

## Summary

**M**ICROFLUIDIC reactor technology is receiving growing interest, as it is believed to provide new opportunities for the chemical and process industries. The highly efficient mass and heat transfer combined with the unprecedented control over the tiny volumes has accelerated development in biochemical, biomedical, and pharmaceutical fields for laboratory automation and industrial manufacturing, especially after the global pandemic in 2020, where research and production of vaccines have been crucial.

The miniaturization of the processes allows the achievement of high reaction yields and selectivities, together with the possibility of taming potentially dangerous reactions safely. The interest is further motivated by the remarkable advances in the field of microfabrication through additive manufacturing (3D printing) techniques.

The present thesis proposes an experimental study joined with numerical modeling of passive microreactors, i.e., where mixing is promoted without the help of external energy sources. The aim is to understand how the flow regimes can influence the mixing and the reaction yield in clever but simple geometries at different operating conditions related to working fluids, reaction kinetics, and flow rate.

The first part of the work foresees the detailed characterization – by using microscope imaging techniques and numerical simulations – of the complex fluid dynamics followed by an analysis of the mixing and the reaction yield in the simplest passive microdevices, i.e., the T- and X-shaped reactors. For this purpose, a test reaction is selected being suitable with the adopted optical techniques and with the chemical times of the same order as the residence time in the fluidic reactors.

For numerical modeling, grid refinement techniques have been implemented in Computational Fluid Dynamics (CFD), increasing the resolution of the calculation grid in areas with high reaction rates. The validation of the methodology is pursued through qualitative and quantitative data analysis techniques. In particular, attention has been paid to the relationship between the mixing and the reaction timescales, identifying a semi-empirical correlation between the Reynolds and Damköhler numbers. Furthermore, a detailed comparison of the performance of the two reactors is also discussed to show the most convenient for the process intensification and the flow chemistry.

The second part of the thesis concerns the optimization of the design of a sequence of mixing elements, called pillars, to enhance mixing in the inertial flow regime. An optimization design software is exploited to predict the combination of the pillars in sequence and improve mixing under narrow working conditions.

Soft-lithography and advanced microscopy imaging are utilized to fabricate and explore the optimally designed microreactors. The experimental data assisted by CFD simulations validate the innovative design strategy of microreactors.

To further investigate the limits of the optimization, the mixing performance achieved within the pillar sequences is tested with varying geometrical and operating parameters, finding one sequence to be notably more robust and efficient than the others.

Then, this microreactor is analyzed for the mixing of water and ethanol, as this process is used to generate, e.g., good-quality lipid nanoparticles for the encapsulation of active pharmaceutical ingredients (APIs). The findings suggest that the optimal sequences of pillars could satisfy the requirements of these new emerging engineering processes.

## State of the Art

**T**RADITIONAL synthetic chemistry has predominantly consisted of macroscopic components during the past century, including round-bottomed flasks, beakers, test tubes, distillation columns, and reflux condensers. The fundamental practical techniques and associated equipment have remained essentially unchanged. The strategies acquired in semiconductor industries have allowed the creation of a new instrumental platform able to manipulate molecular reactions from the micro- to the nanoscale. Although the first example of a microfluidic device can be traced back to 1940, the development of micro total analysis systems ( $\mu$ -TAS) began years later. In particular, in the lately 1990s, the research for chemical synthesis in microfluidic reactors commenced spreading quickly in multiple establishments

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at GlaxoSmithKline (UK), Massachusetts Institute of Technology (USA), the Institut für Mikrotechnik Mainz (Germany), and Imperial College London (UK), amongst others.

Therefore, it is believed the miniaturization of chemical processes and analysis in microfluidic reactors has effective advantages that may affect molecular synthesis similarly to the way that the integrated circuit has defined the computer revolution over the past 50 years [1].

**Process intensification and sustainability** The microfluidic reactor, or so-called *microreactor*, is a device in which chemical reactions take place in volumes 5–9 orders of magnitude smaller than those associated with traditional synthetic chemistry. The channel diameter is less than a few millimeters and confines the fluids within the device to guarantee the advantages of downsizing. One peculiar feature of a microreactor is the high ratio surface/volume. For instance, consider a microreactor and a traditional stirred reactor having a diameter of 0.1 mm and 1 m, respectively. The ratio surface/volume of the former is four orders of magnitude higher than the macroscale reactor.

In general, a microreactor operates as follows. The reagents are fed into the device through two or more inlet channels, and, then, the streams meet together in an outlet channel – also called mixing channel – in which they mix and react within a residence time, depending on the channel length and the averaged flow velocity.

