and a square capillary channel.

The milk fat globules visible are observed to align within one to a few seconds. The field strength is evaluated by alignment of 1 µm diameter PS beads, corresponding to the alignment of all MFGs larger than 2 µm diameter. This corresponds to 99 % of the fat content present in the milk sample, indicating that this is an efficient system. We identify the milk sample as high concentration and suggest that for this case the gathering of particle into close range occurs mainly by the primary acoustic radiation force.

Acknowledgments

This work was funded by the Australian Research Council (ARC LP110200499) and the Geoffrey Gardiner Dairy Foundation.

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