Lab on a Chip

SUPPLEMENTARY INFORMATION

3D Micromixer for nanoliposomes synthesis: A promising advance in high mass productivity

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S1. Reynolds Number (Re) calculation

Reynolds number representing the ratio between inertial and viscous forces.

$$Re = \frac{inertial\ force}{viscous\ force} = \frac{\rho D v}{\mu} \quad (1)$$

In equation 1, ρ represents the density, v the flow velocity, D the pipe diameter, and μ, the dynamic viscosity. For cases in which the cross-sectional diameter is non-circular, as is the case for the HFR-MD, the equivalent diameter (D_{EQ}) is used.

Table S.1. D Density and viscosity obtained according to the FRR variation at TFR of 5 ml/min to determine the Reynolds number in the conditions evaluated.

FRR	**10**	**7**	**5**	**3**	**2**	**1**
Ethanol fraction	0.09	0.13	0.17	0.25	0.33	0.50
Water fraction	0.91	0.88	0.83	0.75	0.67	0.50
Density (kg m^{-3})	979.00	971.88	963.17	945.75	928.33	893.50
Viscosity (Pa.s)	0.0010	0.0010	0.0010	0.0010	0.0011	0.0011
Re	203.47	200.69	197.33	190.77	184.42	172.27

S2. Equivalent diameter (D_{EQ})

The D_{EQ} represents the diameter where the fluid flows, and it is defined as four times the ratio between the wet surface area (A_W) of the section and the wetted perimeter (P_W). As described below in Equation (2):

$$D_{EQ} = \frac{4A_W}{P_W} \tag{2}$$

The cross-section image was obtained (similar to Figure 3E) and using the Fiji ImageJ software to perform the DEQ calculation from the 3D construction of the microdevice. Then, the area and perimeter values were obtained. Then, the central microchannel showed DEQ = 303.81 μm so that a flow velocity of approximately 700 μm s^{-1} was generated for TFR of 5 ml/min.

S3. Mixing and Residence time comparison

Karnik *et al.*[1] estimated the mixture time scale (τ_{mix}) in a rectangular microchannel whose flow rate was based on hydrodynamic focusing. This model was developed for flows with low Reynolds numbers (laminar flow), considering that the sides would compress the central flow, leading to a rapid mixing through diffusion. The mathematical model is presented in Equation (3):

$$\tau_{mix} \sim \frac{{w_f}^2}{4D} \approx \frac{w^2}{9D} \frac{1}{(1 + {}^1/_R)} \tag{3}$$

In this model, ‘w’ represents the width of the channel (excluding the equivalent diameter - D_{EQ}), D is the diffusion coefficient of the solvent, and R is the inverse of the FRR (R = 1/FRR).

According to the results presented in Table S.2., it is clear that all FRR values evaluated, the theoretical time required for mixing (τ_{mix}) is significantly higher than the residence time of the fluid in the microdevice. Such fact was observed because the ratio between τ_{mix} and $\tau_{residence}$ is always greater than 1. Therefore, in this first analysis, the conditions applied indicate that the time required for complete mixing is greater than the residence time for all cases (Table S.2.). However, this behavior is inconsistent because liposomes were appropriately formed at FRR 1 and 10, with complete self-aggregation occurring within the microchannel. Thus, we understand that the hypotheses associated with Equation (3) are not valid for the estimation of τ_{mix} for the HFR-MD, suggesting that additional fluid-dynamic or phenomenological components favor the mixture.

Table S.2. Comparison between theoretical mixing time (τ_{mix} - Equation 3) and residence time ($\tau_{residence}$) at FRR of 5 ml/min and DEQ = 303.81 μm.

FRR	τ_{mix} (s)	τ_{mix} / $\tau_{residence}$
10	0.93	12.98
7	1.28	17.85
5	1.71	23.80
3	2.56	35.70
2	3.42	47.60
1	5.13	71.39

The residence time was calculated based on the average flow velocity in the microchannel. After joining three entries, the HFR-MD has a linear length of 5 cm, and the fluid residence time was estimated at 72 ms for TFR 5 ml/min. This time parameter

allows us to deduce that for liposomes to form properly, in a system operating in a laminar regime, the ethanol/water mixture must be thoroughly produced within a period equal to or less than the estimated residence time in the microchannel.