The microreactor size and material may change based on the purposes of the application and the type of reactive process, e.g., the desired productivity, the properties of the chemicals, or the reaction timescale. The reactor material usually is transparent for research and laboratory analysis to explore the microenvironment. For the industrial scale, microreactors may need more robust and high-resistant confinement. One of the most active players in this field is the Swiss-based company Lonza [2], but others can be listed including Alfa Laval, Corning, Microinnova, Chemtrix, Syrris, Precision NanoSystems, Dolomite Microfluidics, and FutureChemistry.

Since the 2000s, microreactor technology has gained increasing interest for a broad range of applications [3], especially the intensification of chemical reactions in pharmaceutical and fine chemical industries. The main reason for preferring microreactors over conventional batch-stirred reactors is the extraordinary control of the operating conditions that microdevices offer due to the enhanced mass and heat transfer [4].

Microreactors work with a well-defined residence time (strictly dependent on the flow rate and on the microreactor length), which is probably the main factor to be taken into account for the reaction optimization, especially in presence of unstable or short-lived reactive intermediates [5]. Due to the large surface-to-volume ratio that microdevices offer, the superior temperature control allows them to safely handle highly exothermic or dangerous reactions without the need of diluting the reactants, as conversely often required in conventional batch stirred vessels [4]. The above features result in improved yields and selectivities, recognizing the microreactors as the main route to *process intensification* and a *green and sustainable chemistry* [6, 7].

Moreover, microfluidic reactor technology can solve one of the main problems of conventional process design, namely the scale-up of chemical reactors from laboratory to industrial size, by using many microfluidic devices working in parallel (i.e., the so-called “numbering-up”) [8]. Therefore, as large volume production can be attained through massive parallelization of the micro-unit processes, reactor miniaturization has found many applications in diverse branches of engineering (e.g., in the bioengineering field for parallelization of drug encapsulation [9]). In particular, the pharmaceutical industry is naturally interested in microreactor technology as it reduces the time that is needed to bring a new compound or a new synthesis route from the research lab to its industrial production stage [10].

Roberge et al. [11] estimated that around 50% of the homogeneous reactions involved in pharmaceutical and fine chemistry industries would benefit from continuous processes in microreactors. Among them, we can identify: very fast reactions (i.e., with chemical timescale  $\tau_{\text{per}} < 1$  s) which are then controlled by mixing; rapid reactions (i.e., with  $1 \text{ s} < \tau_{\text{per}} < 10$  min) which are kinetically controlled but would benefit from the enhanced heat transfer; dangerous reactions. As concerns the former types of reactions, microreactors well allow exploring the potentialities of flash chemistry [12], through the conduction of extremely fast reactions in a highly controlled manner to produce desired compounds with high selectivity. Flash chemistry is indeed a novel concept in both organic and sustainable synthesis as its chemical transformations are practically impossible to be conducted using conventional batch conditions.

**Challenges** Fundamental research is needed to fully characterize and understand microreactor behavior under different operating conditions and, hence, to optimize their design and efficiency. In particular, the efficient mixing of the reactants is a critical step for many chemical processes [3], which significantly affect the production of organic chemicals (e.g., aromatic and organometallic products, biodiesel) and the quality of lipid, gold, and chitosan particles [4].

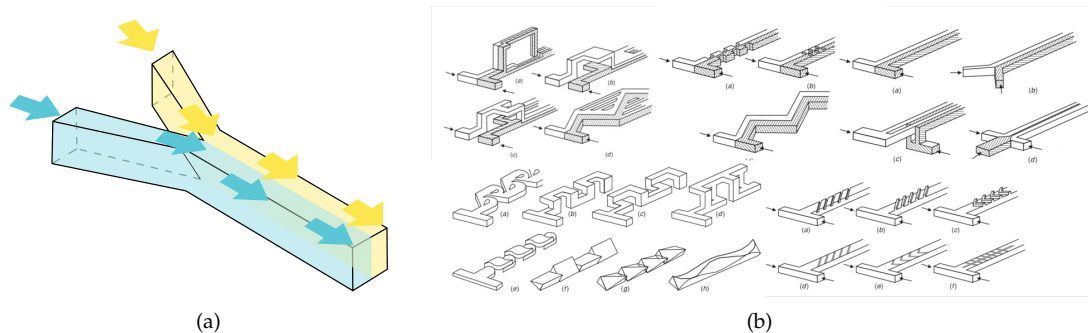


Figure 1: (a) Laminar flow of two liquid fluids in a microchannel. (b) Several sketches of micromixers analyzed in the literature (images from [16]).

