

NOVEL MIXING METHOD FOR CROSS LINKER INTRODUCTION INTO DROPLET EMULSIONS

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ABSTRACT

Microfluidic devices are commonly utilised for the manufacture of particles. In certain applications, cross linkers are required to manufacture these particles, and when introduced in continuous form will foul the microfluidic channels.

We show the introduction of cross linker after droplet formation, together with the utilisation of topological microfluidic channel structures, allowing for the **novel manufacture of particles**. Flow over these structures has been simulated in order to choose the **most efficient structures**. The method has been successfully demonstrated by manufacturing **self-immobilised enzyme particles**, which retain a high percentage of their activity while also producing **highly monodisperse particles**, a considerable advantage for recovery of the particles after catalytic reaction.

KEYWORDS: Microfluidics, Emulsion, Simulation, Droplet coalescence

INTRODUCTION

Microfluidic flow in micro channels is almost exclusively laminar. As a result, mixing is slow and occurs by means of diffusion. A number of techniques have been proposed to overcome this limitation [1], utilising both active and passive methods. A very useful example of passive mixing is the introduction of channel structures to aid with mixing, and this has been demonstrated many times in the case of single phase liquid mixing [2].

Droplet based microfluidics has also received considerable attention due to the many advantages which can be achieved by encapsulating reagents into droplets [3]. In particular, droplets allow for the efficient direct synthesis of particles [4]. Typically, reagents required for cross linking are introduced either during droplet formation, by merging individual droplets or by subsequent UV cross linking. These methods have the potential to work very efficiently, but utilisation of cross linkers during droplet formation can also result in the rapid fouling of the microfluidic channel, making the device unusable.

This work explores a new method for introducing cross linker into the droplets, by rapidly and passively mixing two emulsion streams. This method also introduces a novel application for channel structures, while also having the advantages of allowing for variable time delays between the introduction of the reagents in the droplet and coalescence with the cross linker.

THEORY AND EXPERIMENT

Twelve different structures were investigated. These are shown in Figure 1 and were chosen based on the effective structures in literature together with considerations specific to this work. Designed structure widths are 71 μm (X), except for (k) and (l), where the width is 134 μm . The distances between structures (Y) are 78 μm , except for (i) and (j), where the distance is 141 μm . Figure 1 also shows the position of the structures in the typical microfluidic device. Droplets are formed at a standard flow focusing junction (Detail A). The aqueous solution (bovine serum albumin/lipase solution) is introduced in the central channel (flow rate typically 1 $\mu\text{l}/\text{min}$), while the mineral oil continuous phase is introduced via the side channels (4 $\mu\text{l}/\text{min}$). The droplets flow towards junction B where a pre-emulsified cross linker (glutaraldehyde and ethylenediamine) emulsion is introduced at a flow rate of 1 $\mu\text{l}/\text{min}$.

All structures are simulated by solving the Navier-Stokes equations. For this purpose SolidWorks Flow Simulation was utilised. Figure 2 shows the results of two of these simulations, namely for structures A and C. Modeling is performed over a total distance of 26 mm. Figure 2, for clarity, shows sections of this total distance. Actual parameters being shown are given in the caption. A number of observations can be made by studying these images. Firstly, an indication of distance over which complete mixing takes place can be estimated by looking at Figures 2 f and m, and determining at what distance along the channel the concentration profile plateaus. This occurs at 13 mm for structure A and 17mm for structure C. Figure 2 g also shows that some mixing across the channel center line occurs, which does not happen in figure 2 n, showing that the latter design keeps the two cross linker streams separate. Referring to Figure 1, it is interesting to note that structures I – L result in complete mixing, but this takes longer than structures A and C. Structures B and D, where the structure direction is changed, results in flow diverging towards the channel walls, and could be useful for applications requiring sorting.