Although this model is only applicable to flows in two dimensions (such as D-MD and herringbone), other authors extended the evaluation for 3D microchannels that presented periodicity in space. In this sense, an intermittent and periodic flow could be considered two-dimensional due to a constant cross-section, precisely the case for the HFR-MD.[2-5]

S4. Comparison between experimental and simulated profiles

The fluorescence intensity profile obtained and analyzed represents the integration of the fluorescence effects found along with the entire height of the microchannel cross-section. As the image acquisition considers the sum of all fluorescence effects and the relative height is not constant, the HFR-MD fluorescence profile compatible with complete mixing (Figure S1).

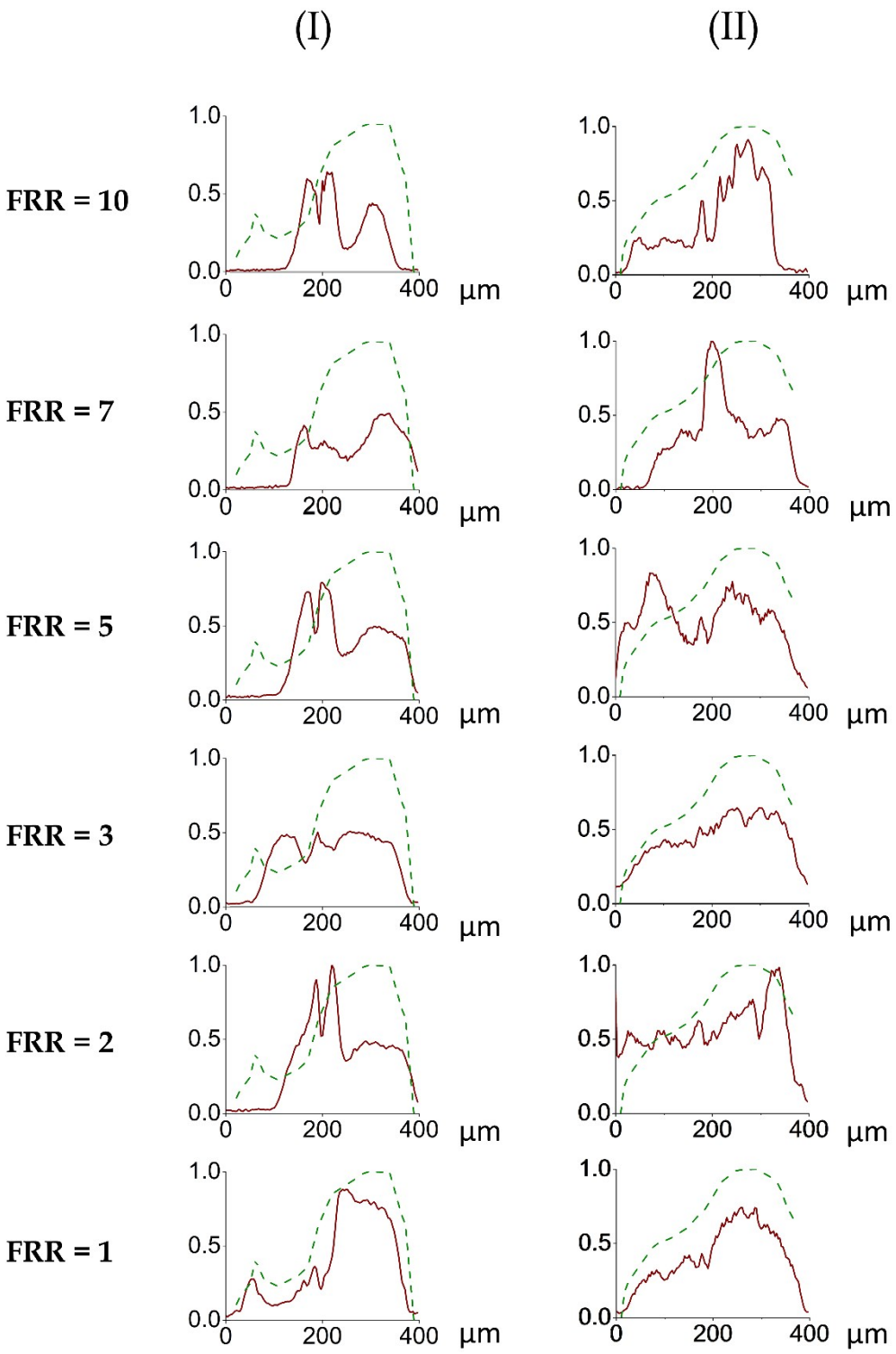

Figure S1. Comparison between the expected theoretical profile of the complete mixture (green dashed line) in the HFR-MD and the experimental fluorescence profiles (solid red

line) at the respective FRR evaluated. X and Y axes represent the relative fluorescence profile intensity and channel width (μm), respectively.

S.5. Computational Fluid Dynamics

1. Geometry

Geometry was created in Solid Edge, a CAD software. Regions E is a cylinder measuring 0.38 mm (external diameter). Region D comprises three circumferences measuring 0.20 mm in diameter (external) whose centers are placed at 0.18 mm and spaced by 120 degrees (geometry and lengths are presented in Figure S2- A). Regions A, B, and C were created as a single circumference (Figure S2-B) that goes from region D until the perpendicular plane at the end of Region C (Figure S2-C).

2. Meshing

The mesh has been built using ANSYS Meshing 19.2. Tetrahedron elements have been considered in order to capture the details of the geometry (Figure S3-D). The selection of the geometry of the mesh element was based on the challenging feature of particular regions of the computational domain where the curvature of the topology of the microchannel posed an additional challenge (Figure S2-E).

The minimum element size was $1.6x10^{-6}$ cm, and the maximum element size was around 1.0 cm. The mesh was generated automatically. The maximum element size was set to $1x10^{-2}$ cm, and the element size was set to $2x10^{-3}$ cm. The growth rate was set as 20%. The viscous mesh was applied at the region close to the solid wall to model the laminar boundary layer properly.

The proper meshing of the regions near the wall was attained using the inflation feature of ANSYS Meshing, where the maximum thickness was modeled as $1x10^{-4}$ cm

with a uniform growth rate and 10 layers. For all the edges on the flow inlet and outlet, it has been considered 100 divisions. The growth rate was set as 20%.

The selection of the optimal mesh was based on the comparison with available experimental data in which a compromise between accuracy and computational time was applied.