In such small devices, the flow is laminar as the microfluidic system is characterized by a small Reynolds number (see Fig. 1a), i.e.,  $Re = d_h \cdot U/\nu \ll 2100$ , where  $d_h$  is the hydraulic diameter of the channel,  $U$  is the average velocity of the flow, and  $\nu$  is the fluid kinematic viscosity. When two homogeneous and miscible fluids are co-flowing in a channel of length  $L_o$ , molecular diffusion governs mass transport, and any mixing process will depend on the competition between flow speed and the diffusion rate. For such a microfluidic system, the required channel length to achieve full mixing  $L_{mix}$  can be estimated as  $L_{mix} \approx d_h \cdot Pe$ , with the Péclet number  $Pe = U \cdot d_h/D$  (which represents the ratio of convective to diffusive transport rate), where  $D$  is the diffusion coefficient.

If the convective transport rate dominates the rate of diffusion ( $Pe \gg 1$ ), the mixing length will be much longer than the microchannel size, i.e.,  $L_{mix}/d_h \gg 1$  [13], making microfluidic mixing especially challenging. This condition can occur either when large molecules are present (for example DNA molecules [14]); or where high flow rates are necessary (e.g. high-throughput production of lipid nanoparticles [9]); or to reach rapid mixing and manage fast and competitive reactions (for instance, cation-pool polymerization [15]).

Over the past two decades, a plethora of microfluidic mixers have been designed and studied (see Fig. 1b) [16]. There are generally two main categories of micromixers: active mixers, where mixing is enhanced *via* some external force, which disturbs and stirs the fluid; and passive mixers, where convective recirculations and vortices are induced to fold and extend the fluid-fluid interface, thereby reducing the lengthscale over which molecular diffusion must act to fully mix the fluid.

Active approaches can achieve high mixing performance, but they are typically more complicated and expensive to integrate and fabricate than passive mixers. On the other hand, passive mixers can also reach a high degree of mixing, but they may have significant pressure requirements to drive flow, and they should have a simple structure both for ease of manufacture and to avert channel clogging.

For instance, Stroock et al. [17] designed the gold-standard three-dimensional chaotic mixer, which performs rapid passive mixing thanks to the staggered herringbone grooves, which induce secondary flow that stretch and fold the co-flow interface. Although the chaotic mixer is an efficient micromixer [9, 17], the complex and small structure of grooves can become clogged, jeopardizing the mixing performance as reported by Maeki and Kimura's coworkers [18, 19] in the production of lipid nanoparticles. Because of this, Kimura et al. [19] suggested that a micromixer with more simple extruded two-dimensional structures may be more suitable for passive micromixers to avoid clogging.

For these reasons, planar and simple designs are usually preferred over complex ones. Among them, shaped micromixers and two-dimensional obstacle sequences within a microchannel are the most studied.

**T- and X-junctions** Several investigations have characterized the flow regimes and the mixing performance on T- and Y-jet reactors, where the inlet channels joined perpendicularly to the outlet one. In shaped reactors (a detailed overview is provided by Camarri et al. [20]), different laminar flow regimes are triggered with the increase of the volumetric flow rate or the Reynolds number  $Re$ , such as segregated, vortex, and engulfment steady flow regimes. At higher fluid velocities, the flow becomes unsteady and periodic, and eventually chaotic. The most used reactor is the T-shaped microreactor, in which the engulfment flow regime can enhance significantly its performance. However, considering water as the working fluid, X-shaped microreactors provide a superior mixing enhancement compared to T-shaped devices [21], as the engulfment regime can be triggered for lower inlet velocities and form a strong central vortex [22], while it features only two co-rotating vortices in the T-shaped channel [23]. Only a few studies investigate the flow regimes occurring in the T-shaped micromixer when mixing

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liquids different from water [24], which are necessary to conduct chemical processes. Even lesser literary works deal with the flow regimes of impinging fluids in the X-shaped micromixer. Furthermore, not much is available concerning the optimization of microreactors, despite the fact that the coupling between mixing and chemical reaction has been widely investigated in general [13]. Remarkable exceptions are the works of Bothe et al. [25] and Plouffe et al. [26] where they studied for specific reactive systems how different flow regimes affected the reaction yield depending on the amount of formed interphase and the fluid residence time.

**Sequences of mixing units (MUs)** The steady flow regimes in the T- and Y-shaped channels can achieve lower mixing efficiencies compared to the unsteady ones found at higher Reynolds numbers ( $Re > 200$ ) [20]. For instance, the steady engulfment flow reaches an efficiency of 27% at  $Re = 160$  in a T-mixer when feeding water, while the unsteady engulfment flow provides about 60% mixing at  $Re = 300$  [20]. Therefore, adding mixing units (e.g., shaped obstacles) in sequence in a T-microchannel at steady flow conditions is a powerful method to improve fluid mixing by altering the flow direction and laminating the two streams with a higher surface area of contact.

Several strategies to design obstacle sequences have been investigated. A typical approach is to periodically repeat a shaped obstacle into the path of the main flow. As reported in the work of Bhagat et al. [27], where the authors carried out experiments on a Y-shaped micromixer with and without shaped obstacles in aligned or staggered configurations, a staggered layout of diamond shaped-obstacles provided the best performance for  $Re < 100$ .