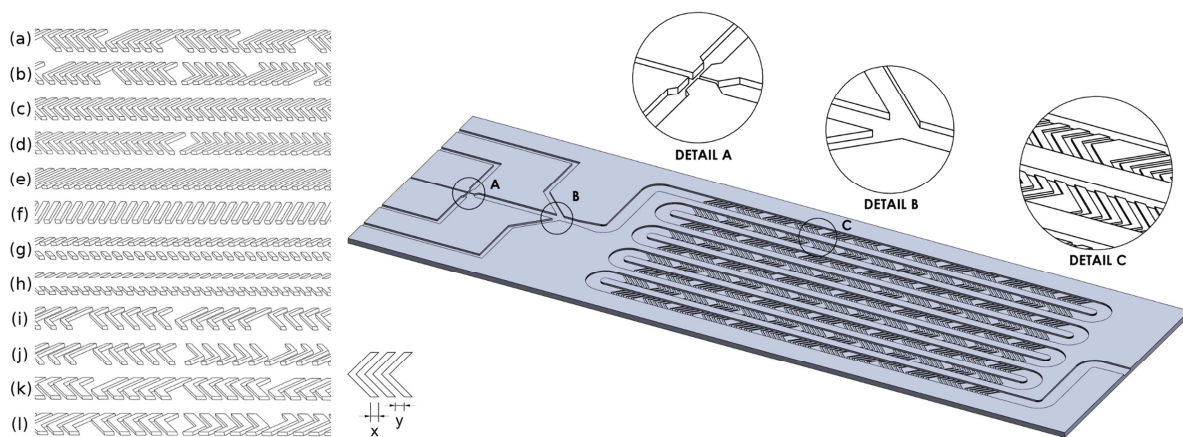


Figure 1: Schematic of the microfluidic structures tested (a – l) [left] and the position of the various structures in the microfluidic device [right]. In the text, the structures are referred to by their capitalised letter, (a) = A, to avoid confusion.

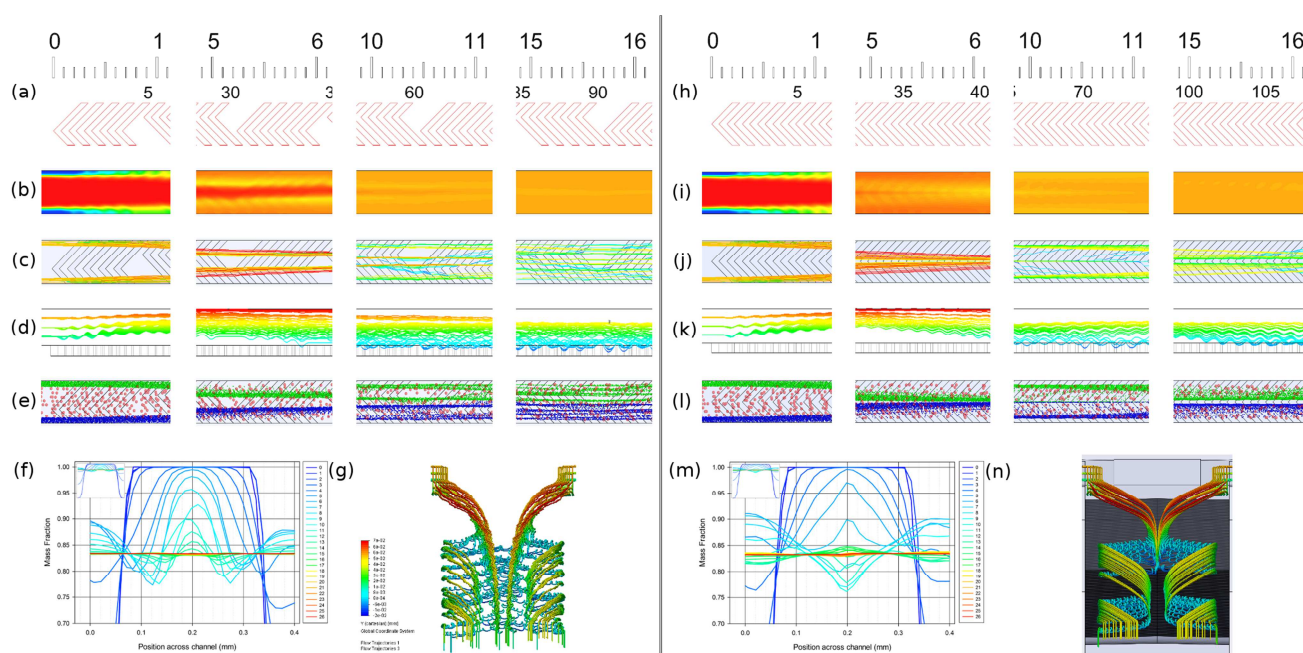


Figure 2: Simulated sections of structure A (left column) and C (right column) of the total distance for the actual structure (a, h), concentration of central flow (b, i), top view of flow lines (c, j), side view of flow lines (d, k), visualisation of droplets and cross linker (e, l). Concentration profiles across the width of the channel, and at intervals of every 1mm down the channel (blue = 0 mm, red = 26 mm), are given in (f) and (m). X axis refers to position across the channel (0 – 0.4mm) while the Y axis refers to the mass fraction of the central flow. (g) and (n) show the flow profile down the length of the channel, with (n) showing the actual channel structure.

RESULTS

Figure 3 shows the results of experiments with structures A and C from Figure 1 together with a typical flow simulation. The experiment shows that flow simulation accurately models these structures, and can be employed to determine the best structures to be utilised for different applications. For this work, asymmetrical structures (e.g. A) are chosen for their ability to achieve rapid mixing. The simulation shows complete mixing after 13mm (Figure 2 f), while the experiment with only cross linker shows mixing over approximately 15mm (Figure 3 (top)), and final experiments with the introduction of droplets shows complete mixing after 10.8mm (Figure 4 f).