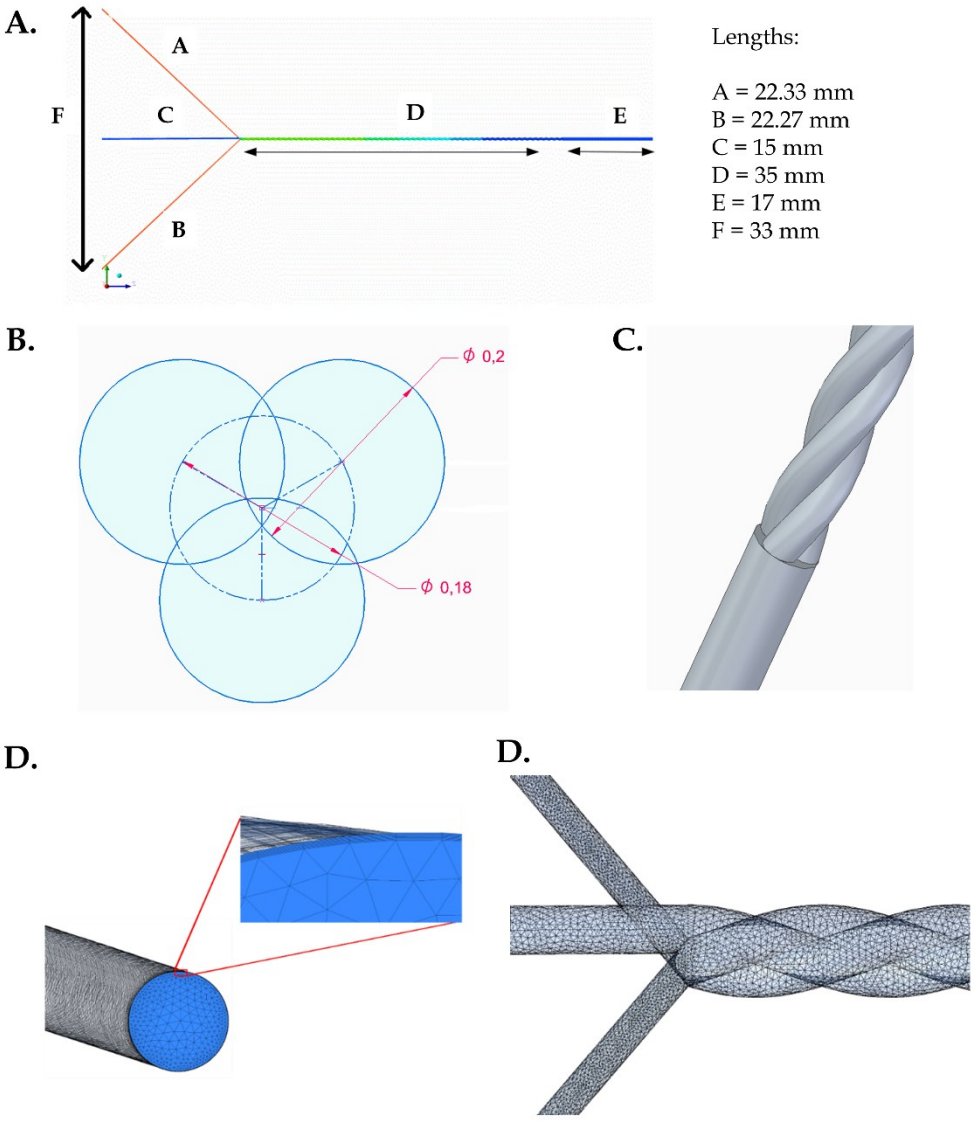

Figure S2. Drawing of HFR-MD dimensions (A); internal view of the microchannel (B); solid structure of the generated numerical model (C).Tetrahedron elements in the geometry (D); Irregular topology of the HFR-MD microchannel (E).

1. Modeling

We have solved the complete set of Navier-Stokes equations. The steady-state model was selected as the study focused on the flow features at steady conditions. The fluid considered in the analysis was a mixture of water and ethanol with no buoyancy effects. The energy equation was not solved in the set of algebraic equations since heat transfer was considered to be negligible.

The chemical species transport equation was solved for water with a kinematic diffusivity calculated according to the ethanol-water ratio, as discussed in the bulk of the current research. However, the mass fraction of ethanol was obtained via the material balance of the chemical species considered. Therefore, we did not solve a transport equation for ethanol.

The flow was set as laminar, and the inlet velocities had been prescribed at then flow inlets. At the flow outlet, a zero gradient was set. In addition, non-slip conditions have been set for solid surfaces.

The convection terms of the transport equations have been discretized using a first-order upwind scheme, and the convergence criterion was set to $1x10^{-4}$. The selection of the convergence criterion was based on the comparison with experimental data. Therefore, it was based on the compromise between computational time and an acceptable level of agreement with available experimental data.

All simulations have been computed in a local workstation with 8 Intel I7 3.4 GHz processors.

Following the research conducted by Glatzel *et al.*[6], all CFD codes applied to microfluidics problems have their strengths and weakness. Hence, considering the nature of the problem (convection-diffusion problem) under investigation, we selected the discretization strategy based on the finite volume method. Moreover, since the drawback

of higher-order reconstructions schemes is not present in the class of problems we are investigating, we selected ANSYS-CFX to model our case.

Supplementary References:

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