A more nuanced design methodology is the optimization of one mixing structure made of more obstacles and then the iteration of this optimal arrangement to enhance the chaotic advection and, thus, the mixing efficiency. This approach was utilized by Wang et al. [28], who optimized a mixing unit composed of cylindrical obstacles through a genetic algorithm based on a database of two-dimensional CFD simulations with 6096 geometries. The results suggested that assembling a sequence of 11 copies of the optimal unit limited the mixing potential, and simulating the interdependences of all connected units could determine the optimal sequence combination.

For this reason, the optimization of multiple combinations of different mixing units seems to enable the full potential for mixing and reaction enhancement [29]. However, the latter method may need high computational power to simulate tens or hundreds of microreactor geometries (particularly for three-dimensional simulations) and as many devices and experiments for result validation. Recent works have investigated innovative predictive tools and models of mixing and micromixing to reduce the computational time of demanding CFD simulations [30, 31]. Still, such promising models require insightful tests about their accuracy and cost-effectiveness when optimizing geometries based on a vast selection of mixing elements or enhancing mixing for applicable reactive systems.

Besides, only a few studies have considered two different fluids with different viscosities/densities and non-ideal behaviors in microreactors [24]. This is a critical aspect to consider to develop practical applications, e.g., particle manufacturing or biofuel synthesis [9, 32]. For instance, water-ethanol mixing was numerically characterized by Alam et al. [33] in T-mixers with curved microchannels and two configurations of cylindrical obstacles. The best design could achieve above 70% of mixing within  $0.1 < Re < 60$ , although it appears they did not consider the composition-dependent physical properties of the mixture. Santana et al. [32] investigated a new millidevice for organic reactions through CFD numerical simulations on the mixing of vegetable oil-ethanol and water-ethanol systems. The "Elis" micromixer could achieve mixing indices above 90% for both mixtures thanks to the DoE (design of experiments) method to optimize internal walls and 14 circular obstacles.

In this context, the advancing research on the flow induced by cylindrical obstacles, i.e., pillars, seems promising to provide accurate passive flow control for mixing (as shown recently by Zhang et al. [34]). In fact, in the presence of finite inertia (i.e.,  $Re > 1$ ), pillars can induce secondary flows similar to the Dean vortices created in curved channels and can be placed at different lateral positions within the channel, enabling more complex manipulation of the fluid's cross-sectional composition [35, 36]. Different pillar sequence arrangements can be optimized to achieve a desired cross-sectional flow shape, which has been used to create microfibers with tailored cross-sectional structures or fabricate shaped 3D microparticles.

## Problem Addressed

**Limits** The limits regarding the chemical process miniaturization and the optimization of design and efficiency of passive milli- and microreactors are listed in the following:

- The mixing of fluids and reagents at the microscale is critical and challenging owing to the condition of high Péclet number ( $Pe \gg 1$ ) that denotes a slow diffusion mass-transfer compared to the

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fluid flow velocity;

- Simple designs of passive microreactors are preferred to simplify the microfabrication and integration of the device and also increase the operative life compared to complex geometries, in which clogging problems are more likely to happen;
- Only a few investigations on the mixing and design optimizations of microreactors consider the effect of composition-dependent physical properties of fluids that are used in practical applications;
- More studies are needed to comprehend the coupling between mixing and chemical reactions, ultimately affecting the reaction yield, for the complex and various flow regimes in the widely used T-shaped microreactor and the more recent X-shaped one;
- Most of the microreactor geometries are conceived by repeating the same geometric pattern (or mixing unit), thus such an aspect limits the potentialities in optimizing different MUs in a sequence leading to more efficient designs;
- The use of three-dimensional CFD is not practical in optimizing the combination of multiple different MUs in sequence.

**Research objectives** This Ph.D. thesis identifies two research objectives to overcome the limits previously shown. It is worth noting that the planar geometries here investigated consist of basic-shaped channels and simple mixing elements to facilitate the microfabrication of a device and its utilization. A description of the two research objectives follows.

- I. Objective I consists of understanding the role of the complex flow regimes on the yield of a chemical reaction in basic geometries, i.e., the T- and X-shaped microreactors. Then, identifying the relation of mixing and reaction timescales varying the chemical kinetics to assess the optimal operating conditions in the considered devices.
- II. Objective II consists of overcoming the dependency of high-demanding numerical simulations to efficiently optimize the design of a sequence made of simple geometrical elements of different sizes and positions. Thus, analyzing the effectiveness and the reliability of the optimization design method when fluids used for a target application are mixed.