Figure 4 (a-c) shows mixing in a typical device where there are no structures and compares this to mixing with structures (Figure 4 (d-f)). Impressively, **complete mixing is achieved 10x faster**. This has a number of advantages:

1. There is a longer contact time of the cross linker with droplets, ultimately resulting in a longer residence time in the serpentine channels.

2. Droplets are all in uniform contact with the cross linker. Figure 4b shows that some droplets can flow against the channel walls, and this flow is at a slower flow rate than the centre channel resulting in uneven cross linking residence times.

The effect of this is clearly demonstrated in Figures 4 g and h, where particles manufactured without structures (g) are clearly less uniform than those manufactured with structures (h). This method has been demonstrated by self-immobilising enzymes into the particle. Monodisperse particles are produced, and these particles are able to retain a high degree of enzyme activity (40%).

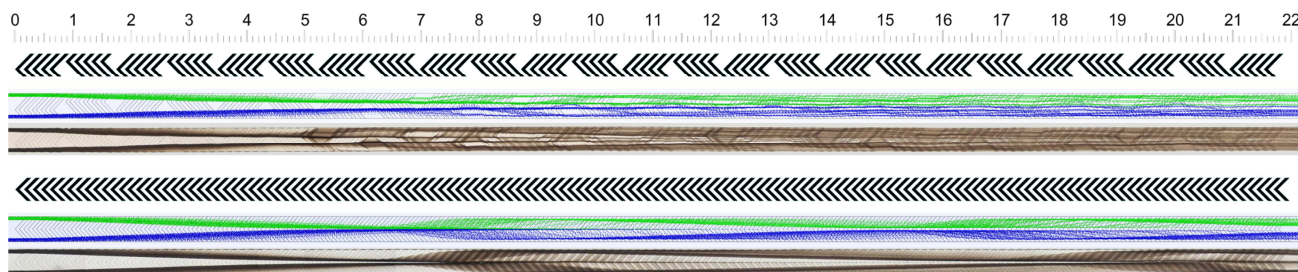


Figure 3: Comparison of simulation and experimental results for structures A and C, showing their close correlation.

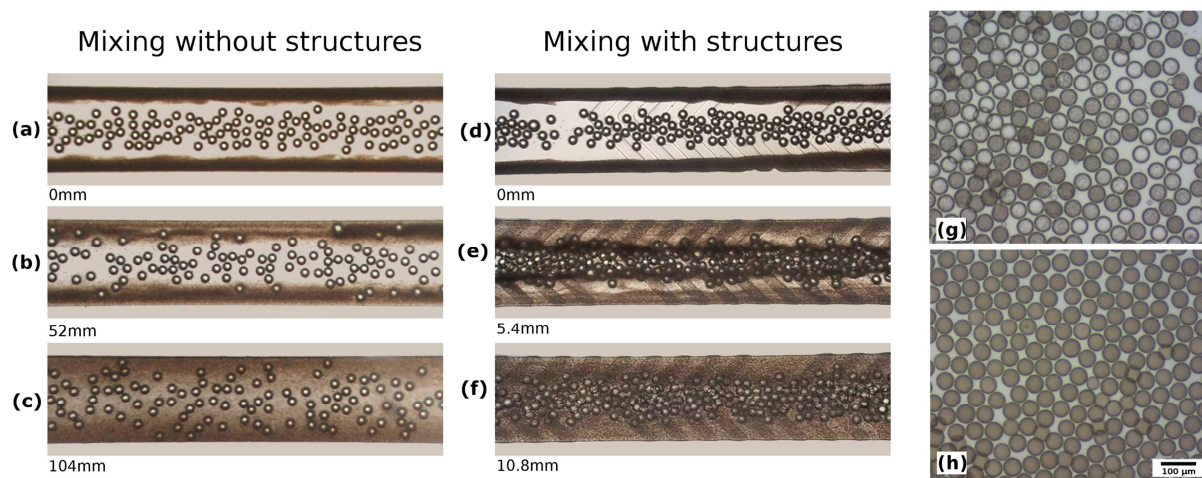


Figure 4: Photographs comparing mixing without any channel structures (a – c) and with channel structures as in figure 1 a (d –f). (g) and (h) show photographs of the droplets after they are collected from the microfluidic device.

CONCLUSION

The device presented has shown a **novel combination of microfluidic structures** with the introduction of cross linker after droplet formation as a separate emulsion. Particles produced with this method have shown the ability to **retain a high percentage of activity**, have produced monodisperse particles with less than 3% standard deviation, and show an even distribution of material through the particle. Future work is aimed at **optimising the structures** and exploring alternative applications for this method.

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