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Supporting information

for

Horseshoe lamination mixer (HLM) sets new standards in the production of monodisperse lipid nanoparticles

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Mesh Benchmark and validation for the simulation of concentration distribution

A parameter study was performed to investigate the influence of mesh on the simulation results of organic phase concentration distributions. The settings for the simulation and the determination of the mixture index are described in chapter 2.3.1 of the article. A model of the HLM system is simulated with different number of mesh elements (Table S1), which is gradually increased for a finer mesh each time a domain is calculated. The HLM system for simulation contained a simplified nozzle without pillar grid inside and with the inlet of the organic phase directly in the nozzle. The nozzle was followed by two HLM elements (Figure S1). The total liquid flow rate Q_L and the flow rate ratio of the organic phase φ_{OP} were set to 100 µl/min and 10%, respectively. The mixing index MI for a certain mesh i was determined at three different positions in the HLM system, namely before the first HLM element, after the first HLM element, and after the second HLM element.



Figure S1: 3D model of the mixing channel with two HLM elements, which is an inverted version of the Computer-aided design (CAD) of the HLM system used for printing by 2PP.

Table S1: Number of elements for	each mesh used for the	mesh benchmark. The	volume of the model
was $9.26 \cdot 10^{-11} m^3$.			

Mesh ⁱ	Number of mesh elements
1	1865661
2	6371881
3	10142752
4	12964834

Figure S2a shows the effect of the meshes i = 1..4 on the mixing index MI_i at three planes in the HLM system, while in Figure S2b the relative change in mixing index from one mesh to the next finer mesh $(1 - MI_{i+1}/MI_i)$ is given. A change is observed at the first plane when the mesh is refined from i = 3 to i = 4, but at the second and third plane changes due to mesh refinements became marginal. At the second HLM element, the change in the mixing index are already less than one 1% and with the complex geometry of the HLM system the available

computer resources were preventing refinements of the mesh beyond i=4. Only before the first element the mixing can be considered slightly overestimated.



Figure S2: a) Influence of the mesh refinement step i on the mixing index. The mixing index values were determined at three positions, namely before the first HLM element, after the first and the second HLM element. b) The relative change in MI from one refinement step to the next calculated as $1 - MI_{i+1}/MI_i$

The accuracy of the numerical model with settings as described in chapters 2.3.1 and 2.3.2 was validated on the basis of experimental data. For the experiment, a simplified system without mixing elements but with the nozzle for the injection of the central phase was selected. After the injection, the channel had a rectangular shape with the dimensions of 250 μ m x 100 μ m and was rotated by 45° so that the channel appeared vertical under the microscope (Figure S3a). In the simulation, the nozzle did not need to contain a pillar grid because homogeneous injection of the central phase through the nozzle was assumed (Figure S3b). The length of the simulated channel was identical to the distance between the nozzle and the observation point under the microscope in the experiment. The working fluids are water and ethanol with a diffusion coefficient of $1.2 \cdot 10^{-9} m^2/s$ at 293.15 K. The mutually influencing properties of the fluids during the mixing are linearly interpolated. 100% water was assigned to the inlet for the surrounding phase and 100% ethanol to the inlet for the central phase.



Figure S3: a) CAD model used for 2PP printing the simplified mixing system for the validation experiment. The end of the CAD model towards the outlet is not shown. b) Inverted CAD model as used for the correlating concentration distribution simulation.

The results of the validation experiment using iodine and sodium thiosulfate solutions at a flow rate ratio of 10% and of a corresponding simulation are shown in Figure S4. Microscope images were taken at the observation point showing iodine which was injected through the nozzle in the form of a centered thin yellow-brown sheet surrounded by sodium thiosulfate at flow rates of 25 µl/min and 50 µl/min (Figure S4a). At 22 µl/min more time is given for the diffusion of the solutions and the iodine has completely decolorized indicating perfect mixing. Therefore, a Q_L of 22 µl/min and flow rate ratio of 10% were assumed for the simulation resulting in the displayed concentration distributions in the nozzle and in the main channel. The streamline cross sectional pattern of the injected phase at the position corresponding to the experimental observation point shows a slight bending at the edges due to the 45° rotation of the channel. As in the mixing experiment, the two liquid phases are separated directly behind the nozzle. The central phase continues to diffuse out as it moves through the channel (Figure S4b) and becomes not perfectly but well mixed with the enveloping phase at the position of the observation point. Even though this rather simplified validation experiment is compared with simulations where the diffusion coefficient differs from the liquids in the experiment and

should therefore not be overinterpreted, it already confirms that the prediction of mixing performance based on our simulation appears reasonable.