The first objective is achieved by finding an appropriate chemical reaction for the T- and X- shaped reactors of mesoscale dimensions (order of 1 mm). This reactor size can better enable industrial-scale applications, as it allows to work at higher flow rates of fluids than the smaller reactors. The progress of the chemical reaction is traceable by using a microscope to evaluate the reaction yield for all flow regimes occurring in the devices. For this reason, the limiting reagent is a dye that fades with the reaction course.

A catalyst is used to tune the reaction kinetics by simply modifying its concentration. Hence, it is possible to describe several reaction timescales at the same operating condition in the microreactors, and, in addition, the chemical reaction can also represent other reactive systems studied in the literature.

Three-dimensional CFD simulations are performed to elucidate the fluid mechanics within the device. To earn reliable solutions by capturing the smallest length scales of the mixing and reactive phenomena [31, 37, 38], grid adaptation techniques are studied and tested. Although time-consuming in terms of computational time, this numerical tool is necessary to obtain consistent results with experiments.

Furthermore, an advanced optical technique is exploited to improve the quality of the validations between the experiments and the simulations for time-dependent flow regimes, i.e., the micro Particle Image Velocimetry ( $\mu$ -PIV).

The collaboration with the bioengineering department at UCLA (University of California, Los Angeles) has allowed exploiting an optimization design software of cylindrical obstacles in a microchannel, which is called FlowSculpt (FS, see [www.flowsculpt.org](http://www.flowsculpt.org)). These obstacles can have several configurations based on the combination of size and position, so they effectively represent multiple MUs. It is worth noting that the software can optimize and simulate the fluid flow neglecting diffusion and one Reynolds number at a time.

Thanks to this joint work, the software functionality is addressed to solve the mixing optimization problem of the same fluids at the microscale. Thus, this advanced numerical tool is used to achieve optimized designs of passive micromixers through the development of a new algorithm in Matlab<sup>®</sup> and C++ programming languages. Confocal laser scanning imaging and CFD simulations are adopted to validate the results obtained in FS and, then, to extend the investigation.

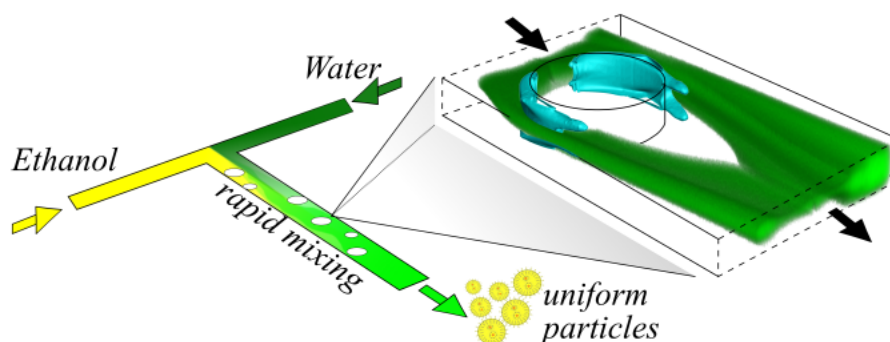


Figure 2: Accurate prediction of the inertial flow induced by pillars enables optimization of mixing and intensification of chemical processes, such as particle production. Note that the green stream is a 3D-experimental flow visualization united with the numerical representation of laminar vortices that amplify the reactive interface.

Soft-lithography techniques are used to fabricate the microscale reactors. Furthermore, the channel dimension is designed to respect the prerequisites for a chosen engineering process, i.e., the production of lipid nanoparticles. Indeed, the particle qualities depend on the rapid dissolution of ethanol in water. The process is relevant for encapsulating active pharmaceutical ingredients (API) for drug delivery and cell targeting purposes, but it has been especially important in the production of vaccines during the pandemic period [39].

## Key Innovations and applications

The strengths of this work are the new methods that have been introduced, such as the characterization of mixing performance with the Interface Stretching Function, which measures the interfacial area per volume available for diffusion and micro-mixing in FS, together with analytical and holistic functions (Greedy, FIF and IAF) which were used to generate an optimal mixing design.

The experiments and simulations have been combined to gain insights that are not possible with just one of the two methods. For example, by using the criteria as the  $\lambda_2$ -vortex indicator [40] for CFD results and combining them with 3D mixing structures measured by laser scanning confocal microscopy, new insights into the mixing process and mixing times were obtained that represent a breakthrough in the understanding of the interaction between fluid dynamics and chemical reactions (see Fig. 2).

The tradeoff between prediction accuracy and cost of CFD simulations was achieved thanks to new grid refinement techniques, which allowed refinement of the calculation grid in the diffusion and reaction zones of the reactants.

An innovative chemical test reaction has been used to characterize the performance of microreactors and the behavior of mixing and reaction timescales.

