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A Hydrodynamic Flow Focusing Microfluidic Device for the Continuous Production of Hexosomes Based on Docosahexaenoic Acid Monoglyceride

Anan Yaghmur[1,*], Aghiad Ghazal[2], Raghib Ghazal[1], Maria Dimaki[2], Winnie Edith Svendsen[2]

[1]*Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark*

[2]*DTU Bioengineering,* Technical University of Denmark, Kgs-Lyngby, Denmark

[*]Corresponding Author: **e-mail address:** anan.yaghmur@sund.ku.dk

Supplementary information

It is important for calculating the diffusive mixing time between lipid ethanol solution and buffer along the exit channel to take into account the dimensions of the microchannel, the total flow rate (TFR), the flow rate ratio (FRR) between the center stream and the two side buffer streams (sheaths), and the diffusion coefficient (D) of ethanol in water.

The width of the focused center stream was calculated using the following equation[1, 2]:

$$w = \frac{width\ of\ channel}{1 + FRR} \qquad (1)$$

where *w* is the width of the focused center stream, and FRR is the flow rate ratio as mentioned above. The diffusion of the molecules from the sheath fluid along the interface with the center ethanol solution containing MAG-DHA to the middle of the center stream through a laminar flow requires a diffusive mixing time that can be estimated using the following equation[3]:

$$t = \frac{x^2}{2D} \qquad (2)$$

where t is the time in s, *x* is the travelled distance measured in cm (which is half of w), and *D* is the diffusion coefficient measured in cm^2/s. The distance travelled down the channel before complete mixing can be estimated by the following equations[1, 2]:

$$D = t \times linear\ velocity\ (mm) \qquad (3)$$

$$linear\ velocity = \frac{TRF}{cross\ sectional\ area} \qquad (4)$$

TFR is measured in µl/min, and the cross sectional area is measured in mm^2 by multiplying the height with the width of the investigated channel. The linear velocity in the exit channel can be calculated by the following equation:

$$TFR/(h \times w) \qquad (5)$$

where h and w are height and width of the microchannel, respectively. For the used microfluidic device, h and w were 125 µm and 90 µm; respectively.

In these calculations, we considered the following:

The width of the center stream for a given FRR ratio can be calculated using eq. (1).

The diffusion length is half the width of the center stream:

x = half of the width of center stream

D = 1.23×10^{-5} cm^2/s (In these calculations, the diffusion coefficient of ethanol in water at 25 ºC was used[4]).

To give an example on the calculations presented in **Table SI1**, we present the estimated diffusive mixing time and distance when applying TFR and FRR of 50 µl/min and 10, respectively.

The width of the focused center stream: $w = \frac{width\ of\ channel}{1 + FRR} = \frac{90}{1 + 10} = 8.18\ \mu m$

x = half the width of the center stream. It was 4.9 µm

The complete diffusive mixing was achieved at the following time: $t = \frac{x^2}{2D} = \frac{4.9^2}{2 \times D} = 6.8\ ms.$ Thus, an estimated time of 6.8 ms was required for the buffer solution to travel from the interface between the sheath and the center fluids to the middle of the center channel.

The linear velocity was calculated using eq. (5), where h and w were 125 µm and 90 µm; respectively. The distance down the channel, where complete diffusive mixing was

achieved: *distance = 74.07 mm/s × 6.8 ms = 0.50 mm*. The width of center stream and linear velocities for different samples are given in **Table SI1**.

Table SI1. The estimated width of center stream and the corresponding linear velocity for samples prepared at different flow rate ratios (FFRs) and total volumetric flow rates (TFRs). For the used microfluidic device, h and w were 125 µm and 90 µm; respectively.

Sample	Volumetric flow rate (TFR, µ/min)	Flow rate ratio (FRR)	Width of center stream (µm)	Linear velocity (mm/s)
S1: FRR10:TFR50	50	10	8.18	74.07
S2: FRR20:TFR50	50	20	4.29	74.07
S3: FRR30:TFR50	50	30	2.90	74.07
S4: FRR10:TFR100	100	10	8.18	148.15
S5: FRR20:TFR100	100	20	4.29	148.15
S6: FRR30:TFR100	100	30	2.90	148.15
S7: FRR10:TFR150	150	10	8.18	222.22
S8: FRR20:TFR150	150	20	4.29	222.22
S9: FRR30:TFR150	150	30	2.90	222.22
S10:FRR40:TFR150	150	40	2.20	222.22

The corresponding mixing times and distances for different samples are also given in **Table SI2**.

Table SI2. The estimated diffusive complete mixing times and the corresponding distance down the polyimide-based microfluidic channel. For the used microfluidic device, h and w were 125 µm and 90 µm; respectively.

Sample	Complete mixing time (ms)	Distance until complete mixing (mm)
S1:FRR10:TFR50	6.80	0.50
S4: FRR10:TFR100	6.80	1.00
S5: FRR20:TFR100	1.80	0.27
S7: FRR10:TFR150	6.80	1.50
S8: FRR20:TFR150	1.80	0.41
S9: FRR30:TFR150	0.85	0.19
S10:FRR40:TFR150	0.48	0.10

References

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