Figure S4: a) Microscopic images taken from the simplified system at the observation point for different flow rates while the flow rate ratio was set to 10%. b) Concentration distributions with false color coding from the simulation at a flow rate of 22 μ l/min while the flow rate ratio was set to 10%. In addition, a cross sectional view at the observation point of the channel is included to display the streamlines of the central phase and the corresponding concentration distribution.

Table S2: Flow rates for system B at different flow rate ratios of the organic phase φ_{0P} and the shield phase φ_{SP} .

Total volume	Aqueous phase	Organic	phase	Shield phase			
flow rate [µl/min]	Flow rate [µl/min]	Flow rateFlow rateFlow[µl/min]ratio φ_{OP} [%][µl/n]		Flow rate ratio ^{<i>φ</i>_{SP}} [%]	Flow rate [µl/min]		
100	89	10	10	1	1		
100	87	10	10	3	3		
100	85	10	10	5	5		
100	83	10	10	7	7		



Figure S5: Cross-sectional views of simulated streamlines of the organic phase obtained at a flow rate ratio φ_{0P} of 10% in system at different total flow rates Q_T which are given in the images together with the resulting Reynolds numbers (Re) and Dean numbers (De). The cross section is positioned in the second HLM element showing the folding at the point with the strongest influence of Dean effect. Only at 400 µl/min and above the centrifugal forces increasingly influence the streamlines of the organic phase.

	Total volume flow rate ^Q _T [µl/min]																
	1	00	2	200	0 300		4	400		500	600		700		8	800	
	t_{RT}	Mixing	t_{RT}	Mixing	t_{RT}	Mixing	t_{RT}	Mixing	t _{RT}	Mixing	t_{RT}	Mixing	t_{RT}	Mixing	t_{RT}	Mixing	
Element	luisi	muex	luisi	muex	luisi	muex	luisi	muex	luel	muex	luisi	muex	liusi	muex	luel	muex	
Nozzle	0	3.2E-7	0	6.4E-7	0	4.5E-7	0	4.0E-7	0	3.7E-7	0	3.6E-7	0	3.6E-7	0	3.5E-7	
Before 1 st element	6.3	0.58	3.2	0.57	2.1	0.56	1.6	0.56	1.3	0.55	1.1	0.54	0.9	0.53	0.8	0.52	
1	14.7	0.76	7.3	0.73	4.9	0.73	3.7	0.73	2.9	0.73	2.4	0.73	2.1	0.73	1.8	0.73	
2	23.0	0.87	11.5	0.85	7.7	0.84	5.8	0.83	4.6	0.82	3.8	0.82	3.3	0.81	2.9	0.81	
3	31.4	0.91	15.7	0.90	10.5	0.90	7.9	0.89	6.3	0.89	5.2	0.88	4.5	0.88	3.9	0.88	
4	39.8	0.92	19.9	0.92	13.3	0.92	9.9	0.92	8.0	0.91	6.6	0.91	5.7	0.91	5.0	0.91	
5	48.2	0.94	24.1	0.93	16.1	0.93	12.0	0.93	9.6	0.93	8.0	0.93	6.9	0.93	6.0	0.93	
6	56.5	0.95	28.3	0.94	18.8	0.94	14.1	0.94	11.3	0.94	9.4	0.94	8.1	0.94	7.1	0.94	
7	64.9	0.96	32.5	0.95	21.6	0.95	16.2	0.95	13.0	0.95	10.8	0.95	9.3	0.95	8.1	0.95	
8	73.3	0.97	36.6	0.96	24.4	0.96	18.3	0.95	14.7	0.95	12.2	0.95	10.5	0.95	9.2	0.95	
9	81.7	0.98	40.8	0.97	27.2	0.97	20.4	0.96	16.3	0.96	13.6	0.96	11.7	0.96	10.2	0.96	
10	90.0	0.98	45.0	0.98	30.0	0.97	22.5	0.97	18.0	0.97	15.0	0.97	12.9	0.97	11.3	0.97	
11	98.4	0.99	49.2	0.98	32.8	0.98	24.6	0.98	19.7	0.97	16.4	0.97	14.1	0.97	12.3	0.97	
12	106.8	0.99	53.4	0.98	35.6	0.98	26.7	0.98	21.4	0.98	17.8	0.98	15.3	0.98	13.3	0.98	
13	115.1	0.99	57.6	0.99	38.4	0.98	28.8	0.98	23.0	0.98	19.2	0.98	16.4	0.98	14.4	0.98	