Possible application impact of the results obtained. The ability to manipulate the fluid reactive interface is of utmost relevance in the optimization and design of microreactors, both on a laboratory scale (in situ creation of drugs for personalized medicine) and an industrial scale. For example, in 2020 Pfizer carried out mass production of the COVID-19 vaccine in modular fluidic reactor chambers, similar to those studied in the present work. In particular, the X-shaped reactor shows higher performances than the common T-shaped one and similarities to the well-known and used confined impinging jet reactor [39, 41]. Overall, the X-shaped millireactor and the IAF microreactor seem promising for the intensification of organic syntheses and particle manufacturing (a list of such processes is available in the supplementary material of Elvira et al. [4]).

The development and testing of a numerical tool for mixing-reaction design and optimization based on interfacial stretching can be used by research centers and industries as an alternative to CFD, which is much more expensive in terms of computational time for the same resources.

## Results

Two parts show the result and discussion of this work. Part I provides an analysis of the relationship between mixing and reaction, changing the flow conditions in two microreactors. Part II deals with the design optimization of multiple MUs (mixing units) for a specific flow rate and the next investigation on the performance of the created mixers for different flow rates and working fluids.

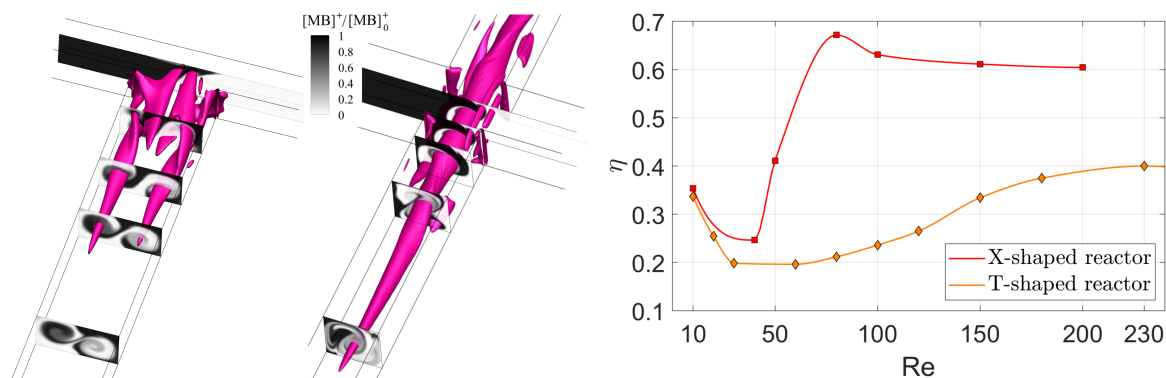


Figure 3: Engulfment flow regime in the T and X-microreactors and reaction yield as a function of Reynolds number.

**Part I** The influence of the flow regimes on mixing and reaction efficiencies are observed for several Reynolds numbers and kinetic constants. The relation between mixing and chemical reaction is analyzed for Reynolds and Damköhler numbers varying about three orders of magnitude within the range  $Re = [5 - 280]$  and  $Da = [0.3 - 95]$ .

A mutual validation is accomplished between the experimental and numerical results. The predicted reaction yield shows remarkable congruence with the experimental results. This match is partially due to the grid refinement strategy adopted in the numerical simulations to better capture the small mixing and reaction length scales in the reaction region, despite the numerical tool being quite time-consuming. T- and X-shaped microreactors are considered. The inlet fluids consist of aqueous solutions of a blue dye (i.e., methylene blue) and ascorbic acid that can react with one another to generate a colorless product. In this way, it is possible to monitor the reaction progress through the flow visualization. In the colored stream, hydrogen chloride is added as the catalyst of the reaction, managing to set different kinetic constants. In particular, three values of the kinetic constant  $k_{r,0}$  have been investigated and, for each one, different values of the Reynolds number in both reactors. The ascorbic acid concentration is kept the same in all cases.

At low Reynolds numbers, the two streams remain segregated with the aqueous solution of ascorbic acid stratifying on the bottom of the mixing channel. In the stratified regime, mixing occurs only by diffusion in T- and X-microreactors and depends on the fluid residence time. More details on the effect of stratification on the mixing and reaction yield in a T-shaped microreactor are shown in [42].

By increasing the Reynolds number, three-dimensional vortical structures are formed, leading to four counter-rotating vortices in the mixing channel in the T-shaped reactor. The symmetric vortices are not equal in strength because of the different physical properties of the fluid streams, which improved the mixing as the flow pattern is asymmetric compared to the single-fluid case.

The mixing efficiency is higher for  $Re > 150$ , where the engulfment regime broke the flow symmetries, and only two co-rotating vortical structures endure in the mixing channel.

Two unsteady regimes, i.e., asymmetric and symmetric, are described with the same features already observed for the non-reactive flows. The unsteady regimes could additionally enhance convection thanks to the increase of the fluid inertia and the intensity of the periodic formation-annihilation of the laminar vortices.