Table S3: Calculated mixing indices and elapsed residence time t_{RT} for system A at different total volume flow rates Q_T . The cells with a mixing index greater than 0.90 and 0.98 were marked in green.

 \leftarrow Mixing index greater than 0.90

← Mixing index greater than 0.98

Table S4: Measured mixing time of the iodine solution with sodium thiosulfate solution at different flow rate ratios φ_{OP} of the organic phase in system A. Four different channel systems were used: The first system contained only the nozzle injection while the other systems additionally contained one, two and three HLM elements. The relative decrease of the mixing time from one channel system to the next channel system were calculated.

	Minimum red	quired flow ra	te $\left[\frac{\mu l}{min}\right]$ / Mixi	Relative decrease of the mixing time				
Flow rate ratio of organic phase φ_{0P}	Only nozzle injection	1 Element	2 Elements	3 Elements	1 Element / Nozzle	2 Elements / 1 Element	3 Elements / 2 Elements	
5%	85 / 67	350 / 16	775 / 7	1200 / 4	76%	56%	37%	
10%	22 / 260	75 / 75	210 / 26	410 / 13	71%	65%	50%	
15%	8 / 714	31 / 180	100 / 55	130 / 41	75%	70%	25%	
20%	3 / 1903	15 / 373	62 / 88	75 / 71	80%	76%	19%	
Volume* $[10^{-11} m^3]$	9.514	9.315	9.116	8.917		Average		
Distance nozzle – observation point $[10^{-3} m]$		3	.7		76%	67%	33%	

* The volume between the nozzle and the observation point varies between the systems with different amounts of HLM elements

Table S5: Influence of total volume flow rate Q_T and lipid concentration c_{Lip} of castor oil on z-average values d_{LNP} , PDI values and yield of lipid processing Y_{LP} in system A. The flow rate ratio φ_{OP} of the organic phase was set to 10%. The yield was calculated as $Y_{LP} = c_{Lip} \cdot \varphi_{OP} \cdot Q_T$. The values of the HLM system were compared with the values from the CLM system.¹

	z-Average diameter ^d _{LNP} [nm] PDI [-]					Lipid processing Y_{LP} [mg/h]						
Lipid concentration $c_{Lip} [mg/ml]$ Flow rate $Q_T [\mu l/min]$	5	10	20	40	5	10	20	40	5	10	20	40
HLM system												
100	71	90	118	166	0.04	0.05	0.06	0.08	3.0	6.0	12.0	24.0
200	64	83	111	165	0.04	0.05	0.05	0.09	6.0	12.0	24.0	48.0
400	55	73	100	156	0.05	0.05	0.07	0.09	12.0	24.0	48.0	96.0
800	48	64	90	142	0.07	0.07	0.09	0.11	24.0	48.0	96.0	192.0
				CLN	system	1		·			•	
50	108	144	174	-	0.04	0.06	0.07	-	1.5	3.0	6.0	-
100	85	116	153	-	0.06	0.07	0.08	-	3.0	6.0	12.0	-
200	77	103	137	-	0.06	0.06	0.09	-	6.0	12.0	24.0	-
400	75	102	140	-	0.07	0.08	0.10	-	12.0	24.0	48.0	-
										← PDI s	maller that	an 0.07

References

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