Noteworthy, the flow becomes engulfed at a lower Reynolds number in the X-shaped microreactor, i.e.,  $Re \approx 50$ , occurring at approximately the same Reynolds number found with water as the working fluid. Here, the impinging streams generate a central vortical structure in the mixing channel extending towards the outlets. For  $Re > 80$ , two counter-rotating lateral vortices joined the central vortex to further improve the mixing of the reagents.

For both reactors, the flow regimes strongly affect both mixing and reactive performances.

In the segregated regime, the reaction yield decreases with the increase of the Reynolds number. Indeed, the mixing occurs mainly by molecular diffusion, and the extension of the reaction is only slightly dependent on the fluid velocity and the  $Re$ . Consequently, the increase in bulk velocity reduces the reaction yield due to the lowering of the residence time that limited the reaction progress.

Confirmation of such a theory is the reaction yield behavior, which fits by  $Da^{0.3}$  without any explicit dependency on reaction kinetics ( $k_{r,0}$ ). Indeed, the residence-to-chemical timescale ratio is the key factor dominating the reaction rate in the segregated regime.

In the T-microreactor, the interface between the two streams becomes larger in the vortex and engulfment regimes, increasing the mixing efficiency and the reaction yield. The growth of the interface and the reaction front is a function of the Reynolds number as the reaction yield in the engulfment regime was found to be  $\eta \propto \tilde{k}_r^{0.5} Re^{0.4}$ . Intrinsically, the gain in efficiency due to better mixing increases for larger kinetic constants.

The same scenario occurs in the periodic asymmetric regime. With increasing  $Re$ , the yield enhancement is larger for higher  $k_{r,0}$ , even higher than the one it would be estimated by considering the relationship  $\eta \propto \tilde{k}_r^{0.1} Da^{0.4}$  developed for the steady regime. Indeed, the yield increases by more than 30% when moving from the steady flow at  $Re = 280$  to the unsteady flow at  $Re = 320$ .

Nevertheless, the onset of the unsteady symmetric regime at higher flow rates achieves  $\eta \approx 11\%$  at  $Re = 650$ , which is lower than the outcomes of the steady segregated regime, as the very low residence time hampers the reaction progress.

The X-microreactor, instead, forms a central vortex that grows significantly for  $Re < 80$ , enhancing the extension of the reaction front that corresponds with the interface amplification. Consequently, the engulfment regime increases the reaction yield thanks to a significant boost of the convective mixing, reaching the highest value at  $Re = 80$ , where the best compromise between mixing degree and residence time is observed inside the device. The intense growth of the central vortex and the associated mixing rate might explain the higher sensitivity to the  $Da$  in the X-micromixer compared to the T-shaped one. It is possible suggesting that the presence of two outlet channels in the X-shaped reactor promotes the development of stronger vortical structures compared to the T-reactor configuration. Indeed, Ait-Mouheb et al. [21] believe that the X-channel configuration has a higher degree of fluid freedom, allowing an easier rotation of the fluid in comparison with the T geometry.

In the X-shaped microreactor, the yield remained approximately constant for  $100 < Re < 200$ . A possible explanation is that, when the Reynolds number is increased in such an interval, the growth rate of the interfacial stretching is almost equivalent to the decrease in residence time, therefore the yield does not significantly change. Furthermore, the reaction yield trend fit again by  $Da^{0.3}$ , similar to the one found in the stratified regime, but it was still dependent on the reaction kinetics.

**Part II** FlowSculpt (FS), a software for the efficient design of flow sculpting devices, is exploited to find optimized designs for mixing in microfluidic devices that are characterized by very small diffusion lengths ( $Pe \gg 1$ ) when the flow is inertial ( $Re > 1$ ). In particular, FS can rapidly predict the convection and the flow pattern (or shapes) in sequences of cylindrical obstacles (i.e., pillars), neglecting any diffusion model, and use these predictions in a genetic algorithm. Thanks to the creation of a fitness function to measure mixing ad hoc – the Interface Stretching Function (ISF) – sequences of 20 cylindrical pillars are designed as individual mixing units using holistic and analytic optimization at  $Re = 40$ . As a result, the clever arrangement of sequential mixing units – rather than simply duplicating a particular unit – can dramatically enhance the mixing. Two holistic objective functions, i.e., FIF and IAF, consider how an entire sequence of MUs affected mixing efficiency using the ISF in the FlowSculpt software’s genetic algorithm. The “Greedy” strategy is adopted to provide a contrast against the holistic methods. The FIF- and IAF-designed devices show a high mixing efficiency and rate, where the analytic function (Greedy) was limited. The optimization and control of predicted fluid flow shapes are confirmed by visualization and analysis of numerical and experimental images on several cross-sections of the mixing channels of the optimal sequences. The performance parameters defined as MI and ISF show a fitting of about 80% as the high Péclet numbers ( $Pe \approx O(10^{5-6})$ ) characterized the system. Despite the mixing performance more quickly deviates from FlowSculpt-predictions in microfluidic systems with lower  $Pe$ , the efficiency becomes higher than the case with higher  $Pe$ , as diffusion is faster maintaining the same fluid residence time.

Given the narrow optimization and operating conditions, additional investigations have been carried out.

The tradeoff between the mixing index and pressure drop is analyzed by modifying the inter-pillar spacing in the CFD simulations, finding an optimal inter-pillar spacing of  $6d_p$  per mixing unit at  $Re = 40$ .

Then, the efficiency of the microreactors is tested by varying the  $Re$  between 10–100, and the holistic designs exhibited superior mixing performance over all the operational intervals than the analytic one. The IAF sequence is particularly effective, holding a high efficiency over the largest span of  $Re$ .

Hence, to explain such a performance in the IAF sequence, experiments and CFD simulations were carried out to gain insight into the fluid dynamics around the pillars spanning the Reynolds number in the interval 10–100. Through the post-processed flow visualizations, symmetric horseshoe vortices are observed in the inertial flow regime that can significantly influence the mixing mechanism by improving the stretching of the fluid contact surfaces and the convective mixing when both inlet streams flow inside the vortical structures. Furthermore, with the increase of the Reynolds number, the laminar vortices



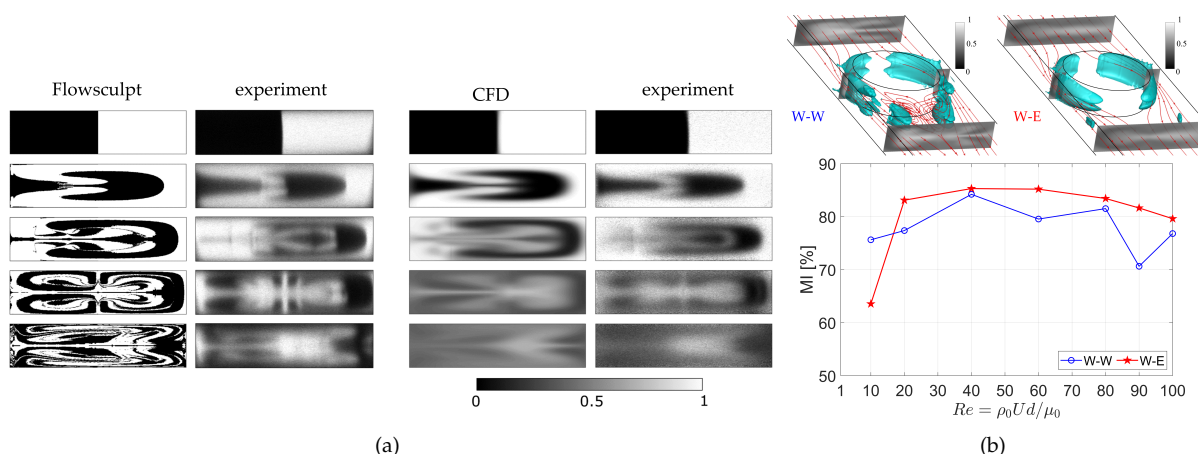


Figure 4: (a) Comparison of confocal images with the FS (on the left) and CFD (on the right) predictions at the inlet section and after pillars 5,10,15, and 20 (from the top to the bottom). (b) Horseshoe vortices at pillar 15 on the top and mixing efficiency of the IAF sequence on the bottom.

become more intense and extended in the post-pillar regions.

Eventually, the flows of water-water (W-W) and water-ethanol (W-E) mixtures are analyzed for  $Re = 10 - 100$  to fulfill the investigation on the limit conditions of FS and evaluate the mixing efficiency for fluids commonly used in lipid nanoparticle production. Indeed, the size and length of the optimal micromixers are designed with the necessary residence time required in this application, ranging approximatively in the interval 0.016-0.16 s.

The experiments and the numerical simulation show consistent results validating the applied methodology. In particular, it is found that an increase in dynamic viscosity occurred in parallel with the mixing enhancement, as the physical properties of the W-E mixture are non-linear and composition-dependent. This effect generated a substantial lowering of the Reynolds number that led to weaker laminar vortices around the pillars. Although the decrease of the fluid inertia could hamper the mixing performance, the diffusion of the W-E mixture allows for achieving high efficiencies reaching values over 80% in the IAF and FIF sequences in the range  $Re = 20 - 100$ .

Pressure drops are quantified in both mixtures, indicating a significant demand in the W-E system. Indeed, they could be related to the increase in dynamic viscosity that affects the distributed term of the pressure drops.

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